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Biodegradation of *N*-Ethyl Perfluorooctane Sulfonamido Ethanol (EtFOSE) and EtFOSE-Based Phosphate Diester (SAmPAP Diester) in Marine Sediments

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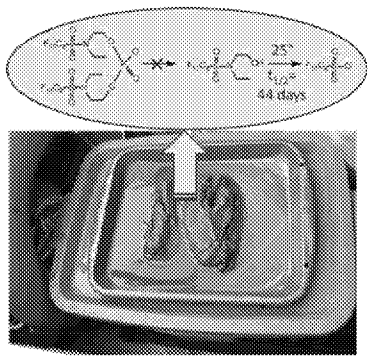
Supporting Information

ABSTRACT: Investigations into the biodegradation potential of perfluorooctane sulfonate (PFOS)-precursor candidates have focused on low molecular weight substances (e.g., *N*-ethyl perfluorooctane sulfonamido ethanol (EtFOSE)) in wastewater treatment plant sludge. Few data are available on PFOS-precursor biodegradation in other environmental compartments, and nothing is known about the stability of high-molecular-weight perfluorooctane sulfonamide-based substances such as the EtFOSE-based phosphate diester (SAmPAP diester) in any environmental compartment. In the present work, the biodegradation potential of SAmPAP diester and EtFOSE by bacteria in marine sediments was evaluated over 120 days at 4 and 25 °C. At both temperatures, EtFOSE was transformed to a suite of products, including *N*-ethyl perfluorooctane sulfonamidoacetate, perfluorooctane sulfonamidoacetate, *N*-ethyl perfluorooctane sulfonamide, perfluorooctane sulfonamide, and perfluorooctane sulfonate. Transformation was significantly more rapid at 25 °C ($t_{1/2}$ = 44 ± 3.4 days; error represents standard error of the mean (SEM)) compared to 4 °C ($t_{1/2}$ = 160 ± 17 days), but much longer than previous biodegradation studies involving EtFOSE in sludge ($t_{1/2}$ ~0.7−4.2 days). In contrast, SAmPAP diester was highly recalcitrant to microbial degradation, with negligible loss and/or associated product formation observed after 120 days at both temperatures, and an estimated half-life of >380 days at 25 °C (estimated using the lower bounds 95% confidence interval of the slope). We hypothesize that the hydrophobicity of SAmPAP diester reduces its bioavailability, thus limiting biotransformation by bacteria in sediments. The lengthy biodegradation half-life of EtFOSE and recalcitrant nature of SAmPAP diester in part explains the elevated concentrations of PFOS-precursors observed in urban marine sediments from Canada, Japan, and the U.S, over a decade after phase-out of their production and commercial application in these countries.



INTRODUCTION

Perfluorooctane sulfonate (PFOS) is an anthropogenic surfactant and widespread contaminant of the global environment.[1−3] In addition to its considerable bioaccumulation and biomagnfication potential in aquatic ecosystems,[3−6] PFOS has been linked to a variety of adverse health effects in marine organisms, including reduced reproduction and offspring development, altered sex ratios, oxidative stress, and growth suppression.[7,8] Following the 2002 production phase-out in North America,[9] PFOS was added to the list of substances regulated by the United Nations Stockholm Convention on Persistent Organic Pollutants (UNSCPOP) in 2009. However, use exemptions listed under UNSCPOP have allowed continued manufacturing and application of PFOS and related substances in some parts of the world (e.g., China).[10]

The historical uses of PFOS in commercial products (e.g., mist suppressants and aqueous film forming foam) are among the potential sources of PFOS measured in the environment.[11] In addition, *N*-alkyl-substituted perfluorooctane sulfonamides (FOSAMs; defined here as all substances containing $C_8F_{17}SO_2N$) may form PFOS through abiotic[12] or biologically catalyzed[13,14] transformation. These substances are referred to

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herein as "PFOS-precursor candidates". Historically, FOSAMs (PFOS-precursor candidates) have been incorporated into a wide range of commercial products (e.g., surface treatments for textiles, carpets, paper, and packaging, and insecticides), either as active ingredients or unintentional residual impurities.[15,16] Assessing the relative contribution of FOSAMs to the overall environmental burden of PFOS is useful for identifying sources of emission and exposure to PFOS, for understanding long-range transport of PFOS to remote locations,[17] and for predicting future burdens of PFOS in humans and wildlife.[18]

Biodegradation studies involving PFOS-precursor candidates have typically focused on activated sludge.[14,19,20] These studies reveal the potential for biotransformation but provide limited insight into the biodegradation rates and stability of PFOS-precursors in the ambient environment. Furthermore, all FOSAM biodegradation studies to date have involved low-molecular-weight substances; little is known about the biodegradation rates of high-molecular-weight FOSAMs such as the *N*-ethyl perfluorooctane sulfonamido ethanol-based phosphate diester (SAmPAP diester). SAmPAP diester was introduced in 1974 for use in food contact paper and packaging[21,22] and was a high production-volume chemical until 2002 when it was phased out in North America. According to documents submitted to the U.S. Environmental Protection Agency, 1997 sales of commercial SAmPAP diester formulation FC-807 represented the largest quantity of "PFOS equivalents" (the total quantity of PFOS formed assuming complete degradation) sold that year out of all 3M PFOS and PFOS-precursor containing commercial products.[23] While SAmPAP diester was only recently detected in humans[24] and environmental samples,[25] polyfluoroalkyl phosphate diesters (diPAPs; structurally similar alternatives to SAmPAP diester) have been observed at concentrations up to 200 ± 130 ng/g in wastewater treatment plant sludge[26] and are also known to biodegrade to perfluoroalkyl carboxylic acids.[27]

