LONG TERM AEROBIC BIOTRANSFORMATION OF PERFLUOROOCTANE SULFONAMIDOETHANOL BASED PHOSPHATE ESTERS USING RIVER WATER/SEDIMENT INCUBATION SYSTEM

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Introduction

N-Ethyl perfluorooctane sulfonamidoethanol based phosphate esters such as *N*-Ethyl perfluorooctane sulfonamidoethanol monophosphate (SAmPAP) and *N*-Ethyl perfluorooctane sulfonamidoethanol diphosphate (diSAmPAP) are a group of fluorinated compound, used as water repellents for food contact paper and packaging[1]. Benskin *et al.* reported that diSAmPAP was highly recalcitrant to microbial degradation in marine sediment after 120 days incubation[2]. On the other hand, Zhang *et al.* clarified that diSAmPAP would be biotransformed to perfluorooctanesulfonic acid (PFOS) in lake sediment[3]. SAmPAP and diSAmPAP were thought to be eventually biotransformed to perfluorooctane-sulfonic acid (PFOS) via several metabolites by aerobic microbial metabolism in the aquatic environment (Fig. 1)[2]. Our previous study showed that 70% of spiked *N*-ethyl perfluorooctane sulfonamidoethanol (EtFOSE) was biotransformed to PFOS in river water/sediment bottle after 200 days incubation[4]. The study also confirmed the biotransformation of EtFOSE to perfluorooctanoic acid (PFOA) and the homologues during the incubation period. It is important to elucidate biotransformation behaviors of SAmPAP and diSAmPAP under natural aquatic conditions using river water and sediment. The objective of this study is to elucidate the ratios of aerobic biotransformation from SAmPAP and diSAmPAP in natural river water/sediment system by using a shake-flask batch test during a long period.

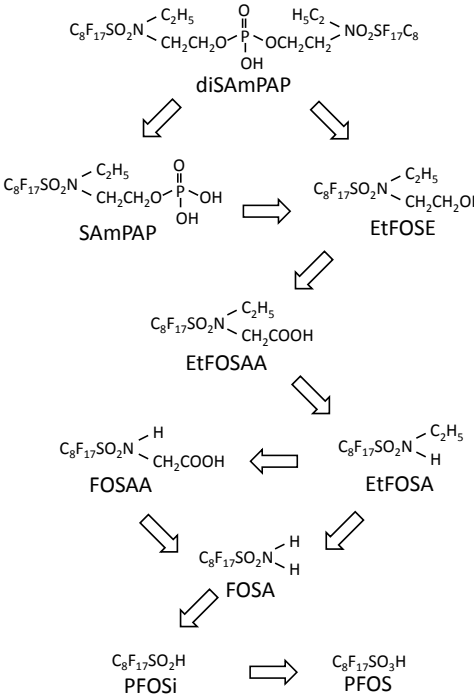

Figure 1: Possible aerobic biodegradation pathway of SAmPAP and diSAmPAP (Data from Ref.2, partially modified)

Materials and methods

Chemicals and reagents

Standard chemicals of PFOS and 11 precursors (perfluorooctane sulfinic acid (PFOSi), perfluorooctane sulfonamide (FOSA), *N*-methyl perfluorooctane sulfonamide (MeFOSA), *N*-ethyl perfluorooctane sulfonamide (EtFOSA), perfluorooctane sulfonamidoacetic acid (FOSAA), *N*-methyl perfluorooctane sulfonamidoacetic acid (MeFOSAA), *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA), *N*-methyl perfluorooctane sulfonamidoethanol (*N*-MeFOSE), EtFOSE, SAmPAP, and diSAmPAP), and PFOA and the 3 homologues (perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA)) were purchased from Wellington Laboratories, Inc. (Canada). $^{13}C_4$-PFOS, $^{13}C_4$-PFOSi, $^{13}C_8$-FOSA, d_3-MeFOSA, d_5-EtFOSA, d_3-MeFOSAA, d_5-EtFOSAA, d_7-MeFOSE, d_9-EtFOSE, $^{13}C_2$-8:2 fluorotelomer phosphate monoester ($^{13}C_2$-8:2 PAP), $^{13}C_4$-8:2 fluorotelomer phosphate diester ($^{13}C_4$-8:2 diPAP), $^{13}C_4$-PFOA, and $^{13}C_2$-PFHxA were also purchased from Wellington Laboratories, Inc., and used as internal standards.

