BIODEGRADATION OF FLUORINATED COMPOUNDS IN RIVER WATER/SEDIMENT SYSTEM BY A SHAKE-FLASK BATCH TEST

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Introduction

Perfluorinated compounds have been widely used for commercial and industrial applications such as surfactants, lubricants, paper and textile coatings, and fire-fighting foams. Many researchers reported a global distribution of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in surface water, groundwater, sediment, and biota[1,2]. PFOS and PFOA are persistent accumulating in the environment, and thus affecting organisms[3-5]. C8-perfluorinated compounds such as *N*-methyl perfluoro-1-octane sulfonamidoethanol (*N*-MeFOSE) and 2-perfluorooctyl ethanol (8:2FTOH) are precursors of PFOS and PFOA, respectively. These precursors are also used for commercial and industrial applications[6-8], and are thought to be breakdown to PFOS or PFOA in the environment. The biodegradation processes of 8:2FTOH and *N*-ethyl perfluoro-1-octane sulfonamidoethanol (*N*-EtFOSE) which related substance of *N*-MeFOSE, were elucidated by aerobic biodegradation experiments[9-11]. Most biodegradation tests have been carried out under artificial incubation media with activated sludge from wastewater treatment plants. A few reports for biodegradation of the precursors were published under natural river water/sediment system. Hence, we investigated the transformation ratios of *N*-MeFOSE and 8:2FTOH in river water/sediment system by laboratory experiments.

Materials and methods

Chemicals and reagents

Standard chemicals of PFOS, and PFOA were purchased from Kanto Chemical Co. (Japan) and Wako Pure Chemical Ind. (Japan), respectively. Nine PFOS precursors (perfluorooctane sulfinic acid (PFOSi), perfluoro-1-octane sulfonamide (PFOSA), *N*-methyl perfluoro-1-octane sulfonamide (*N*-MeFOSA), *N*-ethyl perfluoro-1-octane sulfonamide (*N*- EtFOSA), perfluoro-1-octane sulfonamidoacetic acid (PFOSAA), *N*-methyl perfluoro-1-octane sulfonamidoacetic acid (*N*-MeFOSAA), *N*-ethyl perfluoro-1-octane sulfonamidoacetic acid (*N*-EtFOSAA), *N*-MeFOSE, *N*-EtFOSE), 3 PFOA precursors (8:2FTOH, 2H-perfluoro-2-decenoic acid (8:2FTUCA), and 2-perfluorooctyl ethanoic acid (8:2FTCA)) were purchased from Wellington Laboratories, Inc. (Canada). Twelve kinds of internal standards ($^{13}C_4$- PFOS, $^{13}C_4$-PFOSi, $^{13}C_8$-PFOSA, d_3-*N*-MeFOSA, d_5-*N*-EtFOSA, d_3-*N*-MeFOSAA, d_5-*N*-EtFOSAA, d_7-*N*-MeFOSE, d_9-*N*-EtFOSE, $^{13}C_4$-PFOA, $^{13}C_2$,d_2-8:2FTOH, $^{13}C_2$-8:2FTUCA, and $^{13}C_2$-8:2FTCA) were also purchased from Wellington Laboratories, Inc. Reagents used in this study were of residual pesticide analysis grade.

River surface water and sediment

Surface water and sediment were collected at Iruma river (E139º32'19.2, N35º56'18.4) using a stainless steel bucket or a long-handled stainless steel ladle, and were stored into screw cap polypropylene bottles. The water and sediment collected in January 2013 and February 2013 were used for degradation test of *N*-MeFOSE and 8:2FTOH, respectively.

Experimental design

Aerobic biodegradation test was performed on the basis of 'ISO14592-1; Shake-flask batch test with surface water or surface water/sediment suspensions'[12]. The test was started at the next day of the sample collection. A 100 ml sample of river water and sediment slurry contained 50 mg (dry-weight basis) suspended particle, was added into a 250-ml sterile polyethylene (PE) terephthalate, glycol modified culture bottle with a high-density PE screw cap. The bottles were divided into 3 groups, this includes (i) intact control (no compound added), (ii) active river water/sediment system (only precursor added), and (iii) sterile control (precursor and 1 g each of sodium azide added). After 100 ng of *N*-MeFOSE (180 pM) or 8:2FTOH (216 pM) was spiked to the sample of active river water/sediment system and sterile control groups, the bottles were continuously incubated at 20°C

with 100 rpm shaking using constant temperature incubator shakers (ML-10F or BR-40LF, TAITEC Co., Japan). A bottle of each group was taken from the incubators after 0, 3, 7, 14, 21, and 28 days for analysis.