The objective of the present work was to assess whether two FOSAMs, *N*-ethyl perfluorooctane sulfonamidoethanol (EtFOSE) and SAmPAP diester are degradable by bacteria in marine sediments and can produce PFOS. These data are important for assessing the fate and behavior of FOSAMs in the environment, but also to help explain previous observations of elevated concentrations of PFOS and perfluorooctane sulfonamido acetates (oxidation products of EtFOSE and potentially SAmPAP diester) in some marine sediments.[25,28,29]

EXPERIMENTAL METHODS

Standards and Reagents. Perfluorooctanoate (PFOA) and PFOS were purchased from Sigma-Aldrich (Milwaukee, WI) and perfluorooctane sulfonamide (FOSA) was purchased from SynQuest Laboratories (Alachua, FL). Perfluorooctane sulfonamido acetate (FOSAA), *N*-ethyl perfluorooctane sulfonamidoacetate (EtFOSAA), *N*-ethyl perfluorooctane sulfonamide (EtFOSA), *N*-ethyl perfluorooctane sulfonamido ethanol (EtFOSE), and isotopically labeled standards of perfluorodecanoate (PFDA), PFOS, PFOA, FOSA, EtFOSA, EtFOSAA, and EtFOSE (see Table S1 in the Supporting Information (SI)) were purchased from Wellington Laboratories (Guelph, ON, Canada). Isotopically labeled monoisononylphthalate (^{13}C-MiNP) was purchased from Cambridge Isotope Laboratories (Andover, MA). A solution of FC-807 commercial product containing SAmPAP diester at a concentration of 30% (w/v) in isopropanol/water was acquired from the U.S. Food and Drug Administration. All reported SAmPAP diester concentrations were corrected for % purity.

Sediment Collection. Sediment was collected using a petit ponar from multiple sites within False Creek, an urban marine inlet located in Vancouver, BC, Canada. The bioactive layer (top 0.5−1.0 cm) of sediment was removed with a metal spatula and pooled in a 1-L high-density polyethylene (HDPE) bottle. Sediment was kept on ice in the field and stored at 4 °C in the laboratory until use. Incubations were begun within 2 days of sample collection.

Spiking and Incubation Procedure. Half of the collected False Creek sediment was rendered microbially inactive by a combination of autoclaving (25 min at 121 °C and 25 psi) followed by a one-time addition of 300 μL of 1% mercuric chloride (Fisher Scientific, Ottawa, ON). These autoclaved and mercuric chloride-treated sediments were used as microbially inactive controls. After thorough mixing, sediments were aliquoted into individual 15-mL centrifuge tubes (4 g of wet sediment/tube) with each tube representing a single time point (n = 3 tubes/time point; SI Figure S1). For every active sediment sample, there was a corresponding inactive control. Once aliquoted, sediments were incubated at 4 or 25 °C for 24 h and then spiked with 5 ng of PFDA (10 μL of a 0.5 μg/mL solution in MeOH) as an internal negative control, 350 ng of ^{13}C-MiNP (7 μL of a 50 μg/mL solution in ACN) as an internal positive control, and either 250 ng of EtFOSE (10 μL of a 25 μg/mL solution in MeOH) or 480 ng of SAmPAP diester (20 μL of a 24 μg/mL solution in MeOH). An additional set of active sediments (n = 1 tube/time point) which contained only positive and negative controls (i.e., EtFOSE and SAmPAP diester were not added) were also prepared to monitor background analyte concentrations. The whole spiking procedure took approximately 3 h, with the earliest time points prepared last. To assess the potential inhibitory effect of solvent on sediment bacteria, separate biodegradation experiments were performed whereby various quantities of solvent (MeOH and ACN) were coincubated with EtFOSE and SAmPAP diester in sediments and after 10 days, substrate depletion/product formation was compared. Details of these experiments are provided in the SI. Overall there was no evidence that the solvent vehicle inhibited biodegradation by marine sediment bacteria (Figure S2), consistent with the results of Otton et al.[30] This is not surprising considering the ubiquity of methanogenic bacteria in marine sediments which utilize methane and methanol as a sole carbon energy source.

All incubations were performed concurrently at 4 or 25 °C in the dark for 120 days (n = 10 time points) in a refrigerator or heated water bath incubator. Samples were uncapped every 2 days, then recapped and gently vortex-mixed. This semiclosed system was selected in order to minimize potential off-gassing of semivolatile per-/polyfluoroalkyl substances (PFASs) (relative to a completely open system) while facilitating gas exchange necessary for aerobic conditions. We had considered incorporating a sorbent cartridge to "trap" semivolatiles, but were concerned that this type of setup would lead to considerable loss of moisture over the 120-day incubation period, in particular for the 25 °C sediments.

On days 0, 0.1, 0.75, 3, 6, 13, 26, 53, 107, and 120, three samples from each experiment were removed from the incubation chambers. To each sample, 5 mL of MeOH was added, followed by 10 μL of a mixture of isotopically labeled internal standards. Samples were vortexed and then placed in a freezer at −20 °C until extraction and analysis.

