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Programs and Abstracts

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JEC Japan Society for Environmental Chemistry

Outcome of ICAEC 2014

The expected outcomes are as follows;

- To declare importance to prevent pollution by micro-pollutants (declare a statement)
- To make clear environmental pollution by micro-pollutants in South East Asia, East Asia and South Pacific region
- To strengthen relationship of researchers, particularly young researchers between Thailand and Japan

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BIOTRANSFORMATION OF PERFLUORINATED COMPOUNDS BY THE ACTION OF MICROBIAL COMMUNITY ISOLATED FROM POLLUTED ENVIRONMENT - ROAD TO SUCCESSFUL BIOREMEDIATION

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Keywords : PFOS, PFOA, biotransformation, microbial consortia

Abstract : Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are manmade chemicals that can be found in the environment because of their wide use in chemical production since the 1950s. Their unique properties such as surface activity, water and oil repellency, thermal and acid resistance made them popular for usage in many industrial processes such as in protective coatings for textiles, carpets, leather, food containers, wiring insulations for telecommunications. PFASs are components of many important consumer products such fire-fighting foams, surfactants in cosmetics, electronics and medicals [1].

The focus of this study was to confirm biotransformation of PFASs by the action of microbial community isolated from locations known for long term pollution with PFOS and PFOA. Microorganisms that inhabiting polluted environment are already naturally adapted to higher concentrations of pollutant chemicals. For some of those microorganisms we can expect that they can degrade some particular pollutant. For example, the microbial community from PFOA-polluted site is expected to biotransform/biodegrade PFOA. For the isolation of microbial consortia, sediment samples from Saitama (PFOS polluted) and Osaka (PFOA polluted) were used.

Two microbial communities were enriched and isolated from each sample. Total bacteria were enriched using Bushnell Haas medium with glucose and Malt extract broth was used for enrichment of yeast and molds. In both media, PFOS and PFOA were respectively added to Saitama and Osaka samples to stimulate the growth of zymogenous microorganisms and to inhibit the growth of microorganisms sensitive to PFASs.

There are two main mechanisms for microbial biotransformation/biodegradation of any organic substance: use as only carbon and energy source, and cometabolism. When the substance is used as only carbon and energy source, the microorganisms can synthesize all the cellular materials and obtain all the energy necessary for growth using only that substance plus, nitrogen, phosphorus and oxygen as external electron acceptor under aerobic conditions. Most oil hydrocarbons are used in this way. However, when a substance is used in a cometabolism, as a growth substrate, a primary carbon source is needed, and organic substance or xenobiotic used for cometabolism is oxidized or reduced in an unintentional and coincidental process [2]. It is considered that cometabolic process is not beneficial to the microorganisms directly. Cometabolism may result in compounds that have changed polarity compared to the precursor compound. Furthermore, ecotoxicity and toxicity of precursor compounds can be changed after cometabolic activity of microorganisms.

Bacterial and Yeast microbial consortia were incubated with PFOS and PFOA in biotic tests. After centrifugation, the solution was loaded to Solid Phase Extraction cartridge (Presep PFC-II, Wako Pure Chemical Industries) preconditioned with 10mL of 0.1% methanolic ammonia, 10mL of methanol, 15 mL of Milli-Q water. For elution of the target compounds, 0.1% methanolic ammonia was used. The eluted solution was concentrated to 1mL under nitrogen stream and analyzed by LC-MS/MS. Abiotic tests were used as a control. Although there have been many reports on biodegradation of crude oil and POPs chemicals, only a small number of studies had focused on the biotransformation of PFASs with microorganisms isolated from polluted environment [3]. Our study suggests that microbial community isolated from environment polluted with PFOS and PFOA is a source of microorganisms who can conduct biotransformation of these emerging contaminants.

1. Zareitalabad, P., et al., Chemosphere 91 (2013) 725-732;
2. Dionisi, D., ChemBioEng Rev 1, (2014) 67–82;
3. Kwon, B.G., Chemosphere (2014) in press;

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Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are manmade chemicals that can be found in the environment because of their wide use in chemical production since the 1950s. Their unique properties such as surface activity, water and oil repellency, thermal and acid resistance made them popular for usage in many industrial processes such as in protective coatings for textiles, carpets, leather, food containers, wiring insulations for telecommunications. PFASs are components of many important consumer products such fire-fighting foams, surfactants in cosmetics, electronics and medicals [1].

