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6:2 and 8:2 Fluorotelomer Alcohol Anaerobic Biotransformation in Digester Sludge from a WWTP under Methanogenic Conditions

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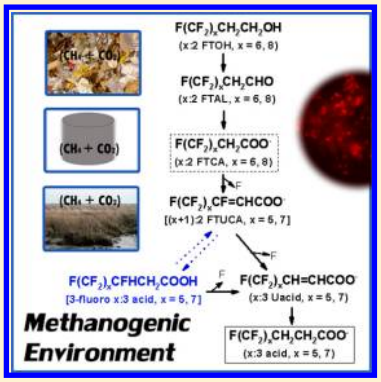
Supporting Information

ABSTRACT: 6:2 FTOH and 8:2 FTOH [FTOHs, $F(CF_2)_nCH_2CH_2OH$, n = 6, 8] are the principal polyfluorinated raw materials used to manufacture FTOH-based products, which may be released to WWTPs during their product life cycle. For the first time, anaerobic biotransformation of FTOHs and key biotransformation intermediates in WWTP digester sludge under methanogenic conditions was investigated. 6:2 FTOH was transformed to 6:2 FTCA, [$F(CF_2)_6CH_2COOH$, 32−43 mol %], 6:2 FTUCA [$F(CF_2)_5CF{=}CHCOOH$, 1.8−8.0 mol %], and 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$, 18−23 mol %] by day 90 and day 176 in two separate studies. 8:2 FTOH was transformed by day 181 to 8:2 FTCA (18 mol %), 8:2 FTUCA (5.1 mol %), and 7:3 acid (27 mol %). 6:2 and 8:2 FTOH anaerobic biotransformation led to low levels of perfluorohexanoic acid (PFHxA, ≤0.4 mol %) and perfluorooctanoic acid (PFOA, 0.3 mol %), respectively. 6:2 FTUCA anaerobic biotransformation led to a newly identified novel transient intermediate 3-fluoro 5:3 acid [$F(CF_2)_5CFHCH_2COOH$] and 5:3 acid, but not 5:2 sFTOH [$F(CF_2)_5CH(OH)CH_3$] and α−OH 5:3 acid [$F(CF_2)_5CH_2CH(OH)COOH$], two precursors leading to PFPeA (perfluoropentanoic acid) and PFHxA. Thus, FTOH anaerobic biotransformation pathways operated by microbes in the environment was likely inefficient at shortening carbon chains of FTOHs to form PFCAs (perfluorinated carboxylic acids). These results imply that anaerobic biotransformation of FTOH-based products may produce polyfluorinated acids, but is not likely a major source of PFCAs detected in anaerobic environmental matrices such as anaerobic digester sludge, landfill leachate, and anaerobic sediment under methanogenic conditions.



INTRODUCTION

Perfluoroalkane sulfonates [PFSAs, $F(CF_2)_nSO_3^-$, n = 4, 6, 8] and perfluoroalkyl carboxylates [PFCAs, $F(CF_2)_nCOO^-$, n = 4−12] are perfluoroalkyl acids (PFAAs) detected in the environment and biota[1−5] due to their historical uses in consumer and industrial products. PFAAs found in the environment may come from direct emission or from indirect precursor degradation.[6−8] Substantial information is available on abiotic[9,10] and aerobic microbial biodegradation of PFSA precursors[11] and PFCA precursors.[12−18] In contrast, no published information is available on precursor anaerobic microbial biodegradation in the environment. Knowledge of precursor biodegradation potential under both aerobic and anaerobic conditions is essential to understand the relative contributions of precursor biodegradation versus direct product emission to PFAAs detected in the environment. For example, fluorotelomer alcohol [FTOH, $F(CF_2)_nCH_2CH_2OH$, n = 6, 8] biodegradation was cited as a potential major source of PFCAs detected in landfill leachate[19] based on inference with no knowledge of FTOH anaerobic biotransformation potential or yields to polyfluorinated acids and PFCAs under methanogenic conditions.

6:2 and 8:2 FTOHs are the principal raw materials used to manufacture FTOH-based products.[6,8] During the product life cycle, FTOH-based materials are released to aerobic and anaerobic environments and potentially subject to microbial biodegradation or biotransformation to form poly- and perfluorinated carboxylic acids and other intermediates.[14,16,17] 6:2 FTOH aerobic biotransformation in soil, sediment, and activated sludge formed PFCAs (e.g., PFBA, PFPeA, and PFHxA) and x:3 acids (e.g., 4:3 and 5:3 acids) as two principal classes of biotransformation products, with 5:2 sFTOH as the primary volatile intermediate and direct PFCA precursor.[17,20,21] Similarly, 8:2 FTOH aerobic biotransformation in soil and activated sludge formed PFHxA and PFOA, and 7:3 acid as two principal classes of stable biotransformation products, with 7:2 sFTOH as the major volatile intermediates and direct PFOA precursor.[14−16] Some FTOH-based products can be converted to corresponding FTOHs and subsequently to PFCAs and x:3

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acids under aerobic conditions in soil and activated sludge.[18,22−24]

Global methane emissions from earth biospheres were estimated to be about 0.18−0.38 × 10^{12} kg annually.[25] Methanogenic environments such as anaerobic digester tanks of sewage treatment plants, landfills, and wetlands (anaerobic sediment) account for 5%, 6%, and 22% of total annual global methane production, respectively.[25] These methanogenic environments are also potential entry points for anthropogenic chemicals such as FTOH-based products at the end of the product life cycle. Biochemical redox potential is about −0.24 V under anaerobic methanogenic conditions in the environment.[26] Anaerobic microbes deploy different metabolic strategies from microbes under aerobic conditions to metabolize organic nutrients and to degrade anthropogenic chemicals.[27] Some anaerobic bacteria are able to utilize short-chain (1−2 carbon-chain length) chlorinated chemicals such as chloroform and 1,2-dichloroethene as carbon sources for growth.[28]

PFSAs and PFCAs were detected in anaerobic digester sludge of WWTPs.[29] PFSAs, PFCAs, 6:2 and 8:2 fluorotelomer sulfonates, FTOHs, and polyfluorinated telomer acids were detected in landfill leachates.[19,30,31] These reports suggest that anaerobic biotransformation of FTOH-based products or FTOHs themselves by microbes may represent a source of PFCAs observed in digester tanks of WWTPs and in landfills. It is unknown to what extent FTOH degradation may contribute to PFCAs detected in digester sludge and landfill leachate, since no information is available on FTOH biodegradation rates to PFCAs under anaerobic methanogenic conditions.

The objective of this study was to determine the molar yields of PFCAs and polyfluorinated acids formed from 6:2 and 8:2 FTOH anaerobic biotransformation under methanogenic conditions to discern the sources of PFCAs detected in the environment. A further objective was to establish FTOH anaerobic biotransformation pathways and determine key steps affecting molar yields of PFCAs from 6:2 FTOH. Major and potential 6:2 FTOH transformation products (e.g., 6:2 FTUCA, 5:3 Uacid, 5:3 acid, and α−OH 5:3 acid) were also dosed to the anaerobic digester sludge to follow their further transformation products up to 90 d. This is the first study to investigate FTOH anaerobic biotransformation potential and mechanisms in anaerobic environments. In addition, 3-fluoro 5:3 acid was for the first time identified as a novel transformation product during 6:2 FTUCA anaerobic biotransformation.