Sample treatment for LC/MS/MS analysis

PFOS, PFOA and the 11 precursors (PFOSi, PFOSA, *N*-MeFOSA, *N*-EtFOSA, PFOSAA, *N*-MeFOSAA, *N*-EtFOSAA, *N*-MeFOSE, *N*-EtFOSE, 8:2FTUCA, and 8:2FTCA) were determined by LC/MS/MS (ACQUITY UPLC H-Class / Xevo TQD, Waters Co., USA) for the biodegradation tests. After the incubation, the sample in a culture bottle was alkalized using 0.04 g of sodium carbonate, and 4.5-50 ng internal standards in 50 µl methanol (MeOH) were added into the sample. The sample was centrifuged at 3,000 rpm for 20 min. The supernatant was passed through a Oasis HLB plus cartridge (Waters Co.) and target compounds were eluted with 3 ml MeOH. 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 to a 100-ml glass flask. This extraction process was repeated twice in the same manner. The inner wall surface of the culture bottle was rinsed with 10 ml MeOH, and the rinsed solution was combined with the ultrasonic extract into the 100-ml flask. The solution was concentrated to 2 ml using a rotary vacuum evaporator at 40°C. The combined extract from the supernatant and particle was concentrated to 0.5 ml under a gentle stream of N_2 gas at 40°C, and 0.5 ml of methanol and 0.1 ml of 0.1% formic acid were added. The details of measurement condition for LC/MS/MS were shown in Table 1. Concentrations of PFCs without PFOSAA were quantified using the corresponding internal standard. PFOSAA was quantified using the absolute calibration method because no internal standard. The detection limits (DL) of those compounds ranged from 0.04 to 0.9 ng/l.

Table 1 Measurement conditions for LC/MS/MS

LC	
Instrument	ACQUITY UPLC H-Class (Waters)
Column	ACQUITY UPLC HSS T3 (Waters) ϕ 2.1x50 mm, 1.8 µm
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) – 10 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
Source temp.	90℃
Capillary voltage	1 kV

Compound	Monitor ion (m/z)	Cone (V)	Collision (eV)
PFOA	413.0 > 168.9	18	18
$^{13}C_4$–PFOA	416.8 > 169.0	18	18
8:2FTUCA	456.9 > 393.0	20	14
$^{13}C_2$–8:2FTUCA	458.9 > 394.0	20	14
8:2FTCA	477.1 > 393.1	16	10
$^{13}C_2$–8:2FTCA	479.2 > 394.1	14	15
PFOS	498.8 > 80.0	76	52
$^{13}C_4$–PFOS	502.9 > 79.9	76	52
PFOSi	482.9 > 419.0	24	12
$^{13}C_4$–PFOSi	486.9 > 423.0	24	12
PFOSA	497.9 > 78.0	56	34
$^{13}C_8$–PFOSA	505.9 > 78.0	56	34
N–MeFOSA	511.9 > 169.0	54	28
d_3–N–MeFOSA	514.9 > 169.0	54	28
N–EtFOSA	525.9 > 169.0	54	32
d5–N–EtFOSA	531.0 > 169.0	54	32
PFOSAA	555.9 > 498.0	48	30
N–MeFOSAA	569.9 > 419.0	32	22
d3–N–MeFOSAA	572.9 > 419.0	32	22
N–EtFOSAA	584.0 > 419.0	36	20
d5–N–EtFOSAA	589.0 > 419.0	36	20
N–MeFOSE	616.0 > 59.0	24	20
d7–N–MeFOSE	623.0 > 59.0	24	20
N–EtFOSE	630.0 > 59.0	24	20
d9–N–EtFOSE	639.0 > 59.0	24	20

Sample treatment for GC/MS analysis

8:2FTOH was determined by GC/MS (GC; Agilent 6890, Agilent Technologies, Inc., USA / MS; GCmateII, JEOL Ltd., Japan) for the 8:2FTOH biodegradation test. The procedure was according to the purge and trap extraction method[13] with minor modifications. Briefly, the incubated river water/sediment and 300 ml pure water was gently poured into a 1000-ml gas washing bottle. Twenty five ng of internal standard ($^{13}C_2$,d_2-8:2FTOH) in acetone and 40 g of sodium chloride were added into the sample, and the bottle was placed in an ultrasonic water bath at 40°C to accelerate the volatilization of target compounds and prevent the foaming of water. Indoor air through an gas filters (XAD-2 and activated carbon was aerated to the bottle at a rate of 0.75 l/min for 1 hour using a vacuum pump, then extracted target compounds from water phase were trapped on a SPE cartridge (InertSep RP-1 mini, GL Sciences Inc., Japan), which was set at the exit of the gas washing bottle. The target compounds were eluted from the SPE cartridge with 3 ml of dichloromethane, and the extract was concentrated to 0.5 ml under a gentle stream of N_2 gas at 30°C. GC/MS conditions were described on Ref. 13. The DL of 8:2FTOH was 0.4 ng/l[13].