Experimental design

Approximately 10 L of surface water and 500 mL of surface sediment slurry were taken from Iruma River, Japan, on August 2018, and collected into screw cap polypropylene bottles. The surface sediment slurry was filtered through a stainless steel sieve (the mesh opening size is 1 mm) before use.

The collected samples were used for degradation tests of SAmPAP and diSAmPAP. The experimental design for aerobic biotransformation test was performed on the basis of 'ISO14592-1; Shake-flask batch test with surface water or surface water/sediment suspensions'[5], but the experimental period was extended for 252 days.

The test was started on the day after of the river water and sediment collection. One hundred milliliter river water and sediment slurry contained 50 mg (dry-weight basis) suspended solids, were poured into a 250-mL sterile polyethylene (PE) terephthalate glycol modified culture bottle with a high-density PE screw cap. The total of 80 bottles were prepared and equally divided into 8 series, includes (i) intact control (20 µL methanol (MeOH) added), (ii, iii) microbial active series of SAmPAP (100 ng SAmPAP (*i.e.* 154 pmol) in 20µL MeOH added), (iv, v) microbial active series of diSAmPAP (100 ng diSAmPAP (*i.e.* 83 pmol) in 20µL MeOH added), (vi) microbial sterile series of SAmPAP (100 ng SAmPAP (*i.e.* 154 pmol) in 20µL MeOH, 1 g sodium azide added), (vii) microbial sterile series of diSAmPAP (100 ng diSAmPAP (*i.e.* 83 pmol) in 20µL MeOH, 1 g sodium azide added), and (viii) water quality series (100 ng SAmPAP (*i.e.* 154 pmol) in 20µL MeOH added). The series of (viii) was prepared to measure optical dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, and electric conductivity (EC). Each 8 series consisted of 10 bottles. Duplicate sample was prepared for 'microbial active series of SAmPAP (ii and iii) and diSAmPAP (iv and v). The bottles were continuously incubated at 20°C with 100 rpm horizontal shaking using constant temperature incubator shakers (BR-40LF or ML-10F, TAITEC Co., Japan). The incubation periods were set to be 0, 28, 56, 84, 112, 140, 168, 196, 224, and 252 days (every 28 days).

Table 1: Measurement conditions for UPLC/MS/MS

UPLC	
Instrument	ACQUITY UPLC H-Class (Waters)
Column	UHPLC PEEK Column InertSustainSwift C18 ϕ2.1x50 mm, 1.9 µm (GL Science)
Mobile phase	A : 10 mM Ammonium acetate B : Acetonitrile
Gradient	0 min (30%B) – 6 min (95%B) – 7 min (95%B) –7.1 min (30%B) – 11 min (30%B)
Flow	0.4 mL/min
Column oven temp.	40℃
Injection volume	5 µL

MS/MS	
Instrument	XevoTQD (Waters)
Ionization mode	ESI negative
Desolvation gas flow	N_2; 800 L/h
Desolvation temp.	350℃
Cone gas flow	N_2; 50 L/h
Collision gas	Ar
Source temp.	90℃
Capillary voltage	1 kV

Compound	Monitor ion (m/z)	Cone (V)	Collision (eV)
PFOS	498.8 > 80.0	88	50
[$^{13}C_4$]–PFOS	502.8 > 79.9	86	52
PFOSi	482.9 > 419.0	24	12
[$^{13}C_4$]–PFOSi	486.9 > 423.0	24	12
FOSA	497.9 > 78.0	56	34
[$^{13}C_8$]–FOSA	505.9 > 78.0	56	34
MeFOSA	511.9 > 169.0	54	28
MeFOSA–d_3	514.9 > 169.0	54	28
EtFOSA	525.9 > 169.0	54	32
EtFOSA–d_5	531.0 > 169.0	54	32
FOSAA	555.9 > 498.0	48	30
MeFOSAA	569.9 > 419.0	32	22
MeFOSAA–d_3	572.9 > 419.0	32	22
EtFOSAA	584.0 > 419.0	36	20
EtFOSAA–d_5	589.0 > 419.0	36	20
MeFOSE	616.0 > 59.0	24	20
MeFOSE–d_7	623.0 > 59.0	24	20
EtFOSE	630.0 > 59.0	24	20
EtFOSE–d_9	639.0 > 59.0	24	20
SAmPAP	650.2 > 526.2	56	30
[$^{13}C_2$]–8:2PAP	545.2 > 97.0	40	28
diSAmPAP	1203.3 > 526.2	100	50
[$^{13}C_4$]–8:2diPAP	993.3 > 97.0	64	42
PFPeA	262.8 > 219.1	26	8
PFHxA	312.8 > 269.0	26	10
[$^{13}C_2$]–PFHxA	314.8 > 270.0	28	10
PFHpA	362.8 > 319.0	26	10
PFOA	412.8 > 169.0	28	20
[$^{13}C_4$]–PFOA	416.8 > 171.9	28	18