MATERIALS AND METHODS

Chemicals. 6:2 FTOH was purchased from Sigma-Aldrich (St. Louis, MO) with 99% purity. [3-^{14}C] 8:2 FTOH [$F(CF_2)_7{}^{14}CF_2CH_2CH_2OH$] was custom synthesized by DuPont (Wilmington, DE) with virtually 100% radiochemical purity and ∼94% chemical purity with 7:2 alcohol [$F(CF_2)_7CH_2CH_2OH$] as the major chemical impurity. The use of [3-^{14}C] 8:2 FTOH helped to determine whether 8:2 FTOH parent and potential biotransformation products can be strongly absorbed to digester sludge during 8:2 FTOH anaerobic biotransformation. Other fluorinated chemical standards (>97% purity) used for LC/MS/MS (liquid chromatography/tandem mass spectrometry) analysis were described previously[16,17] and are listed in the Supporting Information (Table SI-1). 6:2 FTUCA [$F(CF_2)_5CF{=}CHCOOH$], 5:3 Uacid $F(CF_2)_5CH{=}CHCOOH$], 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$], and α−OH 5:3 acid [$F(CF_2)_5CH_2CH(OH)COOH$] were all custom synthesized by DuPont with chemical purity ≥96%. Stable isotope internal standards used for quantitative analysis by LC/MS/MS were [1,1,2,2-D;3-^{13}C] 6:2 FTOH [$F(CF_2)_5{}^{13}CF_2CD_2CD_2OH$], [1,1,2,2-D;3-^{13}C] 8:2 FTOH [$F(CF_2)_7{}^{13}CF_2CD_2CD_2OH$], [1,2-^{13}C] PFOA [$F(CF_2)_6{}^{13}CF_2{}^{13}COOH$] (DuPont, Wilmington, DE), and [1,2-^{13}C] PFHxA [$F(CF_2)_4{}^{13}CF_2{}^{13}COOH$] (Wellington Laboratories, Ontario, Canada). All solvents used were HPLC grade or higher and all other chemicals used in this study were at least reagent grade. Deionized water (18 MΩ cm) used was from a Barnstead E-Pure system. Omnisov water (EMD Chemicals, Gibbstown, NJ) or deionized water was used for LC/MS/MS analysis.

6:2 FTOH and 8:2 FTOH Biotransformation in Diluted Anaerobic Digester Sludge. The digester sludge used in this study was from a digester tank operated under mesophilic conditions with a sludge residence time of approximately two months. 6:2 FTOH biotransformation was investigated twice, about two years apart, to assess variation in transformation rates to other major products by different microbial inoculums from the same WWTP. A 90-d study is henceforth referred to as Study I and another 176-d study is henceforth referred to as Study II. Anaerobic digester sludge was collected from a domestic wastewater treatment plant in Delaware into 0.5-L polypropylene containers to the rim and immediately sealed with lids. The sludge was divided into several 1-L bottles (200 mL of sludge for each bottle) in an anaerobic chamber filled with nitrogen gas plus ∼1.5% hydrogen gas. The H_2 and the platinum catalyst inside the chamber were able to scrub trace O_2 that may have penetrated through the vinyl chamber wall. The bottles were loosely capped and incubated inside the anaerobic chamber at 29 °C (the maximum achievable temperature inside the anaerobic chamber) for approximately two weeks before the initiation of the experiments. Approximately 100−200 mL of the sludge was transferred into a 250−500 mL glass serum bottle and crimp sealed. The glass bottle containing the sludge was moved out of the chamber and incubated at 35 °C for monitoring the headspace gas pressure. Continuous increases of gas pressure inside the headspace of the glass serum bottle indicated CH_4 and CO_2 gas production and methanogenic conditions. Under such conditions, the sludge was deemed metabolically active and was used for anaerobic biotransformation studies. If no continuous gas pressure increases were observed inside the headspace of the glass serum bottle, the sludge was discarded.

Each experiment included live sludge, sterile control, and live control (dosed with a solvent only) samples. All the experimental manipulations for anaerobic setup were conducted inside the anaerobic chamber. The anaerobic nutrient solution was degassed, flushed with nitrogen gas, tightly sealed for storage in a refrigerator (∼ 4 °C), and transferred to the anaerobic chamber the night before the experiment initiation to remove residual oxygen gas. The sludge inoculums were also preincubated inside the anaerobic chamber prior to the experiment to ensure oxygen-free conditions. For live sludge treatment, one part of the sludge was mixed with 4 parts of the anaerobic nutrient media (see Table SI-2 of the Supporting Information (SI) for detailed components and concentrations) by constantly stirring the mixed solution inside a 1-L bottle. A 20-mL aliquot of the mixed solution was transferred into one of the 70-mL glass serum bottles. Each bottle was then dosed with 20 μL of stock solution of either 6:2 FTOH (1.6 mg L^{-1} final

concentration) made in 50% ethanol (one part ethanol mixed with one part H_2O, v/v) or [3-^{14}C] 8:2 FTOH (182 μg L^{-1} final concentration) made in pure ethanol. The starting concentrations of 6:2 FTOH and 8:2 FTOHs were within or near their aqueous solubility.[32] The starting concentrations of 6:2 and 8:2 FTOHs allowed to detect low levels (0.1−2 mol %) of parent and transformation products but not cause inhibitory effects on microbes. Each bottle was immediately crimp-sealed with a butyl rubber stopper and aluminum cap. For the live control, only 20 μL of 50% ethanol or pure ethanol was dosed to the sample bottles and the rest of the procedures were the same as live sludge samples. The live control was used for monitoring background levels of FTOHs, potential transformation products, pH, gas pressure, CH_4, H_2, and CO_2 concentrations in the headspace. Sterile control samples were prepared by autoclaving the mixture of live sludge and nutrient solution. After cooling inside the anaerobic chamber, triple antibiotics (kanamycin, chloramphenicol, cycloheximide) were added at ∼200 mg L^{-1} final concentration to inhibit potential microbial growth. Each bottle was then dosed with 20 μL of stock solution of 6:2 FTOH (1.4−1.8 mg L^{-1} final concentrations) or [3-^{14}C] 8:2 FTOH (174 μg L^{-1} final concentration). All the sample bottles were transferred into a 35 ± 2 °C constant temperature room and shaken twice daily except weekend and holidays to mix the water condensate on the sample bottle walls and the sample media.

Sampling and Sample Analysis. At each sampling time point (Days 0 to 181), 2−3 live sludge and sterile control bottles and two live untreated control bottles were processed for analysis. The headspace gas pressure in a live control bottle was measured by a manometer with a 23-gauge needle probe. The headspace CH_4, CO_2, and H_2 contents in another live control bottle were measured by an Agilent (Santa Clara, CA) model 3000A MicroGC with a thermal conductivity detector. The pH of the sample media in a live control bottle was measured with a combination pH electrode. The headspace of each live sludge and sterile bottle was purged through a C_{18} cartridge (0.6 g of sorbent, Alltech, Deerfield, IL) with an air pump at ∼2 L min^{-1} for 1−3 min to capture 6:2 FTOH or [3-^{14}C] 8:2 FTOH and potential volatile biotransformation products. Each C_{18} cartridge was eluted with 5 mL of acetonitrile for further analysis by LC/MS/MS or liquid scintillation counting to measure ^{14}C radioactivity. The butyl rubber stopper (septum) from each sample bottle was extracted overnight at 50 °C with 5 mL of acetonitrile in a 20-mL glass vial (upright position) with 200 rpm shaking in an orbital shaker. The remaining sample media in each of the sample bottles was extracted with 30 mL of acetonitrile after crimp-sealed with a fresh septum and an aluminum cap. The extraction was conducted at 50 °C in the dark for 1−3 days in an orbital shaker with 200 rpm horizontal shaking. The sludge aqueous extract in each sample bottle was centrifuged at 1500 rpm (∼ 400g) for 15−25 min, and the supernatant was decanted and filtered by a 0.45-μm pore nylon filter. All the processed sample solutions were stored at ∼ −15 °C in a freezer before analysis.