Microorganisms that inhabiting polluted environment are already naturally adapted to higher concentrations of pollutant chemicals. The focus of this study was to confirm biotransformation of PFASs by the action of microbial community isolated from locations known for long term pollution with PFOS and PFOA.

Material and Methods

For the isolation of microbial consortia, sediment samples from Saitama (PFOS polluted) and Osaka Ajifu watercourse (PFOA polluted) were used.

Fig 1. Saitama – PFOS polluted sediment

Fig 2a-b. Osaka – PFOA polluted sediment

PFOS

PFOA

8:2 FTOH

Total bacteria were enriched using Bushnell Haas broth ($MgSO_4$ 0.2g/L; $CaCl_2$ 0.02g/L; KH_2PO_4 1.0g/L; K_2HPO_4 1.0 g/L; NH_4NO_3 1.0 g/L; $FeCl_3$ 0.05 g/L; pH 7.0 +/- 0.2 at 25°C) with glucose (2g/L) and Malt extract broth was used for enrichment of yeast and molds. In both media, PFOS and PFOA were respectively added to Saitama and Osaka samples to stimulate the growth of zymogenous microorganisms and to inhibit the growth of microorganisms sensitive to PFASs.

Biotransformation/biodegradation experiment

Bacterial and yeast microbial consortia were incubated with PFOS and PFOA in biotic tests. After centrifugation, the solution was loaded to Solid Phase Extraction cartridge (Presep PFC-II, Wako Pure Chemical Industries) preconditioned with 10mL of 0.1% methanolic ammonia, 10mL of methanol, 15 mL of Milli-Q water. For elution of the target compounds, 0.1% methanolic ammonia was used. The eluted solution was concentrated to 1mL under nitrogen stream and analyzed by LC/MS. Abiotic tests were used as a control.

Table 1. Biotransformation experiment – model systems

	Dominant microorganisms	PFAS	Time - day of the experiment				
			0	7	14	21	28
1.	Total chemo organo heterotrophs	PFOS	**BT-1-0**	BT-1-7	**BT-1-14**	BT-1-21	**BT-1-28**
2.		PFOA	**BT-2-0**	BT-2-7	**BT-2-14**	BT-2-21	**BT-2-28**
3.		8:2 FTOH	**BT-3-0**	BT-3-7	**BT-3-14**	BT-3-21	**BT-3-28**
4.	Hydrocarbon degrading bacteria	PFOS	**BT-4-0**	BT-4-7	**BT-4-14**	BT-4-21	**BT-4-28**
5.		PFOA	**BT-5-0**	BT-5-7	**BT-5-14**	BT-5-21	**BT-5-28**
6.		8:2 FTOH	**BT-6-0**	BT-6-7	**BT-6-14**	BT-6-21	**BT-6-28**
7.	Yeast and molds	PFOS	**BT-7-0**	BT-7-7	**BT-7-14**	BT-7-21	**BT-7-28**
8.		PFOA	**BT-8-0**	BT-8-7	**BT-8-14**	BT-8-21	**BT-8-28**
9.		8:2 FTOH	**BT-9-0**	BT-9-7	**BT-9-14**	BT-9-21	**BT-9-28**

BT-Biotic test (Media+PFCs+Microorganisms); AC - Abiotic control (Media+PFCs); BC-Biotic control (Media+Microorganisms)

Results and Discussion

Table 2. PFASs concentration in sediment samples sampled in Saitama and Osaka, Japan

	Concentration [ng/kg-dry]																	
	PFBA (C4)	PFPeA (C5)	PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	PFHxDA (C16)	PFOcDA (C18)	PFBS (C4)	PFHxS (C6)	PFOS (C8)	PFDS (C10)
Saitama	100	<300	<300	<300	<300	<300	<100	<100	<100	<100	<100	<100	<100	<200	<200	**1000**	<200
Osaka	390	1100	2300	1100	**9500**	1900	2600	1500	16000	4600	18000	<300	<300	<600	<600	<600	<600