Sample analysis

Water quality parameters (DO, ORP, pH, and EC) of incubating samples in 'water quality series (viii)' were measured by a multi-parameter meter (HQ40d, Hach, USA) at the end of the each incubation day. Sixteen fluorinated compounds (PFOS, PFOSi, FOSA, MeFOSA, EtFOSA, FOSAA, MeFOSAA, EtFOSAA, MeFOSE, EtFOSE, SAmPAP, diSAmPAP, PFPeA, PFHxA, PFHpA, and PFOA) were determined by an ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS (ACQUITY UPLC H-Class / Xevo TQD, Waters Co., USA)). After the incubation, the sample in a culture bottle was alkalized using 0.04 g of sodium carbonate, and 4.5-5.0 ng internal standards in 50 µL MeOH were added into the sample. The sample was transferred into a 100-mL glass centrifuging tube, and centrifuged at 3,000 rpm for 20 min. A pre-conditioned Presep® PFC-II solid phase extraction (SPE) cartridge (FUJIFILM Wako Pure Chemical Co., Japan) with 5 mL of 0.1% ammonium hydroxide in MeOH, 5 mL MeOH and 20 mL ultrapure water, was equipped onto a Sep-Pak concentrator system (Waters Co.), then the supernatant was passed through the cartridge at a flow rate of 10 mL/min. The cartridge was centrifuged at 3,000 rpm for 20 min for dehydration. Target compounds were eluted with 3 mL of 0.1% ammonium hydroxide in MeOH from the cartridge to a 10-mL glass test tube. Target compounds remained in the centrifuged sediment were extracted by ultrasonic vibration for 10 min with 10 mL MeOH. After centrifugation at 3,000 rpm for 10 min, the supernatant was transferred into a 100-mL glass eggplant flask. This extraction process was repeated twice in the same manner. The inner wall surface of the culture bottle and a SPE reservoir were rinsed with 10 mL MeOH, and then combined with the ultrasonic extract in the 100-mL glass eggplant flask. The solution was concentrated to 2 mL using a rotary vacuum evaporator at 36°C, and mixed to the eluate

in the 10-mL glass test tube. The combined extract was concentrated to 0.5 mL under a gentle stream of N_2 gas at 36°C, and added with 0.45 mL of methanol and 0.05 mL of 0.1% formic acid. The extract was passed through a cellulose-acetate membrane filter (DISMIC, 25 mm in diameter and 0.20 μm pore size, Toyo Roshi Kaisya, Ltd., Japan), and transferred into a 2-mL amber glass vial for UPLC/MS/MS measurement. The details of measurement condition for a UPLC/MS/MS were shown in Table 1. Concentrations of target compounds except for FOSAA, SAmPAP, diSAmPAP, PFPeA, and PFHpA were quantified using the corresponding internal standard. FOSAA, SAmPAP, diSAmPAP, PFPeA, and PFHpA were quantified using $^{13}C_4$-PFOSi, $^{13}C_2$-8:2 PAP, $^{13}C_4$-8:2 diPAP, $^{13}C_2$-PFHxA, and $^{13}C_4$-PFOA, respectively. The method detection limits (MDL) of target compounds ranged from 0.1 to 1.9 pmol/bottle, and the average recoveries were ranged from 67 to 115%.

Results and discussion

Surface river water and sediment

Water quality parameters of the collected surface river water were 6.8 of pH, 20.7 mS/m of EC, 8.6 mg/L of SS, and 1.4 mg/L of VSS. SS and VSS of the collected surface sediment slurry were 180,500 and 17,237 mg/L, respectively.

Aerobic condition of biotransformation experiment

Aerobic conditions in samples at 0-252 days were confirmed in 'water quality series (v)' which incubated in the same manner. PH, EC, DO, and ORP ranged 5.9-9.0, 19.4-21.0 mS/m, 4.6-8.4 mg/L, and 195.3-315.1 mV, respectively. Aerobic conditions were kept during the incubation period.