Results and discussion

Aerobic biodegradation of N-MeFOSE

PFOA, 8:2FTUCA, PFOS, PFOSi, PFOSA, *N*-EtFOSA, and *N*-MeFOSE were detected in the intact control samples during the test period, and their concentrations ranged <0.4-3.0, <0.3-0.3, 0.8-1.1, <0.2-0.4, 0.1-0.5, <0.2-0.2 and <0.3-0.8 pM/bottle, respectively. Other PFCs were not detected in the samples at this period. The concentrations of PFCs in active and sterile groups shown in Fig. 1 were after deduction of corresponding concentration of the intact control. The concentration of *N*-MeFOSE ranged from 136 to 206 pM/bottle in sterile control samples. Little or no biodegradation of *N*-MeFOSE was observed during the test period.

The spiked *N*-MeFOSE decreased from 165 pM/bottle at Day 0 to 6.8 pM/bottle at Day 28 in the active river water/sediment system (Fig. 1). The first-order half-life of *N*-MeFOSE was calculated to 6.7 days. The primary and dominant metabolite *N*-MeFOSAA gradually increased from 1.1 pM/bottle at Day 0 to 133 pM/bottle at Day 28. Lai *et al*. reported that *N*-EtFOSAA was detected from 33% of 83 river and surface runoff water in Japan[14]. It was thought that *N*-MeFOSAA and *N*-EtFOSAA are one of the major residual metabolite of perfluorooctanesulfonamido ethanol. PFOS, PFOSi, PFOSA, and PFOSAA also gradually increased from Day 3 to Day 28. PFOS concentration increased to 26.6 pM/bottle at Day 28 and 15% of spiked *N*-MeFOSE transformed to PFOS. The biodegradation process of *N*-MeFOSE was similar to those of *N*-EtFOSE using municipal wastewater treatment plant (WWTP) sludge[10,11]. *N*-EtFOSE can be transformed to *N*-EtFOSAA, PFOSAA, PFOSA, PFOSi, and PFOS in WWTP system. As PFOS was determined to be a persistent organic pollutant by the Stockholm Convention in May 2009, the production, import, export, and use of PFOS were strictly regulated. Nevertheless, PFOS precursors can be a source of PFOS in the environment.

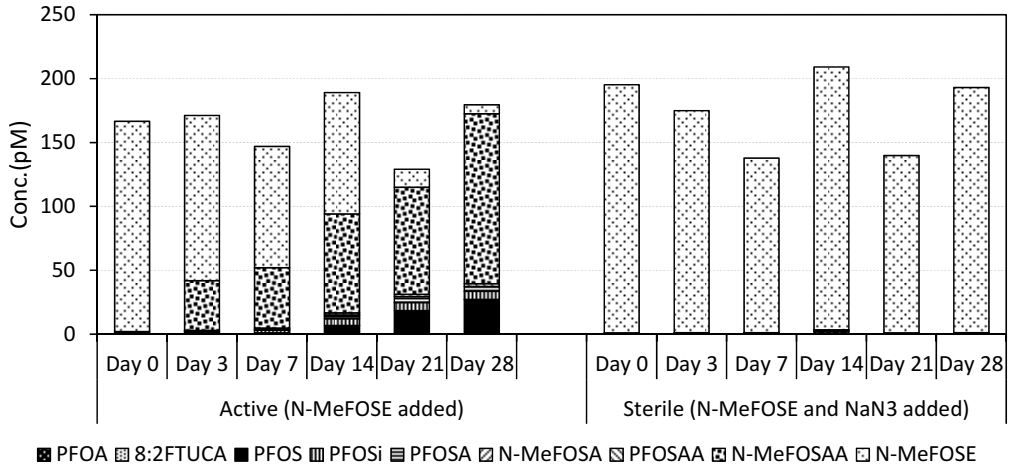

Fig. 1 Transformation profiles of *N*-MeFOSE in the biodegradation test

Aerobic biodegradation of 8:2FTOH

PFOA, 8:2FTOH, PFOS, and PFOSA were detected in the intact control samples during the test period, and their concentrations ranged 1.8-3.4, <0.9-1.3, 0.8-1.1, and <0.1-0.3 pM/bottle, respectively. Other PFCs were not detected in the samples at this period. The concentrations of PFCs in active and sterile groups shown in Fig. 2 were after deduction of corresponding concentrations of the intact control samples. The concentrations of 8:2FTOH ranged from 132 to 191 pM/bottle in sterile control samples. Little or no biodegradation of 8:2FTOH was observed during the test period.

