Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Aerobic BTEX biodegradation Increases Yield of Perfluoroalkyl Carboxylic Acids from Biotransformation of a Polyfluoroalkyl Surfactant, 6:2 FtTAoS

Electronic Supplementary Information

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Supplemental Methods

Total oxidizable precursor (TOP) assay

The total oxidizable precursor (TOP) assay was performed as described previously.[10] Briefly, 100 µL of aqueous microcosm slurry was added to 6 mL of 60 mM $K_2S_2O_8$ and 200 mM NaOH, and incubated at 85 ^oC overnight. At the end of the reaction, samples were cooled to room temperature and their pH was adjusted to 5-8 with 4M HCl. 1 mL methanol was added to quench any residual oxidants. Samples were then prepared for LC-MS/MS as described in the main text (internal standard addition, dilution in LCMS grade methanol).

GC-FID

BTEX compounds were measured by injecting 100 µL of headspace air into a gas chromatograph (GC) coupled to flame-ionization detection (FID) equipped with a GS-GasPro column (Agilent Technologies). The oven was set initially at 50 ^oC for two minutes, followed by a 50 ^oC min^{-1} ramp to 260 ^oC and held at 260 ^oC for two minutes. The retention times for benzene, toluene, ethylbenzene, and o-xylene were 3.9, 4.8, 5.6, 5.8 min, respectively. The injection port and detector temperatures were kept at 250 ^oC. The limit of detection (LOD) was determined as 3× the height of the baseline and the limit of quantification (LOQ) was determined as 2× LOD. The LOQs were (in µg): benzene (0.10), toluene (0.14), ethylbenzene (0.12), and o-xylene (0.39).