Concentrations and ratios of the parent compound and the biotransformation compounds

PFOS, PFOSi, FOSA, SAmPAP, diSAmPAP, PFPeA, PFHxA, PFHpA, and PFOA were detected in the 'intact control samples (i)' during each incubation period, at the concentrations ranging 0.5-1.2, <0.1-0.3, <0.1-0.2, <1.9-6.7, <0.1-0.1, <0.3-23.8, 0.9-2.2, <0.2-1.0, and 0.5-1.4 pmol/bottle, respectively. MeFOSA, EtFOSA, FOSAA, MeFOSAA, EtFOSAA, MeFOSE, and EtFOSE were not detected in all intact control samples.

The concentrations of PFCs in samples of active and sterile series were corrected by deduction of the intact control concentration at the corresponding incubation periods.

The results of active and sterile incubation of SAmPAP were shown in Figure 2(a, b). The total molar on microbial active (ii, iii) and sterile series (vi) to initial spiked SAmPAP (154 pmol/bottle) ranged 81.7-166.1 pmol (53-108%), and 92.6-193.3 pmol (60-126%), respectively. MeFOSA, MeFOSAA, MeFOSE, and diSAmPAP were not detected in these series.

The concentration of SAmPAP drastically decreased from 153.8 (Initial spiked amount) to 51.1 pmol/bottle at Day 0 in active series samples (ii, iii), and the primary dominant metabolite EtFOSE was generated. EtFOSE gradually decreased from 113.7 pmol/bottle at Day 0 to 0.2 pmol/bottle at Day 252. The first-order half-life of EtFOSE was calculated to 24.3 days. The results was similar to those of EtFOSE (33.6 days) by our previous biodegradation test in same manner[4]. EtFOSAA was also found to be one of major metabolite, and the highest concentration was 53.3 pmol/bottle at Day 28. EtFOSAA was frequently detected in river and surface runoff waters in Japan[6]. It was thought that EtFOSAA is one of the major residual metabolite of EtFOSE. Concentrations of FOSAA, EtFOSA, FOSA, PFOSi, and PFOS were detected at the peak concentration of 5.0 (Day 56), 1.6 (Day 28), 30.5 (Day 56), 4.5 (Day 56), and 75.3 (Day 252) pmol/bottle, respectively. This study demonstrated that at least 49% of initial spiked SAmPAP was biotransformed to PFOS under the microbial active condition in river water/sediment system about 250 days incubation. Interestingly, PFPeA, PFHxA, PFHpA, and PFOA were also detected in this biotransformation test, with the ranges of <0.3-14.3, <0.1-0.2, <0.2-0.8, and 0.2-7.5 pmol/bottle, respectively. Lange conducted a similar aerobic biotransformation experiment for EtFOSE with artificial mineral salts medium containing municipal waste treatment facility sludge in a culture flask[7]. The results showed that EtFOSE was mostly degraded with small portion of biotransformed PFOA (0.6%) in 35 days incubation, suggesting the aerobic biotransformation from SAmPAP to PFOA requires long biodegradation period, and an unknown biotransformation pathway from EtFOSE to PFOA can be considered.

The concentrations of SAmPAP decreased from 103.5 (Day 0) to 2.6 (Day 56) pmol/bottle in sterile series samples (vi), and SAmPAP was not detected till the end of this incubation period. Instead of the disappearance of SAmPAP, EtFOSE was generated and remained in the sterile bottles. The result suggests EtFOSE may be generated from SAmPAP by hydrolysis in this sterile condition.

The results of active and sterile incubation of diSAmPAP were shown in Figure 2(c, d). To compare initial molar concentration, the metabolite concentrations of diSAmPAP presented at a half concentration measured because diSAmPAP consists of two EtFOSE units. The total molar on microbial active (iv, v) and sterile series (vii) to initial spiked diSAmPAP (83 pmol/bottle) ranged 53.2-76.5 pmol (64-92%), and 48.9-97.6 pmol (59-117%), respectively. MeFOSA, MeFOSAA, MeFOSE, and SAmPAP were not detected in these series.

The concentration of diSAmPAP decreased from 83.1 (Initial spiked amount) to 49.6 pmol/bottle at Day 0 in active series samples (iv, v) with the primary metabolite EtFOSE.

EtFOSE gradually decreased from 11.7 pmol/bottle at Day 0 to 0.6 pmol/bottle at Day 252. EtFOSAA was also one of major metabolite, and the highest concentration was 22.0 pmol/bottle at Day 56. Concentrations of the other detected metabolites FOSAA, EtFOSA, FOSA, PFOSi, and PFOS were also detected at the peak concentration of 6.0 (Day 84), 0.5 (Day 28), 9.7 (Day 84), 1.4 (Day 56), and 47.7 (Day 252) pmol/bottle, respectively. This study demonstrated that at least 57% of initial spiked diSAmPAP biotransformed to PFOS under the microbial active condition in river water/sediment system about 250 days incubation. Interestingly, PFPeA, PFHxA, PFHpA, and PFOA also detected in this biotransformation test, ranging <0.3-3.8, <0.1-0.1, <0.2-0.4, and <0.2-3.8 pmol/bottle, respectively. It was thought that the aerobic biotransformation from diSAmPAP to PFOA requires long biodegradation period.