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Microbial Activity Test. The presence/absence of microbial activity in sediments was assessed using Easi-Cult TTC dipslides (Orion Diagnostica, Finland) using the following protocol: On days 1 and 120 of the experiment, approximately 20 mg each of both active and inactive sediment (4 and 25 °C) was removed and diluted with 40 mL of HPLC water. A control was also prepared with HPLC water and no sediment. The dip slide was fully immersed in the dilute sediment solution, then removed and incubated at 25 °C. After 48 h the slides were assessed visually for the presence of microbial colonies.

Sediment Extraction and Cleanup. We evaluated three different extraction protocols using spike/recovery experiments (see discussion and Table S2) and found the following procedure (adapted from Powley et al.[31]) to be the most effective in terms of analyte recoveries. All extractions took place in the 15-mL polypropylene centrifuge tubes in which incubations were performed. After addition of 5 mL of MeOH to 4 g wet sediment, samples were vortexed, centrifuged at 3000 rpm, and the MeOH was transferred into a clean 15-mL centrifuge tube. The procedure was repeated twice more for a total of 15 mL of MeOH. The extract was reduced under a steady flow of nitrogen in a warm water bath to 1 mL, after which 50 mg of EnviCarb sorbent was added and the sample was vortexed, centrifuged, and transferred to a clean polypropylene centrifuge tube. The Envicarb pellet was rinsed with an additional 1 mL of ACN (necessary to remove SAmPAP diester, see Results and Discussion), and following vortexing and centrifugation this was combined with the previous 1 mL of MeOH, for a 2 mL final volume (1:1 MeOH:ACN). A portion of this extract was transferred to a μ-vial containing a snap-cap closure with a polyethylene septum for instrumental analysis.

Instrumental Analysis. Analysis of extracts was accomplished by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Dionex HPLC coupled to an API 5000Q triple quadrupole mass spectrometer (Applied Biosystems/Sciex, Concord, ON, Canada). Extracts (10 μL) were injected onto a Waters Xterra C18 column (5 μm, 4.6 mm × 30 mm) which was maintained at 35 °C. Two Waters Xterra columns (each 5 μm, 4.6 mm × 30 mm) connected in series were placed directly upstream of the injector to separate PFASs originating from the LC pump from those injected onto the analytical column. The mobile phase consisted of 100% MeOH (solvent A) and 0.1% ammonium acetate/0.1% acetic acid in water (solvent B) maintained at a flow rate of 300 μL/min. The starting mobile phase composition was 90% B which was held for 1 min, followed by a decrease to 40% B by 2.5 min, then 0% B by 4 min. The column was held at 0% B for 7.5 min before being returned to starting conditions and allowed to equilibrate for 4 min. A diverter valve (VICI Valco Canada, Inc., Brockville, ON) was placed downstream of the analytical column to divert flow to waste for the first 5 min of the run, after which time the flow was redirected to the mass spectrometer. Mass spectral data were collected under negative ion, multiple reaction monitoring (MRM) mode.

Analyte Quantification and QA/QC. Analyte quantification was accomplished using an isotope dilution approach when exact isotopically labeled standard was available, otherwise an internal standard approach (i.e., using a structurally similar isotopically labeled standard) was used. The exception to this was for ^{13}C-MiNP, which was more reliably quantified by external quantification. Calibration curves were run initially, and then a series of blanks and a standard were run after every 12 samples to assess instrument drift. When a given analyte produced more than one product ion with sufficient intensity to monitor (all analytes except for FOSA and EtFOSE) the ratios of primary to secondary product ions were compared in samples relative to standards to rule out the presence of coeluting isobaric interferences. For EtFOSE, sum responses of formate and acetate adducts were monitored, and for FOSA, only a single product ion was generated in sufficient intensity to monitor. For all analytes, observed concentrations were not corrected for the % recoveries obtained from initial spike/recovery experiments (Table S2).

Other QA/QC protocols incorporated into this experiment (mentioned previously) include the following: incubating blank sediment along with real samples to assess the formation of products from substances present in the sediment, the use of a positive control (^{13}C-MiNP; for assessing microbial activity) and a negative control (PFDA; for assessing sediment losses prior to addition of internal standard, for example from a cracked tube during the incubation). ^{13}C-MiNP was chosen as the internal positive control based on previous reports of its biodegradation in False Creek sediments.[30] PFDA was chosen as a negative control due to its chemical and biological stability, properties common to all perfluoroalkyl carboxylates imparted by the strength of carbon−fluorine bonds (450 kJ/mol), and the shielding of the carbon chain by fluorine atoms from nucleophilic attack.[32] Inactive sediments spiked with EtFOSE or SAmPAP diester were also incubated at both temperatures to monitor for losses due to irreversible sorption, volatilization, or abiotic hydrolysis. Spike/recovery experiments were also performed with all test substances, degradation products, and controls prior to beginning experiments.

Data and Statistical Analysis. Apparent biodegradation rate constants for test substances and metabolites were determined by linear regression of the natural logarithm (ln) of concentration versus time over the course of the experiment. Half lives ($t_{1/2}$) were calculated as $0.693/k$, where k is the slope of the regression. All errors reported in the present work represent standard error of the mean (SEM). Student's t tests were performed using SigmaPlot Version 12.0 (Systat Software Inc., Chicago, IL) to determine if the slopes of regression curves obtained from active sediment experiments were statistically different from zero, or from slopes obtained from inactive sediments, or from slopes obtained in active sediments at different temperatures. To assess the potential for formation of products other than those monitored in the present work, sum molar concentrations of products and reactants at each time point were expressed as a percentage of the corresponding value at $t = 0$.