The potential nonextractable fraction in digester sludge dosed with [3-^{14}C] 8:2 FTOH was washed/centrifuged twice, and the resulting sludge solid was air-dried and used for thermal combustion to measure ^{14}C radioactivity. The combustion was done at 900 °C inside a quartz tube filled with cupric oxide catalyst in a Harvey (Tappan, NY) biological oxidizer, and the mineralized $^{14}CO_2$ was captured by a Harvey scintillation cocktail to measure ^{14}C activity by a Beckman (Fullerton, CA) LS 5000 TD liquid scintillation counter.

Biotransformation of 6:2 FTUCA, 5:3 Uacid, α−OH 5:3 Acid, and 5:3 Acid in Anaerobic Digester Sludge. To investigate the rate-limiting step(s) of 6:2 FTOH biotransformation under methanogenic conditions, similar experiments were conducted with known potential biotransformation products including 6:2 FTUCA, 5:3 Uacid, α−OH 5:3 acid, and 5:3 acid as starting precursors, respectively. The experimental procedures and sample processing were identical to 6:2 FTOH biotransformation experiments except that the starting concentrations of these compounds ranged from 2.3 to 2.7 mg L^{-1}.

LC/MS/MS Quantitative Analysis. The sludge extract, septum extract, and C18 cartridge eluent were analyzed separately by LC/MS/MS. Prior to analysis of 6:2 FTOH and transformation products, 50 μL of internal standards containing 0.200 mg L^{-1} of [1, 2-^{13}C] PFHxA and 5 mg L^{-1} of [1,1,2,2-D; 3-^{13}C] 6:2 FTOH were spiked to 1-mL sample solution for quantifying 6:2 FTOH precursor and transformation products. The analysis was done with a model 2795 HPLC/Waters Quattro Micro tandem mass spectrometry system (Waters, Milford, MA) performed in negative electrospray ionization mode with multiple reaction monitoring. Detailed information on instrumental parameters, HPLC column and mobile phase conditions, analyte ion transitions, sample injection volume, and limit of quantitation are described in Table SI-3.

Prior to analysis of [3-^{14}C] 8:2 FTOH and transformation products, each 0.40-mL sample solution was spiked with 20 μL of internal standards containing 25 μg L^{-1} of [1,2-^{13}C] PFOA and 3 mg L^{-1} of [1,1,2,2-D; 3-^{13}C] 8:2 FTOH for quantifying [3-^{14}C] 8:2 FTOH precursor and transformation products. Acetonitrile (0.18 mL) and 0.4 mL of acidified water containing 0.30% glacial acetic acid and HCl were then added to the sample solution to a final volume of 1.0 mL. The addition of acidified water was found to improve the chromatographic peak shape of each analyte. The analysis was done with an Agilent model 1100 HPLC/Sciex API 4000 tandem mass spectrometry system (Applied Biosystems MDS, Foster City, CA) performed in negative electrospray ionization mode with multiple reaction monitoring. Detailed information on instrumental parameters, HPLC column and mobile phase conditions, analyte ion transitions, sample injection volume, and limit of quantitation are described in Table SI-4.

Structural Elucidation of Novel Biotransformation Products. LC/MS/MS quantitative analysis of digester sludge samples dosed with 6:2 FTUCA indicated the presence of potential novel biotransformation products. The acetonitrile extracts from 6:2 FTUCA-dosed samples were directly analyzed to identify novel polyfluorinated acids or derivatized with DNPH (2,4-dinitrophenylhydrazine) to identify polyfluorinated aldehydes with an LTQ Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) in electrospray negative ionization. Detailed instrumental methods (Table SI-5) including DNPH derivatization and HPLC column conditions were described previously.[17,33] A full scan (100−1800 m/z) mass data was used to identify potential transformation products. Mass defect data filter was applied to the raw data to filter out signals that did not meet the range of mass defect expected for polyfluorinated biotransformation products. Mass defect data filtration helped detect ions representing deprotonated molecules of biotransformation

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products present in live sludge samples. Molecular identification was performed based on the elemental composition of deprotonated molecules determined from the accurate mass measurement and product ion spectra (MS/MS) of the transformation products.

RESULTS AND DISCUSSION

Experimental System Conditions and Study Mass Balance. The anaerobic incubation systems used to monitor 6:2 and 8:2 FTOH biotransformation and intermediate biotransformation products were methanogenic, with steady increases of CH_4 and CO_2 gases and gas pressure in the headspace over time (Table SI-6). The sum of CH_4 and CO_2 partial pressure accounted for 77−93% of the headspace gas pressure. The pH of the aqueous anaerobic digester sludge remained slightly alkaline, close to the optimal pH of 7.0−7.2[34] for methanogenic archaea.

Total recovery of 6:2 FTOH and transformation products from sludge extracts, headspace, and septum extracts in Study I (90-d duration) averaged 96% and ranged from 86% to 104% (Figure 1A). For the sterile control in Study I, total 6:2 FTOH recovery averaged 104% and ranged from 87% to 121% (Figure 1A). Total recovery of 6:2 FTOH and transformation products from live sludge extracts, headspace, and septum extracts in Study II (176-d duration) averaged 83% and ranged from 66% to 111% (Figure 2A). For the sterile control in Study II, total 6:2 FTOH recovery averaged 80% and ranged from 60% to 98% (Figure 2A). The lower 6:2 FTOH recovery after 30 days in sterile controls in study II could be attributed to instrumental variations and also lower efficiency of C18 SPE cartridges (due to batch variations) used to capture volatile 6:2 FTOH in the headspace.

Total recovery of 8:2 FTOH and transformation products from live sludge extracts, headspace, and septum extracts averaged 97% and ranged from 90% to 102% (Figure 3). For the sterile control, total 8:2 FTOH recovery averaged 98% and ranged from 80% to 108% (Figure 3). Nonextractable ^{14}C recovered through thermal combustion accounted for 4.6% of initially applied [3-^{14}C] 8:2 FTOH in live digester sludge and 4% in sterile digester sludge on day 181, indicating that 8:2 FTOH was not strongly absorbed by digester sludge under the study conditions. In contrast, 8:2 FTOH can be absorbed to soil and activated sludge,[14,32] which has a different organic component profile compared with digester sludge due to the additional anaerobic microbial treatment process.[34]

6:2 FTCA and 5:3 Acid Are Major Products of 6:2 FTOH Anaerobic Biotransformation. The half-life of 6:2 FTOH primary anaerobic biotransformation to other products is about 30 days in Studies I and II (Figures 1A and 2A). Approximately 6 mol % of 6:2 FTOH still remained by day 176 (Figure 2A). The trend of major transformation product formation is similar in Study I and Study II, although molar yields of individual transformation products varied during 6:2 FTOH anaerobic biotransformation (Figures 1 and 2). The anaerobic primary biotransformation of 6:2 FTOH was much slower than that in aerobic soil, sediment, and activated sludge where the 6:2 FTOH half-life was 1−2 days.[17,20,21] Table 1 summarizes average molar yields of poly- and perfluorinated carboxylic acids from 6:2 FTOH and 8:2 FTOH biotransformation under aerobic and anaerobic conditions in different environmental matrices based on published information.

6:2 FTCA was the most abundant biotransformation product observed, accounting for 44 mol % of initially applied 6:2 FTOH by day 90 in Study I (Figure 1A) and 32 mol % by day 176 in Study II (Figure 2A). In contrast, 6:2 FTCA was only observed in days 2−3 in aerobic activated sludge dosed with 6:2 FTOH[21] and was not observed in soils due to rapid biotransformation to other products (Table 1).