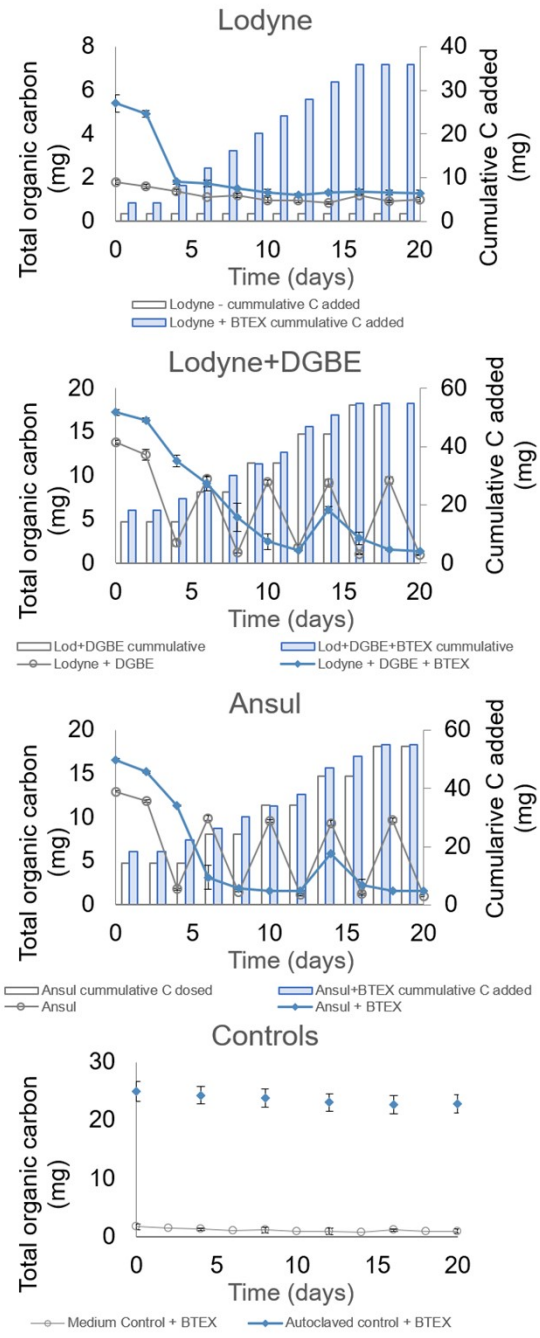

Figure S1. Organic carbon mass balances in biotransformation microcosms

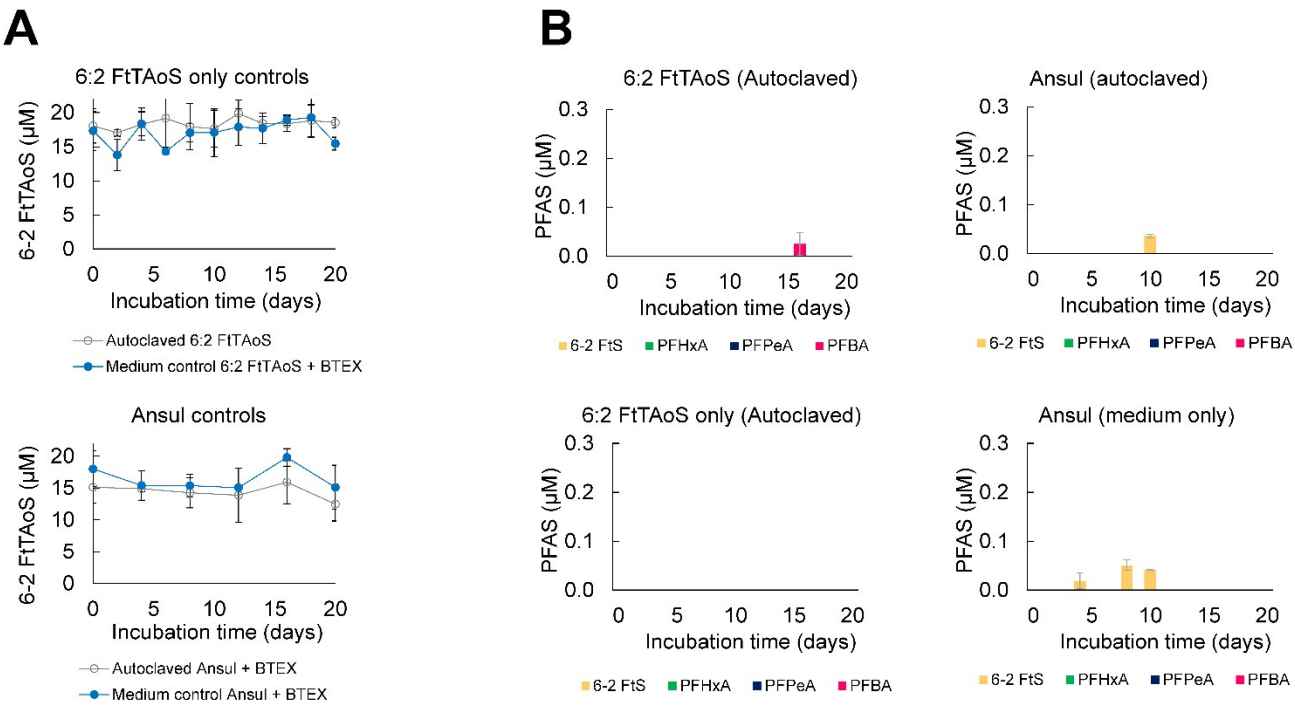

Figure S2. Autoclaved and medium-only controls. Panel A: Organic carbon mass balance, Panel B: Transformation products detected.