RESULTS AND DISCUSSION

Recoveries from Sediment. A section detailing the optimization of the sediment extraction protocol can be found in the SI. Percent recoveries ($n = 3$) were 105 ± 6.0%, 88 ± 3.4%, and 87 ± 5.2% for ^{13}C-MiNP, EtFOSE, and SAmPAP diester, respectively, and ranged from 78 to 107% for remaining analytes (Table S2). The internal negative control/performance spike, PFDA, which was incubated along with each test substance, and not expected to degrade, was recovered quantitatively in all experiments, with average ($n = 30$) recoveries of 120 ± 3.4% (inactive 4 °C SAmPAP diester incubations), 99 ± 4.7% (active 4 °C SAmPAP diester incubations), 110 ± 3.8% (inactive 4 °C EtFOSE incubations), 120 ± 5.8% (active 4 °C EtFOSE incubations), 92 ± 3.7%

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Table 1. Biodegradation Half Lives (Days ± SEM) of EtFOSE, SAmPAP Diester, and ^{13}C-MiNP by Bacteria in Active Marine Sediments and Comparison to Literature Data

	biodegradation half lives (days ± SEM)				
substrate	EtFOSE incubation: 4 °C	SAmPAP diester incubation: 4 °C	EtFOSE incubation: 25 °C	SAmPAP diester incubation: 25 °C	literature
^{13}C-MiNP	8.8 ± 1.0	9.0 ± 1.6	1.0 ± 0.1	1.2 ± 0.2	1.0 ± 0.1 (22 °C)[a] 8.3 ± 0.9 (5 °C)[a]
EtFOSE	160 ± 17	-	44 ± 3.4[e] (33 ± 1.2[f])	-	≤2 (47 ng/mL; 28 °C)[b] 2–3 (2380 ng/mL; 28 °C)[b] ~4.2 (1.50 μg/mL; 25 °C)[c] 0.7 ± 0.05 (100 ng/mL; 30 °C)[d]
SAmPAP diester	-	>3400[g]	-	>380[g]	-

[a]False Creek sediments (Otton et al.[30]). [b]Sludge (Lange[20]). [c]Sludge (Boulanger et al.[19]). [d]Sludge (Rhoads et al.[14]). [e]Corrected for losses from volatilization. [f]Not corrected for volatilization. [g]Estimated from the lower bounds 95% confidence interval of the slope.