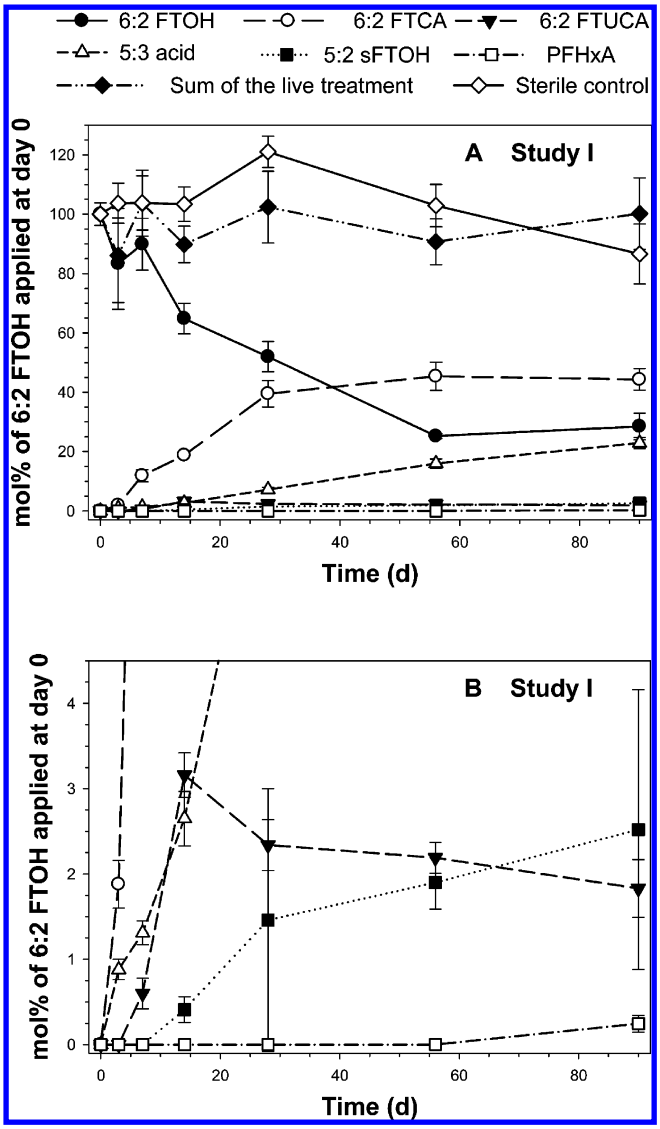

Figure 1. Molar yields of individual biotransformation products during 6:2 FTOH anaerobic biotransformation in anaerobic digester sludge under methanogenic conditions for a 90-d study (Study I, n = 3). The molar yields were calculated based on total moles of recovered 6:2 FTOH and biotransformation products from aqueous phase, septum, and headspace versus moles of 6:2 FTOH applied at day 0. Panel B is a zoom view of A, showing the time trends of biotransformation products with low molar yields. 6:2 FTOH starting concentration was 1.6 mg L^{-1} for live and 1.8 mg L^{-1} for sterile control digester sludge.

5:3 Acid was also a principal biotransformation product and was formed steadily over time, accounting for 23 mol % of initially applied 6:2 FTOH by day 90 in Study I (Figure 1A) and 18 mol % by day 176 of in Study II (Figure 2A), slightly higher than 5:3 acid molar yield in aerobic activated sludge at 14 mol % (Table 1). 5:3 Acid was stable under methanogenic conditions in this study and was not further biotransformed (Figure 4 and Figure SI-1). In contrast, 5:3 acid can be further degraded to 5:3 Uacid, 4:3 acid, PFBA, PFPeA, and other products in aerobic activated sludge.[33]

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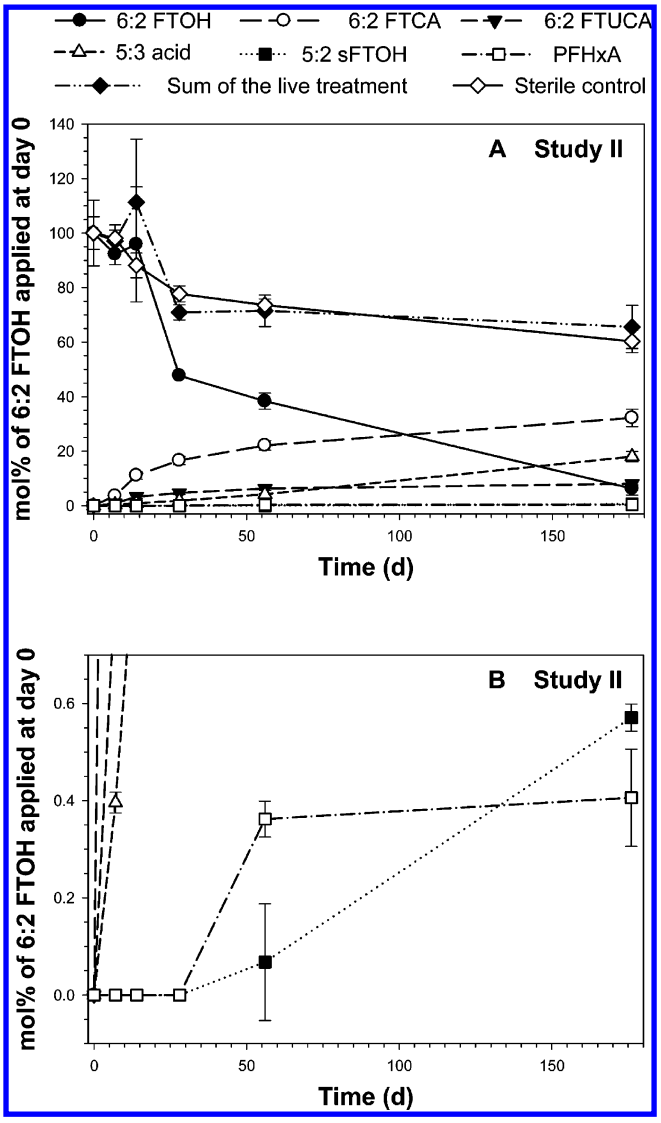

Figure 2. Molar yields of individual biotransformation products during 6:2 FTOH anaerobic biotransformation in anaerobic digester sludge under methanogenic conditions for a 176-d study (Study II, n = 3). The molar yields were calculated based on total moles of recovered 6:2 FTOH and biotransformation products from aqueous phase, septum, and headspace versus moles of 6:2 FTOH applied at day 0. Panel B is a zoom view of A, showing the time trends of biotransformation products with low molar yields. 6:2 FTOH starting concentration was 1.6 mg L^{-1} for live and 1.4 mg L^{-1} for sterile control digester sludge.

6:2 FTUCA was also observed, accounting for 2 mol % of initially applied 6:2 FTOH by day 90 in Study I (Figure 1A) and 8 mol % by day 176 in Study II (Figure 2A). High levels of 6:2 FTUCA were observed in days 2−3 in aerobic activated sludge dosed with 6:2 FTOH and was not observed after two months.[21] 6:2 FTUCA was not observed in aerobic soils due to fast biotransformation to other products (Table 1).

5:3 Uacid was not observed in digester sludge dosed with 6:2 FTOH in both Study I and II (Figures 1 and 2) due to its fast biotransformation to 5:3 acid (Figure SI-2). The half-life of 5:3 Uacid was less than 3 h in anaerobic digester sludge and it was biotransformed exclusively to 5:3 acid at ~95 mol % by day 56. No α−OH 5:3 acid[33] was observed (Figure SI-2).