The spiked 8:2FTOH decreased from 153 pM/bottle at Day 0 to <0.9 pM/bottle at Day 14 in the active river water/sediment system (Fig. 2). The first-order half-life of 8:2FTOH was calculated to 1.7 days. The primary metabolite 8:2FTCA and the secondary metabolite 8:2FTUCA were already detected at Day 0. Because these metabolites were not detected in spiked 8:2FTOH solution, it indicates that the biodegradation of 8:2FTOH can be immediately occurred in river system. 8:2FTCA was detected at Day 0 and 3, and the concentrations were 7.5 and 10.0 pM, respectively. 8:2FTUCA was detected from at Day 0 to 21, but the maximum concentration was 12.5 pM at Day 3. The findings that the rapid transformations from 8:2FTOH to 8:2FTCA, then from 8:2FTCA

to 8:2FTUCA, suggest the intermediates from 8:2FTOH were rarely detected in river water. PFOA, the biodegradable final product of 8:2FTOH was detected at Day 3 and the concentration gradually increased until Day 14. The concentrations were maintained at an almost constant rate (91-107 pM) from Day 14 until Day 28. The results showed that 42-50% of 8:2FTOH was transformed to PFOA under aerobic biodegradation. Total concentrations of 8:2FTOH and the derivatives were not matched with the initial concentration, suggesting another intermediate or end product can be transformed from 8:2FTOH. Further study is needed to identify the unknown biodegradable substances.

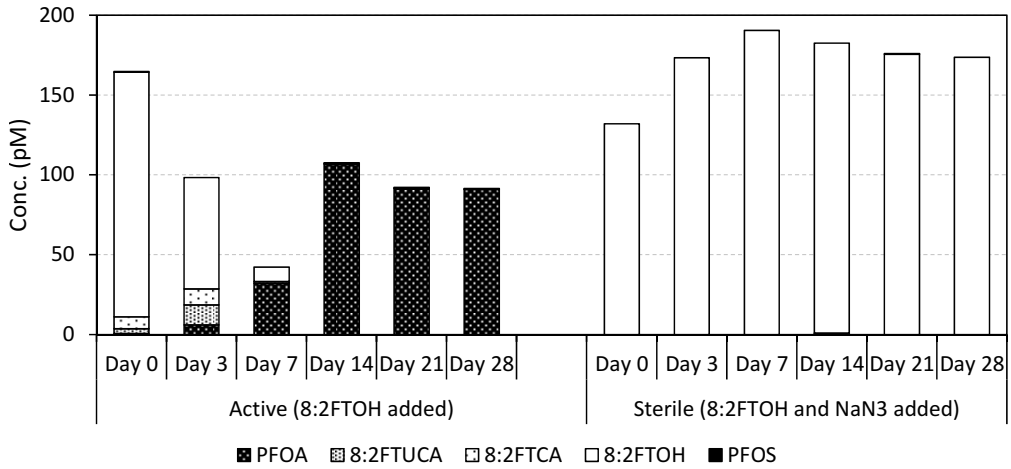

Fig. 2 Transformation profiles of 8:2FTOH in the biodegradation test

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References

1. Schultz MM, Barofsky DF, Field JA. (2003); *Environ. Eng. Sci.* 20 (5): 487-501
2. Higgins CP, Field JA, Criddle CS, Luthy RG. (2005); *Environ. Sci. Technol.* 39 (11): 3946-3956
3. Kennedy Jr. GL, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG. (2004); *Crit. Rev. Toxicol.* 34 (4): 351-384
4. Nakayama S, Harada K, Inoue K, Sasaki K, Seery B, Saito N, Koizumi A. (2005); *Environ. Sci.* 12 (6): 293-313
5. Beach SA, Newsted JL, Coady K, Giesy JP. (2006); *Rev. Environ. Contam. Toxicol.* 186: 133-174
6. Dinglasan MJA, Mabury SA. (2006); *Environ. Sci. Technol.* 40(5): 1447-1453
7. Fiedler S, Pfister G, Schramm K. (2010); *Toxicol. Environ. Chem.* 92(9-10): 1801-1811
8. Ye F, Zushi Y, Masunaga S. (2012); *Organohalogen Compounds* 74: 251-254
9. Dinglasan MJA, Ye Y, Edwards EA, Mabury SA. (2004); *Environ. Sci. Technol.* 38 (12): 2857-2864
10. Rhoads KR, Janssen EML, Luthy RG, Criddle CS. (2008); *Environ. Sci. Technol.* 42: 2873-2878
11. Lange CC. (2000); *U.S. EPA Docket AR-226-058*
12. The International Organization for Standardization (2002); *ISO 14592-1:2002(E)*
13. Motegi M, Horii Y, Hosono S, Nojiri K. (2010); Organohalogen Compounds 72: 880-883
14. Lai FY, Yeung LWY, Taniyasu S, Li P, Horii Y, Kannan K, Lam PKS,Yamashita N. (2009); *Organohalogen Compounds* 71: 2627-2632