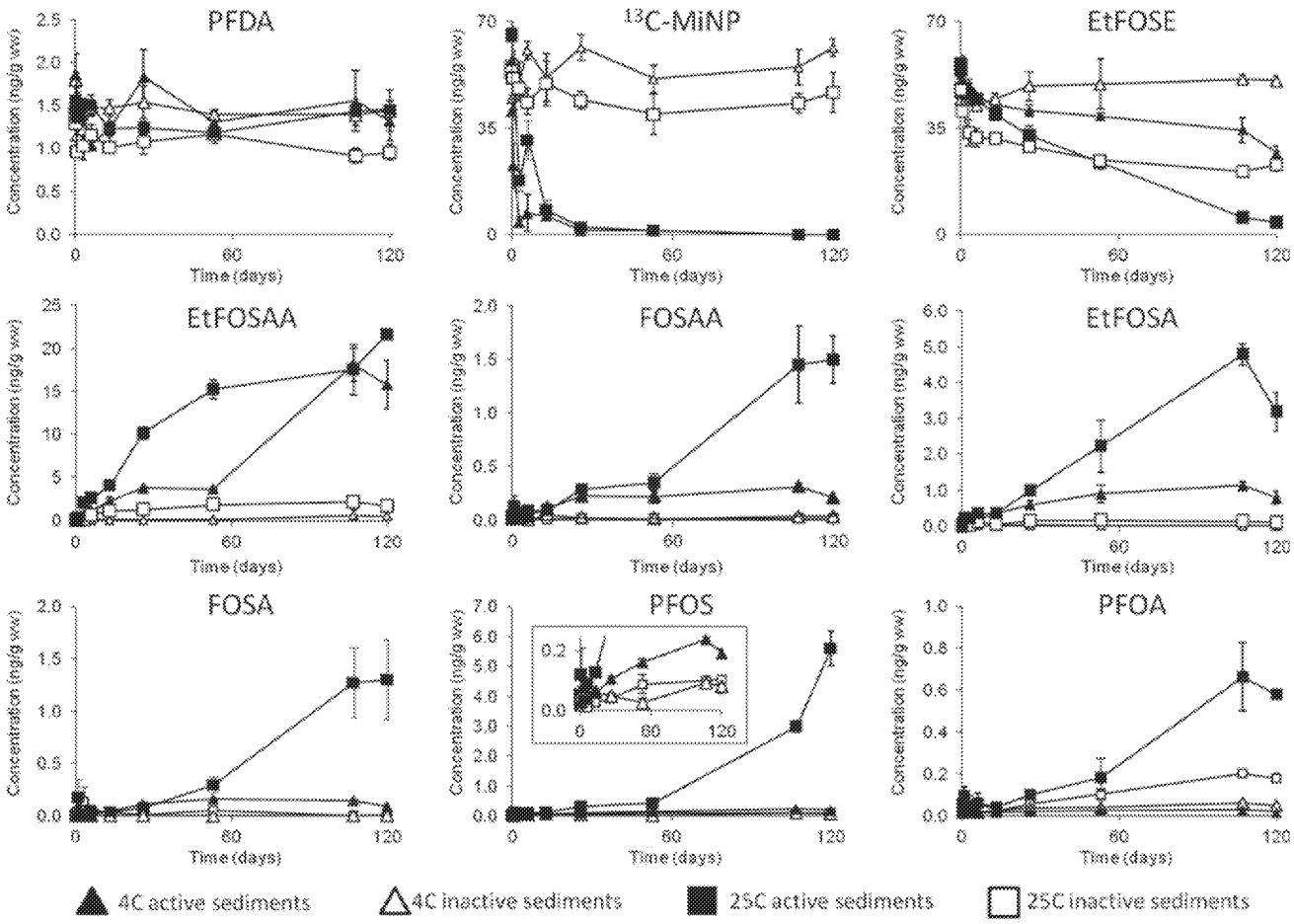

Figure 1. Incubation of EtFOSE with active marine sediments at 25 °C (squares) and 4 °C (triangles). Filled squares and triangles represent incubations with active sediments; hollow squares and triangles represent incubations with inactive sediment. The observation of PFOA in 25 °C active sediments is tentative based on its observation in 25 °C inactive sediments.

(inactive 25 °C SAmPAP diester incubations), 96 ± 3.1% (active 25 °C SAmPAP diester incubations), 88 ± 3.4% (inactive 25 °C EtFOSE incubations), and 110 ± 3.2% (active 25 °C EtFOSE incubations).

Microbial Activity. Results of microbial tests are provided in Figure S3. Dip slide tests performed on day 2 displayed clear presence of bacterial colonies in active sediments (4 and 25 °C) and absence of colonies in the HPLC-water control and inactive sediments (4 and 25 °C). Results of tests carried out on day 120 again indicated the presence of bacterial colonies in active sediments at both 4 and 25 °C and absence of colonies in inactive sediments. Overall these data indicate that a combination of autoclaving and treatment with mercuric chloride was successful at rendering control sediments inactive, while active sediments displayed the presence of microbial activity at the start and end of the study period.

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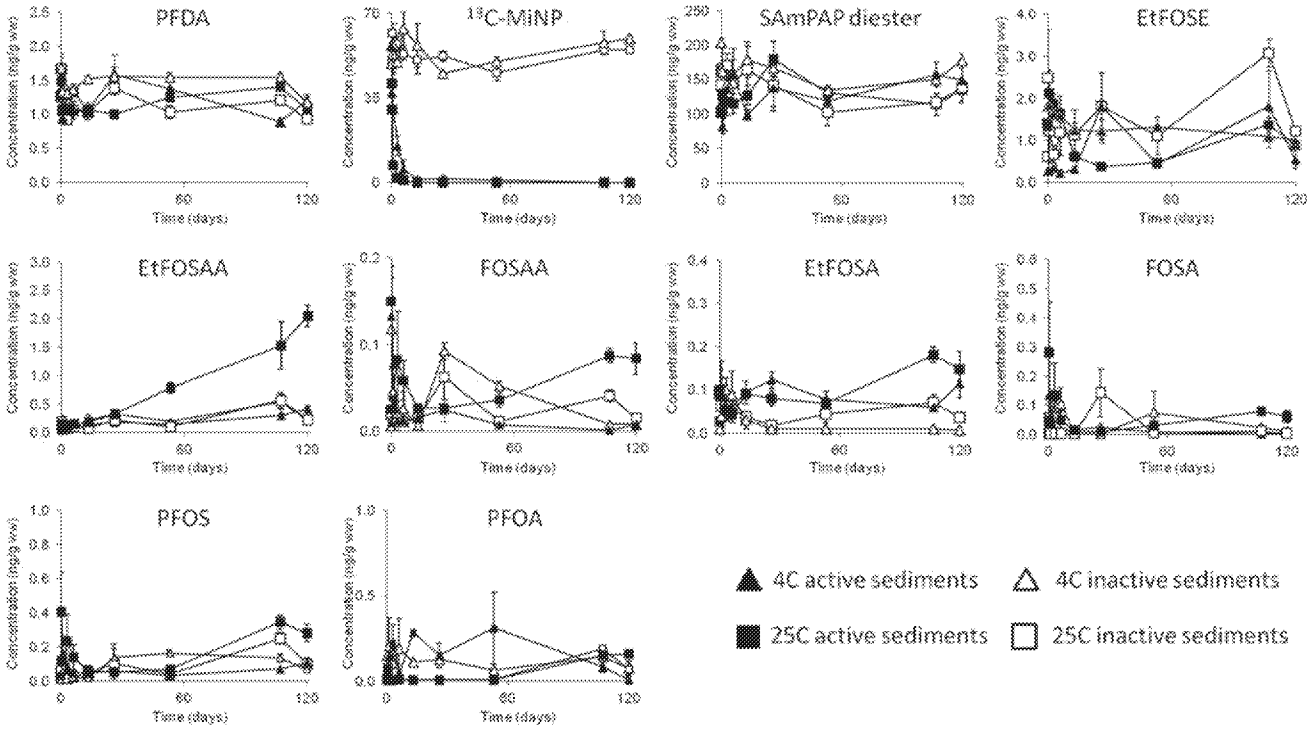

Figure 2. Incubation of SAmPAP diester with active marine sediments at 25 °C (squares) and 4 °C (triangles). Filled squares and triangles represent incubations with active sediments; hollow squares and triangles represent incubations with inactive sediment.