5:2 sFTOH, a direct PFHxA precursor under aerobic conditions,[17] accounted for 2.5 mol % of initially applied 6:2

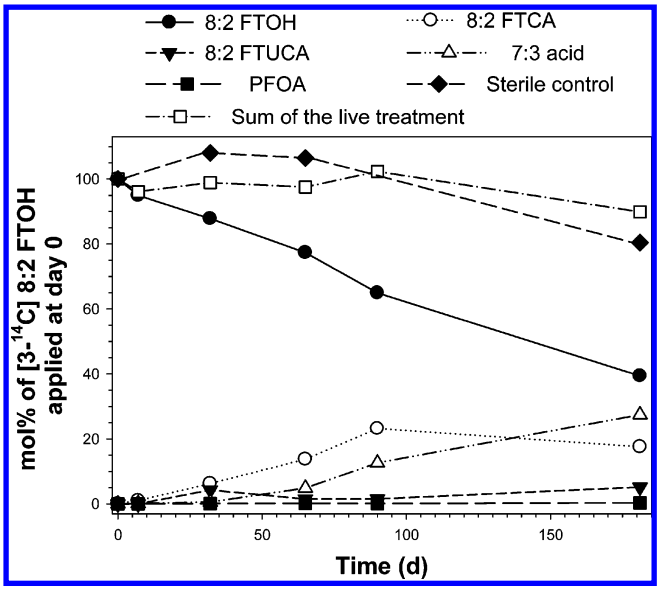

Figure 3. Molar yields of individual biotransformation products during [3-^{14}C] 8:2 FTOH anaerobic biotransformation in anaerobic digester sludge under methanogenic conditions for a 181-d study (n = 2). The molar yields were calculated based on total moles of recovered [3-^{14}C] 8:2 FTOH and biotransformation products from aqueous phase, septum, and headspace versus moles of [3-^{14}C] 8:2 FTOH applied at day 0. [3-^{14}C] 8:2 FTOH starting concentration was 182 μg L^{-1} for live and 174 μg L^{-1} for sterile control digester sludge.

FTOH by day 90 in Study I (Figure 1A) and 0.6 mol % by day 176 in Study II (Figure 2A), which is at least 16 times lower than in aerobic activated sludge dosed with 6:2 FTOH.[21] PFPeA, PFHpA, and other potential intermediate products of 6:2 FTOH aerobic biotransformation such as 5:2 ketone [$F(CF_2)_5C(O)CH_3$], 5:2 acid [$F(CF_2)_5CH_2COOH$], 5:2 Uacid [$F(CF_2)_4CF{=}CH_2COOH$], α−OH 5:3 acid, and 3:3 acid [$F(CF_2)_3CH_2CH_2COOH$] were not detected in either Study I or II.

PFHxA in 6:2 FTOH-dosed anaerobic digester sludge was barely detected only after day 56 (Figures 1B and 2B) above the background levels of live sludge control without addition of 6:2 FTOH. Assuming such low levels were indeed from 6:2 FTOH anaerobic biotransformation, PFHxA accounted for 0.2 mol % of initially applied 6:2 FTOH by day 90 in Study I (Figure 1B) and 0.4 mol % by day 176 in Study II (Figure 2B). Such PFHxA molar yields were at least 28 times lower than that in aerobic activated sludge dosed with 6:2 FTOH at 11 mol % (Table 1).

8:2 FTCA and 7:3 Acid Are Major Products of 8:2 FTOH Anaerobic Biotransformation. Figure 3 shows the time trend of 8:2 FTOH biotransformation products. The half-life of 8:2 FTOH primary anaerobic biotransformation to other products is about 145 days. Approximately 39 mol % of 8:2 FTOH still remained by day 181. The anaerobic primary biotransformation of 8:2 FTOH was much slower than that in aerobic activated sludge and soil with an 8:2 FTOH half-life less than 30 days.[14−16]

7:3 Acid was the most abundant biotransformation product and was formed steadily over time, accounting for 27 mol % of initially applied 8:2 FTOH by day 181, at least two times higher than that in aerobic soil (Table 1). 8:2 FTCA was formed steadily and peaked (23 mol %) at day 90 and then declined to 18 mol % of initially applied 8:2 FTOH by day 181. 8:2 FTCA

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Table 1. Molar Yield Comparison of Poly- and Perfluorinated Carboxylic Acids from 6:2 FTOH and 8:2 FTOH Biotransformation under Aerobic and Anaerobic Conditions in Different Environmental Matrices[a]

environ. matrix	6:2 FTOH biotransformation (mol %)								8:2 FTOH biotransformation (mol %)								
	PFBA	PFPeA	PFHxA	PFHpA	6:2 FTCA	6:2 FTUCA	5:3 acid	ref	PFHxA	PFHpA	PFOA	PFNA	8:2 FTCA	8:2 FTUCA	7:3 acid	ref
aerobic sludge (6:2 FTOH)[b] or highly diluted sludge (8:2 FTOH)[c]	<0.5	4.4	11	ND	ND	2.8	14	21	ND	ND	2.1	ND	27	6.0	2.3	13
aerobic soil[d]	1.8	30	8.1	ND	ND	ND	15	17	2.5	ND	25	ND	ND	ND	11	16
aerobic sediment[e]	1.5	10	8.4	ND	ND	ND	22	20	not available							
anaerobic digester sludge[f]	ND	ND	≤0.4	ND	38	4.9	21	this study	ND	ND	0.3	ND	18	5.1	27	this study

[a]ND: Not detected. [b]Average molar yields by day 56 and 60. [c]Average molar yields by day 28. [d]Average molar yields by day 28 and 208. [e]Molar yields by day 90. [f]Average molar yields of Study I and II by day 90 and 176.

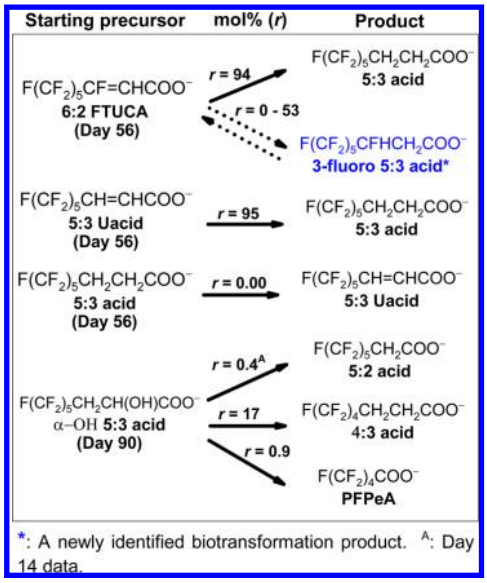

Figure 4. Summary of molar yields on day 56 or day 90 of potential biotransformation products (6:2 FTUCA, 5:3 Uacid, α–OH 5:3 acid, and 5:3 acid) as starting precursors during anaerobic biotransformation in anaerobic digester sludge under methanogenic conditions (n = 3 for live and n = 1 for sterile control). The molar yields were calculated based on total moles of recovered starting precursor and biotransformation products from aqueous phase, septum, and headspace versus moles of starting precursor applied at day 0. Detailed time trends of anaerobic biotransformation for these four starting precursors are included in Figures SI-1, 2, 3, and 4, respectively.

was not observed in soil dosed with 8:2 FTOH in a 7-month study (Table 1). 8:2 FTUCA was observed in anaerobic digester sludge and accounted for 5.1 mol % of initially applied 8:2 FTOH by day 181, whereas no 8:2 FTUCA was observed in soil dosed with 8:2 FTOH in a 180-d study.[16] 7:3 Uacid was not observed in anaerobic digester sludge dosed with [3-^{14}C] 8:2 FTOH probably due to its fast biotransformation to 7:3 acid, as also occurred in aerobic soil.[16] 7:2 sFTOH [$F(CF_2)_7CH(OH)CH_3$], a direct PFOA precursor under aerobic conditions,[14,16] and perfluorononanoic acid (PFNA) were not observed during 8:2 FTOH anaerobic biotransformation in digester sludge.