The percentages of diSAmPAP were ranged from 84 to 99% in sterile series samples (vi), but 1 to 15% of EtFOSE also detected during the incubation period. The result suggests that river water/sediment had biotransformation properties from SAmPAP and diSAmPAP to PFOS at the rate of 49-57% at 252 days incubation. The biodegradation patterns of SAmPAP and diSAmPAP were similar, but diSAmPAP was tolerable to hydrolysis and more persistent in river than SAmPAP. Moreover, SAmPAP fully transformed to EtFOSE by hydrolysis in the sterile conditions of this study, but the reaction was limited for diSAmPAP.

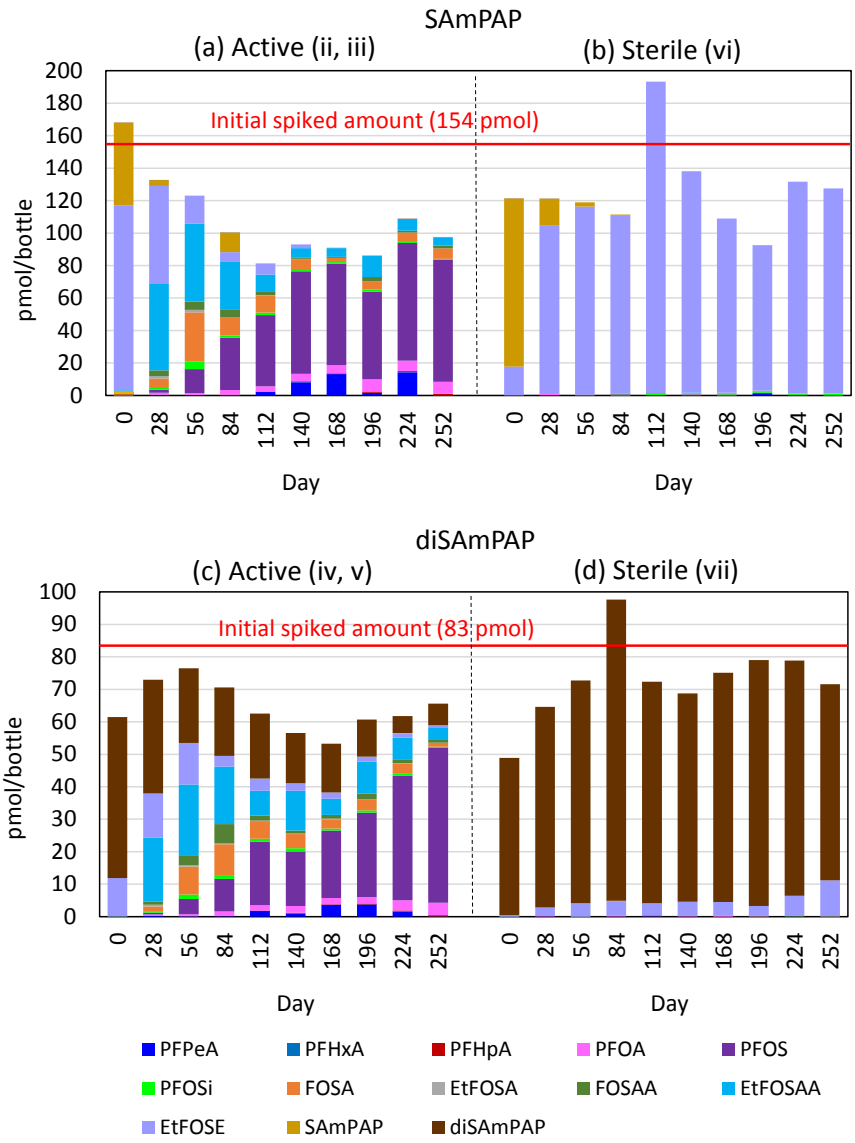

Figure 2: Biodegradation patterns of SAmPAP (a: Active, b: Sterile) and diSAmPAP (c: Active, d: Sterile) in river water/sediment system during incubation period.
Concentrations of chemicals in Figure 2(c, d) except for diSAmPAP were presented at a half concentration measured.

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