Microbial activity in sediments was also assessed in situ using an internal positive control (^{13}C-MiNP), which had been previously observed to undergo biodegradation by bacteria in False Creek sediments.[30,33] At 25 °C in active sediments, the half-life of ^{13}C-MiNP was 25 ± 1.9 h for the EtFOSE incubation and 28 ± 3.9 h for the SAmPAP diester incubation (Table 1), consistent with the measurements of Otton et al.[30] (23 ± 2.5 h at 22 °C). Similarly, at 4 °C, half-lives of ^{13}C-MiNP were 210 ± 25 h for the EtFOSE incubation and 220 ± 39 h for the SAmPAP diester incubation, compared to 200 ± 22 h at 5 °C reported by Otton et al.[30] In contrast, inactive sediments incubated at 4 or 25 °C showed negligible decrease in ^{13}C-MiNP over 120 days (Figures 1 and 2), indicating an absence of microbial activity in negative control sediments. The only inconsistency between these studies was that the biodegradation lag time of 20−70 h (which was accounted for in calculation of half-lives from that study) reported by Otton et al. was not observed in the present work. We attribute this primarily to the use of fresh sediment in the present work, compared to previously frozen sediments in the latter study.

EtFOSE Incubations. Low concentrations (i.e., <0.1 ng/g ww) of potential EtFOSE biodegradation products (EtFOSAA, FOSAA, FOSA, PFOS, PFOA) observed in $t = 0$ inactive and active blank (i.e., unspiked) sediments were attributed to ambient levels in False Creek sediments. Of these substances, only EtFOSAA and PFOS displayed minor increases in negative controls over time, but concentrations were always <8% of corresponding concentrations in active sediments. This is likely due to a small amount of bacteria in negative controls which was not detected by the dip slides.

In active sediments, EtFOSE was transformed to a suite of products, including EtFOSAA, FOSAA, EtFOSA, FOSA, and PFOS (Figure 1). PFOA was also observed to form in 25 °C active sediments but based on its observation in 4 °C and 25 °C inactive controls (at concentrations higher than in 4 °C active sediments) its formation was attributed to background precursors in the sediment, rather than biodegradation of EtFOSE. The proposed biodegradation pathway of EtFOSE by bacteria in marine sediments is provided in Figure 3 (adapted from Lange[20]) and is consistent with the pathway proposed in sludge. Mole balance of products and reactants relative to $t = 0$ was achieved for sediments incubated at 4 °C, ranging from 87 to 107% over all time points in EtFOSE + active sediment incubations and 90−110% over all time points in EtFOSE + inactive sediments (Table S3). However, at 25 °C, mole balance was not achieved for all time points. For example, on day 120 in EtFOSE active and inactive experiments, the sum molar concentrations of reactants and products equated to only 70 ± 2.0% and 52 ± 9.0%, respectively, of the sum molar concentrations observed on day 0 (Table S3). EtFOSE has a dimensionless air−water partition coefficient of 0.79,[14] thus we attribute these losses primarily to volatilization of EtFOSE. This is consistent with a previous study which predicted that 76% of EtFOSE in an activated sludge aeration basin would be lost to the atmosphere.[14] The absence of significant losses of EtFOSE during the 4 °C experiments is not surprising considering the vapor pressure of a substance decreases nonlinearly with decreasing temperature. In active sediments at 25 °C, losses may also be attributable to minor formation of 2 aldehyde intermediates (structures provided in Figure 3), perfluorooctane sulfonamido ethanol and/or perfluorooctane sulfinate ($C_8F_{17}SO_2^-$), none of which were monitored in the present work, but which were previously observed and/or hypothesized products in sludge biodegradation experiments.[14,20]

Even after accounting for losses from volatilization, transformation of EtFOSE was significantly ($p < 0.05$; t test) more rapid at 25 °C ($t_{1/2}$ = 44 ± 3.4 days) compared to at 4 °C ($t_{1/2}$ = 160 ± 17 days). These half-lives are considerably longer than