PFOA in [3-^{14}C] 8:2 FTOH-dosed anaerobic digester sludge was detected slightly above the background levels of live sludge control without addition of 8:2 FTOH (Figure 3). Assuming such low levels were from 8:2 FTOH anaerobic biotransformation, PFOA accounted for 0.3 mol % of initially applied [3-^{14}C] 8:2 FTOH by day 181 (Figure 3). Such PFOA molar yield was 83-fold lower than that in aerobic soil dosed with [3-^{14}C] 8:2 FTOH at an average of 25 mol %[16] under experimental conditions with >400 mg organic carbon kg^{-1} soil and large numbers of microbes (>10^7 bacterial cells), similar to the conditions in anaerobic digester sludge. Furthermore, 8:2 FTOH aerobic biodegradation in 200-fold diluted activated sludge containing limited organic carbons (~1 mg L^{-1}) and low microbial populations (~ 10^5 bacterial cells) produced 2.1 mol % PFOA.[13]

3-Fluoro 5:3 Acid Was Identified As a Novel Biotransformation Product during 6:2 FTUCA Anaerobic Biotransformation. 3-Fluoro 5:3 acid [$F(CF_2)_5CFHCH_2COOH$] for the first time was identified as a novel biotransformation product during 6:2 FTUCA anaerobic

biotransformation in digester sludge. Its identification is based on observed deprotonated molecule ion (m/z 359) with the elemental composition of $C_8H_3F_{12}O_2$ determined based on experimental accurate mass of 358.9960, which represents 0.3 mDa (0.8 ppm) deviation from theoretical accurate mass for this elemental formula (Figure SI-5). The m/z 359 product ion spectrum (Figure SI-5) contains two fragment ions m/z 275 and 255. The m/z 275 represents a combined neutral loss of (CO_2 + 2 HF) from the precursor ion m/z 359, and m/z 255 represents a consecutive loss of HF from m/z 275. 3-Fluoro 5:3 acid was not quantified because of a lack of authentic standard. Instead, its time trend was qualitatively estimated by plotting peak areas of extracted ion chromatograms for m/z 359 (Figure SI-6). 3-Fluoro 5:3 acid was formed at 2 h (0.08 day) during 6:2 FTUCA anaerobic biotransformation and peaked at day 7 and then declined thereafter, coincident with the lowest recovery of 5:3 acid at day 7 (Figure SI-3). The decline of 3-fluoro 5:3 acid (Figure SI-6) and increased 5:3 acid recovery after day 7 in live sludge (Figure SI-3) indicate that 3-fluoro 5:3 acid may be converted back to 6:2 FTUCA, which was further transformed to 5:3 acid. Alternatively, 3-fluoro 5:3 acid can be converted to 5:3 Uacid first, which was subsequently transformed to 5:3 acid. We are currently in the process of synthesizing 3-fluoro 5:3 acid to be used to investigate its biotransformation potential and pathways under both anaerobic and aerobic conditions in the future. Polyfluorinated aldehydes were not observed during 6:2 FTUCA anaerobic biotransformation based on high-resolution mass spectrometry analysis. The possibility of 6:2 FTUCA conjugate formation with glucose or other polar molecules was also ruled out because such conjugate reactions with nonfluorinated chemicals were not reported in prokaryotes such as bacteria[35] and also were not observed in aerobic activated sludge and soils dosed with ^{14}C-labeled 6:2 and 8:2 FTOHs.[16,36]

Rate-Limiting Steps Prevent FTOHs Conversion to PFCAs under Anaerobic Methanogenic Conditions. Enzymatic inefficiency at decarboxylating 6:2 FTUCA to 5:2 sFTOH under anaerobic conditions most likely limited subsequent PFCA production. 6:2 FTUCA, 5:3 Uacid, α−OH 5:3 acid, and 5:3 acid were observed as biotransformation products of 6:2 FTOH aerobic biodegradation in activated sludge.[21,33] Under such aerobic conditions in activated sludge, 6:2 FTUCA can be either decarboxylated to 5:2 sFTOH, which was subsequently degraded to PFHxA and PFPeA, or it can be converted to 5:3 acid.[17] 6:2 FTUCA was rapidly converted under anaerobic conditions to 5:3 acid via the intermediate, 5:3 Uacid, which was only observed at 2 h after the initiation of the experiment at about 3 mol % (Figure 4 and Figure SI-3). PFCAs and 5:2 sFTOH, the direct precursor of PFPeA and PFHxA and a product of 6:2 FTUCA aerobic decarboxylation,[17] were not observed during 6:2 FTUCA anaerobic biotransformation. This indicates that enzymatic decarboxylation of 6:2 FTUCA to form 5:2 sFTOH under anaerobic methanogenic conditions was one of the rate-liming steps in converting 6:2 FTOH to PFCAs.

Inability for microbial enzyme(s) to hydroxylate 5:3 Uacid to form α−OH 5:3 acid was another possible rate-limiting step restricting PFCA production from 6:2 FTOH in digester sludge under anaerobic methanogenic conditions. 5:3 U acid was an intermediate product of 6:2 FTOH aerobic biotransformation in activated sludge and pure bacterial culture[21,37] and it can be further hydroxylated in activated sludge to form α−OH 5:3 acid.[33] α−OH 5:3 acid can be rapidly decarboxylated in aerobic activated sludge to form PFBA, PFPeA, and 4:3 acid.[33] Thus, hydroxylation of 5:3 Uacid to form α−OH 5:3 acid may be another necessary enzymatic step leading to PFCAs. During 6:2 FTOH anaerobic biotransformation in digester sludge, 5:3 U acid was not observed due to its rapid and exclusive conversion to 5:3 acid as shown in Figure 4 and Figure SI-2, and no α−OH 5:3 acid was formed. Under anaerobic methanogenic conditions, enzymatic reduction of 5:3 Uacid to 5:3 acid was much more favorable thermodynamically compared to 5:3 Uacid hydroxylation, which is an oxidation reaction much more likely to happen aerobically. On the other hand, α−OH 5:3 acid, if present in an anaerobic system, could be further decarboxylated to shorter-chain poly- and perfluorinated acids as occurred in aerobic activated sludge.[33] Indeed, α−OH 5:3 acid was biotransformed anaerobically to three products over 90 d (Figure 4 and Figure SI-4). 4:3 Acid was formed steadily from α−OH 5:3 acid and accounted for 17 mol % of initially applied α−OH 5:3 acid by day 90 (Figure 4 and Figure SI-4A). PFPeA was also generated from α−OH 5:3 acid and accounted for 0.9 mol % on day 90 (Figure 4 and Figure SI-4B). 5:2 Acid [$F(CF_2)_5CH_2COOH$] was only observed transiently in days 14−28 and peaked at day 14 at 0.4 mol % (Figure 4 and Figure SI-4B). These results demonstrated that anaerobic decarboxylation of α−OH 5:3 acid under methanogenic conditions was less efficient compared to that under aerobic conditions in activated sludge.[33]

x:2 FTCAs and x:3 Acids Are Potential Indicators of FTOH Anaerobic Biotransformation to Discern the PFCA Sources in Anaerobic Environments. PFCAs were detected in anaerobic environmental matrices such as landfill leachate[19,30,31] and digester sludge.[29] Signature or indicator biotransformation products can be used to discern the extent that precursor (e.g., FTOH) biotransformation or direct PFCAs discharge contribute to PFCAs detected in such anaerobic environments. Our work clearly illustrates that 6:2 and 8:2 FTOH anaerobic biotransformation generated overwhelmingly x:2 FTCAs [$F(CF_2)_xCH_2COOH$, x = 6, 8] and x:3 acids [$F(CF_2)_xCH_2CH_2COOH$, x = 5, 7], and little PFHxA or PFOA, respectively. Thus, x:2 FTCAs and x:3 acids may be better indicators of FTOH or FTOH-based product anaerobic biotransformation. For example, the molar yields of 6:2 FTCA are 177 and 79 times higher than that of PFHxA at day 90 (Figure 1) and day 176 (Figure 2), respectively. Similarly, the molar yield of 8:2 FTCA is 59 times higher than that of PFOA at day 181 (Figure 3). In comparison, 6:2 FTCA is about 9 times lower and 8:2 FTCA is only 3.5 times higher than that of PFHxA and PFOA, respectively, in landfill leachate,[19] suggesting that substantial amounts of detected PFHxA and PFOA were from their direct discharge in addition to probably smaller contributions from 6:2 and 8:2 FTOH anaerobic biotransformation in landfills.

