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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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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

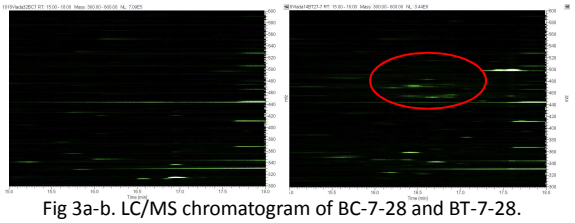
	Dominant microorganisms	PFAS	Time - day of the experiment				
			0	7	14	21	28
1.	Total chemo organo heterotrophs	PFOS	**BT-1-0**	BT-1-7	**BT-1-14**	BT-1-21	**BT-1-28**
2.		PFOA	**BT-2-0**	BT-2-7	**BT-2-14**	BT-2-21	**BT-2-28**
3.		8:2 FTOH	**BT-3-0**	BT-3-7	**BT-3-14**	BT-3-21	**BT-3-28**
4.	Hydrocarbon 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

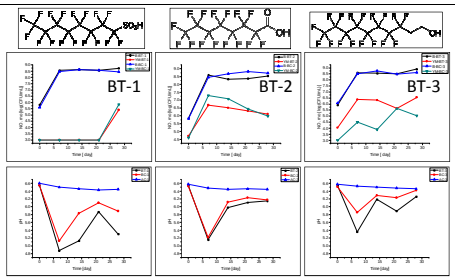
Table 2. PFASs concentration in sediment samples sampled in Saitama and Osaka, Japan

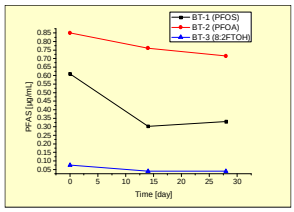
	Concentration [ng/kg-dry]																	
	PFBA (C4)	PFPeA (C5)	PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	PFHxDA (C16)	PFOcDA (C18)	PFBS (C4)	PFHxS (C6)	PFOS (C8)	PFDS (C10)
Saitama	100	<300	<300	<300	<300	<300	<100	<100	<100	<100	<100	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



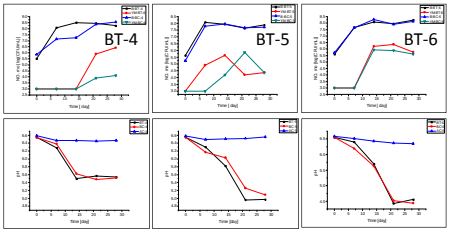
***m/z* 343.1891**
$C_{21}H_{27}O_4$
(343.1915)
or
$C_{18}H_{28}O_5F$
(343.1926)
???

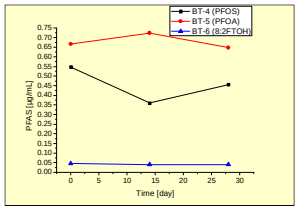
Fig 3a-b. LC/MS chromatogram of BC-7-28 and BT-7-28.





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.

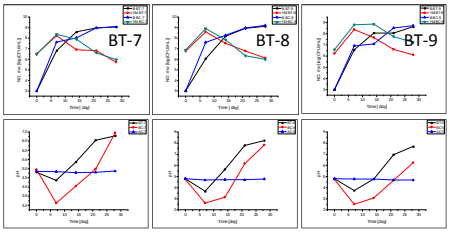


Fig 4a-r. Change of number of microorganisms and pH in BT, and BC model systems (B-bacteria; YM-yeast and molds).

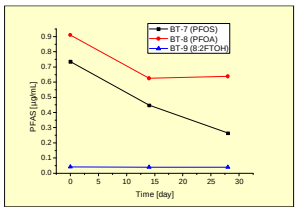


Fig 5a-c. Reductions in PFAS concentrations

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.

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Reference

1. Zareitalabad, P., et al., Chemosphere 91 (2013) 725-732; 2. Dionisi, D., ChemBioEng Rev 1, (2014) 67–82;
3. Kwon, B.G., Chemosphere 109 (2014) 221–225;