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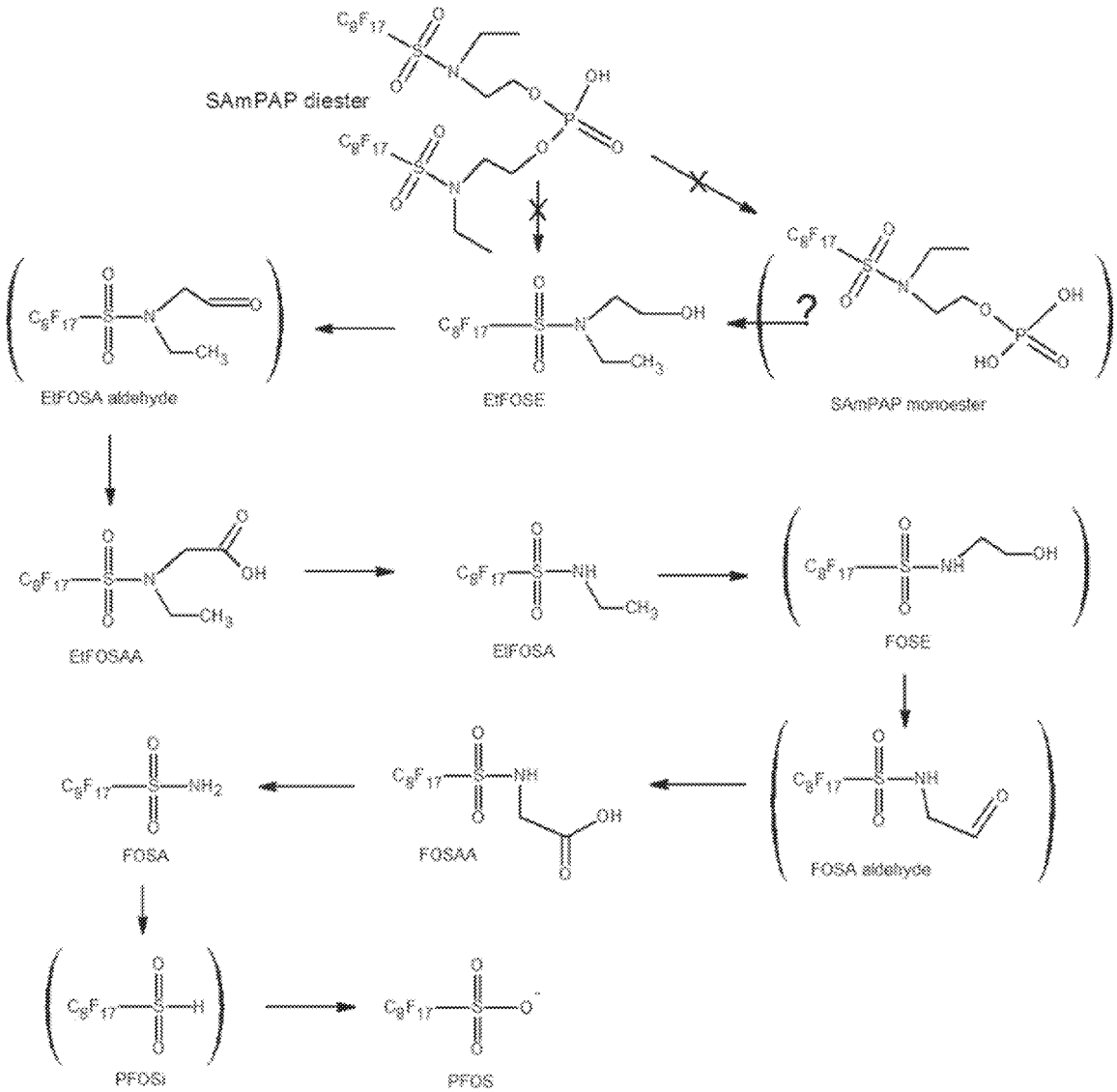

Figure 3. Biodegradation pathway of FOSAMs in marine sediments. Structures in parentheses were not monitored in the present work and are hypothesized products based on the biodegradation pathway proposed by Lange.[20]

previous reports of EtFOSE biodegradation in activated sludge (≤ 48−100 h;[14,19,20] Table 1). After 120 days in active sediments at 4 °C, EtFOSE accounted for 53% of the original dose, followed by EtFOSAA (31%), EtFOSA (1.7%), PFOS (0.44%), FOSAA (0.43%), and FOSA (0.21%), with 13.5% of the original dose lost from either volatilization or formation of products not monitored in the present work. In comparison, after 120 days in active sediments at 25 °C, EtFOSE accounted for only 7.2% of the original dose, with the balance attributable to EtFOSAA (39%), PFOS (12%), EtFOSA (6.4%), FOSAA (2.8%), and FOSA (2.8%), with 30% of the original dose attributable to losses from volatilization or formation of products other than those monitored in the present work.

SAmPAP Diester Degradation. Mole balance was achieved for SAmPAP diester biodegradation experiments (averages of 93, 89, 111, and 98% for 25 °C active, 25 °C inactive, 4 °C active, and 4 °C inactive experiments, respectively), albeit with larger variability compared to experiments involving EtFOSE (Table S3). The slopes of ln (concentration) versus time curves for SAmPAP diester were not statistically different from zero ($p < 0.05$, t test) for active or inactive sediments at either temperature. In the absence of observable biodegradation, we estimated SAmPAP biodegradation half-lives using the lower bounds 95% confidence interval of the slopes obtained from active sediment biodegradation experiments. At 4 °C, the half-life of SAmPAP diester was estimated to be >3400 days, while at 25 °C the half-life was estimated at >380 days. It should be noted that small quantities of potential SAmPAP diester degradation products were observable after 120 days (including EtFOSAA, FOSAA, and PFOS) possibly indicating minor biodegradation of the test substance (Figure 2). However, the concentrations of these substances were always <2 ng/g ww and likely arise from transformation of residual EtFOSE or SAmPAP monoester impurities (not monitored) in the commercial SAmPAP diester formulation, some of which were also observed in inactive spiked sediments. Novel residuals in historical 3M commercial products continue to be identified[34] and present a challenge for biodegradation studies. The development of purified (i.e., residual-free) SAmPAP standards will greatly assist future

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assessment of their stability and environmental behavior. To our knowledge, this is the first study to investigate the biodegradation of a high-molecular-weight FOSAM and the first report in which a FOSAM has been recalcitrant over a long-term (120 days) assessment of its biodegradation potential.