6:2 and 8:2 FTOH Anaerobic Biotransformation Pathways. Figure 5 illustrates 6:2 and 8:2 FTOHs (referred to as FTOHs hereafter) major biotransformation pathways leading to stable biotransformation products, 5:3 acid and 7:3 acid, respectively, under methanogenic conditions. The pathways are based on observed products from anaerobic biotransformation of FTOHs and individual potential biotransformation products investigated in this study. Minor pathways (≤0.4 mol %) leading to low yields of PFHxA or PFOA are not shown and likely follow similar routes proposed for aerobic conditions via 5:2 sFTOH and 7:2 sFTOH, respectively.[16,17]

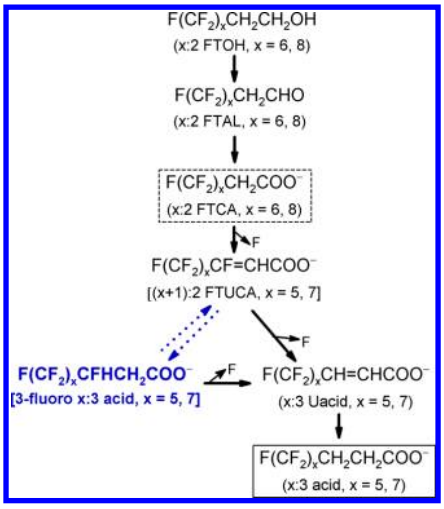

Figure 5. 6:2 FTOH (x = 6) and 8:2 FTOH (x = 8) anaerobic biotransformation pathways in anaerobic digester sludge under methanogenic conditions. All the polyfluorinated acids are presented in deprotonated forms, which are expected under slightly alkaline pH in digester sludge. The dashed rectangular box represents major intermediate biotransformation product and the solid rectangular box represents the major stable biotransformation product under anaerobic methanogenic conditions.

Anaerobic oxidation of FTOHs can occur via either enzymatic addition of oxygen(s) from water/organic carbons or by removing hydrogen(s) along with electron(s) from FTOHs. In digester sludge of this study, FTOHs were first oxidized anaerobically to x:2 FTALs [$F(CF_2)_xCH_2CHO$, x = 6, 8] due to loss of two hydrogens along with two electrons per FTOH molecule as described before.[16,17] x:2 FTALs were then further oxidized to x:2 FTCAs catalyzed by dehydrogenases using nicotinamide adenine dinucleotide (NAD) to accept electrons and protons to form $NADH_2$ (nicotinamide adenine dinucleotide, reduced form), a reducing power (or bioenergy) that can be used to drive many biochemical processes.[38] These first two reactions produced two $NADH_2$ from each FTOH that was oxidized. It is not known whether monooxygenases were involved in these reactions. x:2 FTCAs can be dehydrohalogenated (HF elimination) to (x + 1):2 FTUCAs [$F(CF_2)_xCF{=}CHCOOH$, x = 5, 7] via HF elimination. It is not clear whether this reaction can produce $NADH_2$ from NAD. (x + 1):2 FTUCAs were converted to x:3 Uacids [$F(CF_2)_xCH{=}CHCOOH$, x = 5, 7] by a possible dehalogenase[28] via reductive defluorination using $NADH_2$ to provide electrons and protons. (x + 1):2 FTUCAs can also be converted to the newly identified 3-fluoro x:3 acids [$F(CF_2)_xCFHCH_2COOH$, x = 5, 7] by a reductase also using $NADH_2$ to shuttle electrons and protons. 3-Fluoro x:3 acids may be converted back to (x + 1):2 FTUCAs first, or be converted directly to x:3 Uacids via dehydrohalogenation. Finally, x:3 Uacids were transformed to x:3 acids by a reductase using $NADH_2$ to provide electrons and protons. As discussed earlier, x:3 acids were stable anaerobically and not degraded further under methanogenic conditions in this study. However, when digester sludge is applied to surface soil as a biosolid amendment and subsequently released to sediment via soil erosion where aerobic conditions may be present, x:3 acids could be further decarboxylated to form shorter-chain PFCAs and other polyfluorinated substances.[20,33]

The processes of anaerobic microbial biotransformation of FTOHs to x:3 acids are bioenergy neutral, generating two $NADH_2$ while converting FTOHs to x:2 FTCAs and then consuming the two $NADH_2$ to transform (x + 1):FTUCA to x:3 acids. These bioenergy neutral reactions are also coincident with deficiency in decarboxylation reactions, which are required to form PFCAs from FTOHs as discussed earlier. In contrast, aerobic microbial biodegradation of 5:3 acid to 4:3 acid produced net bioenergy during the processes of removing one $-CF_2-$ from 5:3 acid.[33] Our work suggests that anaerobic microbes under methanogenic conditions lacked efficient biochemical mechanisms to decarboxylate FTOHs to form PFCAs, shifting FTOHs biotransformation pathways toward the production of x:3 acids.

■ ENVIRONMENTAL IMPLICATIONS

PFCAs were widely detected in the environment. PFCAs detected in anaerobic environmental matrices may come from direct PFCA discharge and/or from precursor biodegradation. Our work shows that anaerobic biotransformation of FTOHs under methanogenic conditions generated polyfluorinated acids but little PFCAs. This implies that 6:2 FTOH and 8:2 FTOH anaerobic biotransformation is not likely a major contributor to PFCAs detected in anaerobic environmental matrices such as WWTP anaerobic digester sludge, landfill leachate, and anaerobic sediment under methanogenic conditions.

■ ASSOCIATED CONTENT

Supporting Information

Details of analytical methods, physiological conditions of experimental systems, time trends on anaerobic biotransformation of individual biotransformation products as precursors, product ion mass spectra of newly identified novel transformation product, 3-fluoro 5:3 acid, and time trend of 3-fluoro 5:3 acid formation during 6:2 FTUCA anaerobic biotransformation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Quinete, N.; Wu, Q.; Zhang, T.; Yun, S. H.; Moreira, I.; Kannan, K. Specific profiles of perfluorinated compounds in surface and drinking waters and accumulation in mussels, fish, and dolphins from southeastern Brazil. *Chemosphere* **2009**, *77*, 863−869.

(2) Shoeib, M.; Vlahos, P.; Harner, T.; Peters, A.; Graustein, M.; Narayan, J. Survey of polyfluorinated chemicals (PFCs) in the atmosphere over the northeast Atlantic Ocean. *Atmos. Environ.* **2010**, *44*, 2887−2893.