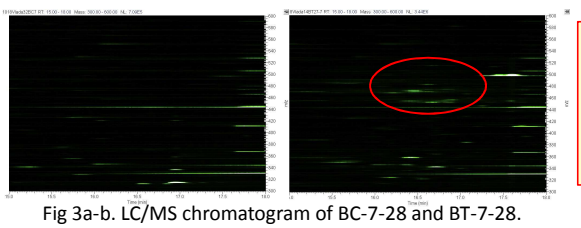
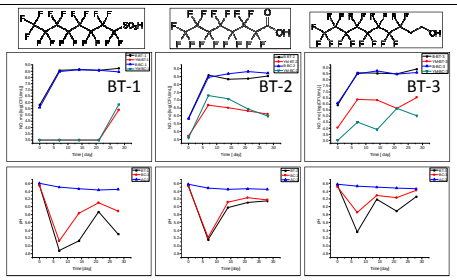
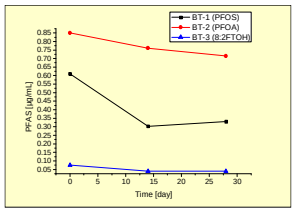


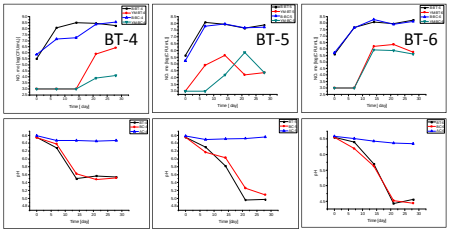
Fig 3a-b. LC/MS chromatogram of BC-7-28 and BT-7-28.

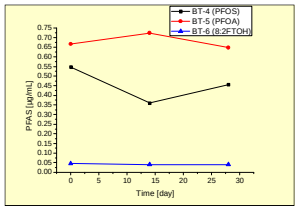
***m/z* 343.1891**
$C_{21}H_{27}O_4$
(343.1915)
or
$C_{18}H_{28}O_5F$
(343.1926)
???





Increase in the number of chemoorganoheterotrophic bacteria (BT-1, BT-2 and BT-3) was followed with decrease in pH during first week. The highest reduction in PFOS concentration was determined during first two weeks.





The concentration of hydrocarbon degrading bacteria (BT-4, BT-5 and BT-6) was stable and decrease in pH was more intensive. However decrease in PFAS concentration was not intensive.

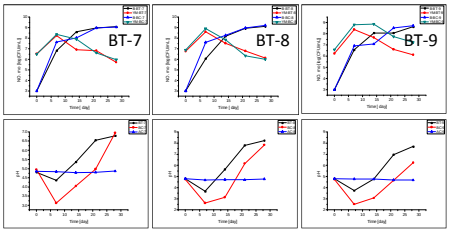


Fig 4a-r. Change of number of microorganisms and pH in BT, and BC model systems (B-bacteria; YM-yeast and molds).

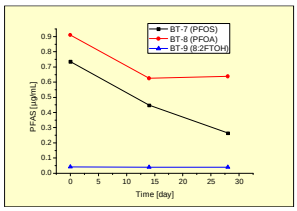


Fig 5a-c. Reductions in PFAS concentrations

The number of microorganisms was stable during biodegradation experiment and the changes within pH values suggested possible changes in the composition of microbial consortia.

Table 3. New peaks detected only in BT-x-28 model systems

	BT-1-28	BT-2-28	BT-3-28	BT-4-28	BT-5-28	BT-6-28	BT-7-28	BT-8-28	BT-9-28
419.2775	+	+			+				
341.1734	+	+					+	+	
343.1891		+	+				+	+	
347.2204	+	+	+				+	+	+
359.184		+	+				+	+	
363.2153			+	+			+	+	+
475.3032	+	+	+				+	+	+
455.2772			+				+	+	+
218.1029	+		+				+		+
412.9637 (PFOA)		+			+			+	
498.9268 (PFOS)	+			+			+		

Conclusion

Although there have been many reports on biodegradation of crude oil and POPs chemicals, only a small number of studies had focused on the biotransformation of PFASs with microorganisms isolated from polluted environment [3]. Our study suggests that microbial community isolated from environment polluted with PFOS and PFOA is a source of microorganisms who can reduce concentration of these emerging contaminants.

Acknowledgements: This research was supported by Japan Society for the Promotion of Science.

Reference

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3. Kwon, B.G., Chemosphere 109 (2014) 221–225;