The lack of biodegradation of SAmPAP diester observed here is consistent with the predictions of environmental persistence for this substance by Howard and Muir[35] and also observations associated with high-molecular-weight PFASs (e.g., fluoroacrylate polymers), where half-lives of 10−1700 years have been reported.[36,37] While Lee et al. reported biodegradation of 6:2 diPAP and 10:2 monoPAP (which are structurally and functionally similar to SAmPAP esters) in sludge,[27] degradation rates decreased considerably with increasing chain length and substitution. Thus it is unclear whether long chain diPAPs (e.g., 10:2 diPAP) would, in fact, be amenable to biodegradation, even in sludge. SAmPAP diester has a shorter perfluoroalkyl chain compared to 10:2 diPAP, similar molecular weight (1204 g/mol versus 1190 g/mol, respectively), but a considerably larger estimated log K_{ow} (16.16[35] versus 12.88,[38] respectively). As mentioned by Lee et al., these properties could favor sorption of both substances to solids, making them less accessible for microbial attack. Nonetheless, the biological activity in sludge is expected to be much greater than that in marine sediments (as evidenced by the much longer half-lives for EtFOSE in the present work compared to previous measurements in activated sludge[14,20]), thus further work is necessary to assess the biodegradation potential of 10:2 diPAP and SAmPAP diester in sludge. Overall, these findings are consistent with previous experiments involving phthalate esters biodegradation in False Creek sediments[33] in which the particulate-bound fraction of the test substance was not degraded or degradable, even though biodegradation was possible for the unbound fraction.

Sources, Fate, and Behavior of FOSAMs in Marine Sediments. According to UNSCPOP, a substance is considered persistent in sediments if its half-life is greater or equal to 180 days.[39] Under this criteria, SAmPAP diester appears to be persistent in marine sediment, even using half-life estimates based on lower bounds confidence intervals of regression slopes (>380 days at 25 °C and >3400 days at 4 °C). Considering its hydrophobicity (field-based log sediment−water distribution coefficient >4.3),[25] and the fact that there are no known precursors of SAmPAP diester (except for potentially SAmPAP triester, which was a minor (~5%) constituent in commercial formulations[16]), the occurrence of SAmPAP diester in sediments is expected to arise from sorption to suspended solids in effluent or urban runoff, which are deposited in sediment following release. This hypothesis is consistent with recent measurements in unfiltered stormwater runoff using advanced oxidation processes, where ∑perfluoroalkyl acid (PFAA)-precursors (possibly including SAmPAP diester) were present at similar concentrations to PFAAs.[40] Once present in sediments, SAmPAP diester is not expected to biodegrade but might be absorbed and biotransformed to PFOS by benthic and higher trophic level organisms. However, data submitted to the U.S. Environmental Protection Agency by the 3M Co. indicated that while SAmPAP diester is biotransformed to low-molecular weight FOSAMs and PFOS in rats, it was poorly (~1%) absorbed after a single oral dose.[41] Therefore it remains unclear if SAmPAP diester would, in fact, be a significant source of PFOS in benthic and higher trophic level organisms.

While EtFOSE is technically not persistent in marine sediments under UNSCPOP criteria ($t_{1/2}$ = 44 ± 3.4 days at 25 °C and 160 ± 17 days at 4 °C), its biodegradation half-life is considerable (in particular under cold conditions), which may in part explain the continued observation of FOSAMs in marine sediments in Tokyo Bay, Vancouver, and San Francisco, over a decade after the phase-out of these substances.[25,28,29] This observation, and the fact that low-molecular-weight FOSAMs are efficiently absorbed and biotransformed in rats,[42] humans,[42] fish,[13] and aquatic worms[43] is suggestive that low-molecular weight FOSAMs are a potentially significant source of indirect PFOS exposure in benthic organisms. Others have drawn similar conclusions from field data. For example, Martin et al. observed higher concentrations of FOSA compared to PFOS in diporeia and slimy sculpin from Lake Ontario,[44] and recent work by Asher et al. suggested that nonracemic profiles of chiral PFOS in benthic organisms from Lake Ontario are indicative of exposure primarily to PFOS-precursors in sediment.[45] Another study measuring both extractable organic fluorine along with 17 known PFASs (13 perfluoroalkyl carboxylates, 6 perfluoroalkyl sulfonates, 4 fluorotelomer acids, FOSA, and EtFOSAA) found that only 10−12% of the total extractable organic fluorine in shrimp could be accounted for by known PFASs, with the balance attributable to unidentified PFASs (possibly including FOSAMs).[46] Clearly there is mounting evidence that precursors in sediment are a potentially significant source of PFAAs in benthic organisms, and potentially also higher trophic-level organisms. Until such time that the contribution of PFAA precursors is considered in PFAA bioaccumulation models, the disposition of PFAAs within marine foodwebs will be difficult to predict.

ASSOCIATED CONTENT

Supporting Information

Further details on instrument parameters, results of spike/recovery experiments, half-lives, and results of microbial activity tests. 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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