(3) Ahrens, L. Polyfluoroalkyl compounds in the aquatic environment: A review of their occurrence and fate. *J. Environ. Monit.* **2011**, *13*, 20−31.
(4) Houde, M.; De Silva, A. O.; Muir, D. C. G.; Letcher, R. J. Monitoring of perfluorinated compounds in aquatic biota: An updated review on PFCs in aquatic biota. *Environ. Sci. Technol.* **2011**, *45*, 7962−7973.
(5) Lau, C. Perfluorinated compounds. *Mol. Clin. Environ. Toxicol., Exper. Suppl.* **2012**, *101*, DOI 10.1007/978-3-7643-8340-4_3.
(6) Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H. Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006**, *40*, 32−44.
(7) Cousins, I. T.; Kong, D.; Vestergren, R. Reconciling measurement and modelling studies of the sources and fate of perfluorinated carboxylates. *Environ. Chem.* **2011**, *8*, 339−354.
(8) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; Leeuwen, S. P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr. Environ. Assess. Manage.* **2011**, *7*, 513−541.
(9) Ellis, D. A.; Martin, J. W.; De Silva, A. O.; Mabury, S. A.; Hurley, M. D.; Sulbaek Andersen, M. P.; Wallington, T. J. Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. *Environ. Sci. Technol.* **2004**, *38*, 3316−3321.
(10) Martin, J. W.; Ellis, D. A.; Mabury, S. A.; Herley, M. D.; Wallington, T. J. Atmospheric chemistry of perfluoroalkanesulfonamides: Kinetic and product studies of the OH radical and Cl atom initiated oxidation of *N*-ethyl perfluorobutanesulfonamide. *Environ. Sci. Technol.* **2006**, *40*, 864−872.
(11) Rhoads, K. R.; Janssen, E. M. L.; Luthy, R. G.; Criddle, C. S. Aerobic biotransformation and fate of *N*-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge. *Environ. Sci. Technol.* **2008**, *42*, 2873−2878.
(12) Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004**, *38*, 2857−2864.
(13) Wang, N.; Szostek, B.; Folsom, P. W.; Sulecki, L. M.; Capka, V.; Buck, R. C.; Berti, W. R.; Gannon, J. T. Aerobic biotransformation of ^{14}C-labeled 8−2 telomer b alcohol by activated sludge from a domestic sewage treatment plant. *Environ. Sci. Technol.* **2005**, *39*, 531−538.
(14) Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Capka, V.; Berti, W. R.; Gannon, J. T. Fluorotelomer alcohol biodegradation. Direct evidence that perfluorinated carbon chains breakdown. *Environ. Sci. Technol.* **2005**, *39*, 7516−7528.
(15) Liu, J.; Lee, L. S.; Nies, L. F.; Nakatsu, C. H.; Turco, R. F. Biotransformation of 8:2 fluorotelomer alcohol in soil and by soil bacterial isolates. *Environ. Sci. Technol.* **2007**, *41*, 8024−8030.
(16) Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Gannon, J. T. 8−2 Fluorotelomer alcohol aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. *Chemosphere* **2009**, *75*, 1089−1096.
(17) Liu, J.; Wang, N.; Szostek, B.; Buck, R. C.; Panciroli, P. K.; Folsom, P. W.; Sulecki, L. M.; Bellin, C. A. 6:2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. *Chemosphere* **2010**, *78*, 437−444.
(18) Dasu, K.; Liu, J.; Lee, L. S. Aerobic soil biodegradation of 8:2 Fluorotelomer stearate monoester. *Environ. Sci. Technol.* **2012**, *46*, 3831−3836.
(19) Benskin, J. P.; Belinda, L.; Ikonomou, M. G.; Grace, J. R.; Loretta, L. Per- and polyfluoroalkyl substances in landfill leachate: Patterns, time-trends, sources. *Environ. Sci. Technol.* **2012**, *46*, 11532−11540.
(20) Zhao, L.; Folsom, P. W.; Wolstenholme, B. W.; Sun, H.; Wang, N.; Buck, R. C. 6:2 Fluorotelomer alcohol biotransformation in an aerobic river sediment system. *Chemosphere* **2013**, *90*, 203−209.
(21) Zhao, L.; McCausland, P. K.; Folsom, P. W.; Wolstenholme, B. W.; Sun, H.; Wang, N.; Buck, R. C. 6:2 Fluorotelomer alcohol aerobic biotransformation in activated sludge from two domestic wastewater treatment plants. *Chemosphere* **2013**, DOI: http://dx.doi.org/10.1016/j.chemosphere.2013.02.032.
(22) Lee, H.; D'Eon, J.; Mabury, S. A. Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. *Environ. Sci. Technol.* **2010**, *44*, 3305−3310.
(23) Wang, N.; Liu, J.; Buck, R. C.; Korzeniowski, S. H.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* **2011**, *82*, 853−858.
(24) Dasu, K.; Royer, L. A.; Liu, J.; Lee, L. S. Hydrolysis of fluorotelomer compounds leading to fluorotelomer alcohol production during solvent extractions of soils. *Chemosphere* **2010**, *81*, 911−917.
(25) U.S. National Aeronautics and Space Administration (NASA). *Global Methane Production*; 2012. http://icp.giss.nasa.gov/education/methane/intro/cycle.html (accessed November 3, 2012).
(26) Madigan, T. M.; Martinko, J. M., Parker, J. *Brock Biology of Microorganisms*, 10th ed.; Prentice Hall: Upper Saddle River, NJ, 2003.
(27) Ferry, J. G. How to make a living by exhaling methane. *Annu. Rev. Microbial.* **2010**, *64*, 453−473.
(28) Häggblom, M. M.; Bossert, I. D. Halogenation: Microbial Processes and Environmental Applications. In *Halogenated Organic Compounds − A Global Perspective*; Häggblom, M. M., Bossert, I. D., Eds.; Kluwer Academic Publishers: Norwell, MA, 2003.
(29) Sun, H.; Gerecke, A. C.; Giger, W.; Alder, A. C. Long-chain perfluorinated chemicals in digested sewage sludges in Switzerland. *Environ. Pollut.* **2011**, *159*, 654−662.
(30) Huset, C. A.; Barlaz, M. A.; Barofsky, D. F.; Field, J. A. Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere* **2011**, *82*, 1380−1386.
(31) Ahrens, L.; Shoeib, M.; Harner, T.; Lee, S. C.; Guo, R.; Reiner, E. J. Wastewater treatment plant and landfills as sources of polyfluoroalkyl compounds to the atmosphere. *Environ. Sci. Technol.* **2011**, *45*, 8098−8105.
(32) Liu, J.; Lee, L. S. Effect of fluorotelomer alcohol chain length on aqueous solubility and sorption by soils. *Environ. Sci. Technol.* **2007**, *41*, 5357−5362.
(33) Wang, N.; Buck, R. C.; Szostek, B.; Sulecki, L. M.; Wolstenholme, B. W. 5:3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel "one-carbon removal pathways". *Chemosphere* **2012**, *87*, 527−534.
(34) Bitton, G. *Wastewater Microbiology*, 4th ed.; Wiley-Blackwell: Hoboken, NJ, 2011.
(35) Alexander, M. *Biodegradation and Bioremediation*, 2nd ed.; Academic Press: New York, 1999.
(36) Liu, J.; Wang, N.; Buck, R. C.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M.; Bellin, C. A. Aerobic biodegradation of [^{14}C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system. *Chemosphere* **2010**, *80*, 716−723.
(37) Kim, M. H.; Wang, N.; McDonald, T.; Chu, K. H. Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane degrading *Pseudomonas* Strains. *Biotechnol. Bioeng.* **2012**, *109*, 3041−3048.
(38) Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry*, 3rd ed.; Worth Publishers: New York, 2000.