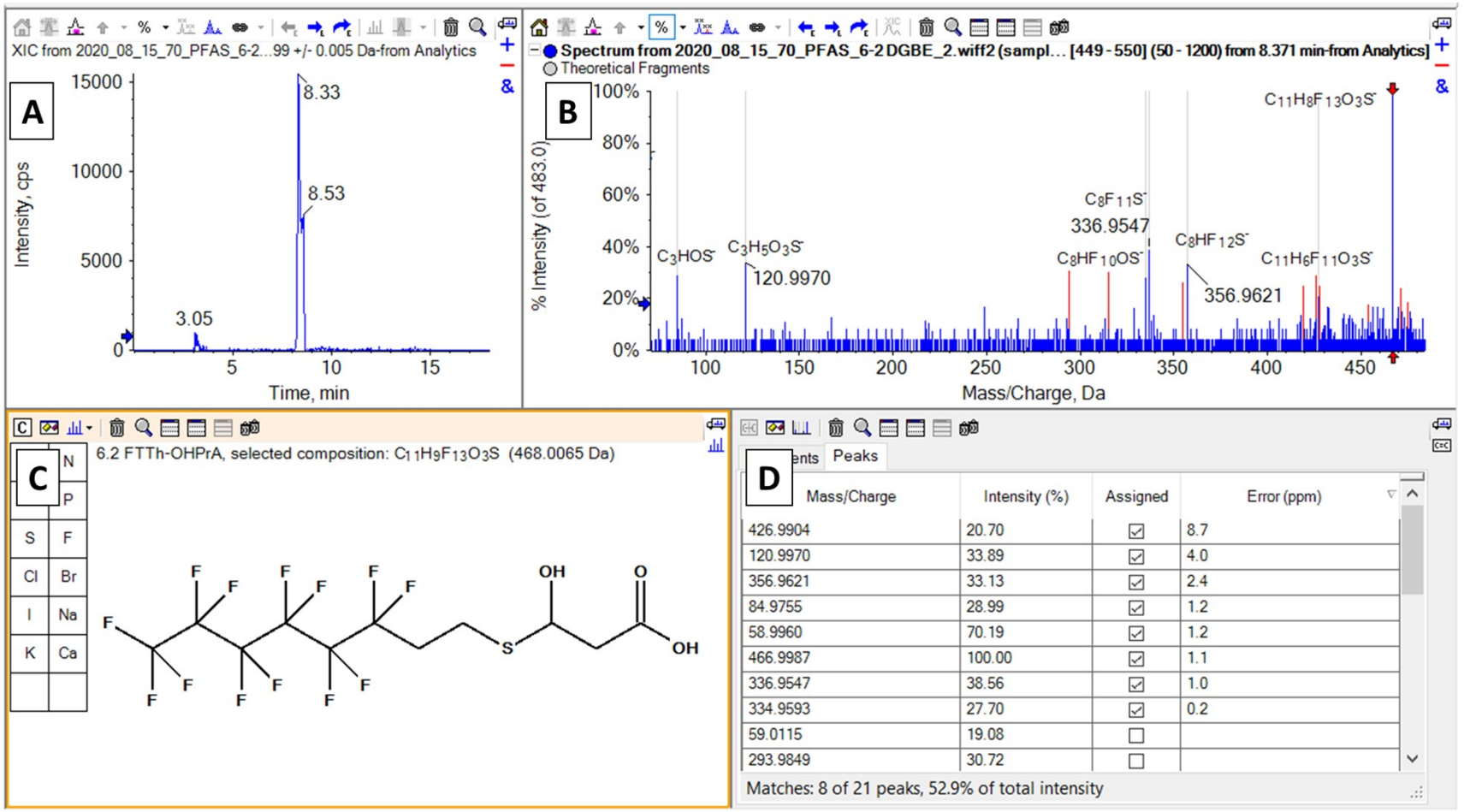

Figure S3. Chromatography and MSMS spectrum for confirmation of 6:2 FTTh-OHPrA. A: Extracted ion chromatography at precursor mass 499.999 m/z; B: Fragmentation (MS/MS) spectrum at retention time 8.37 min for precursor mass 499.999 m/z, captions on the fragment are possible fragment predicted from its tentative structure; C: structure of 6:2 FTTh-OHPrA; D: Predicted fragment from the structure. Mass tolerance for the fragment to MSMS spectra in C was 20 ppm. 6:2 FTSO-PrA generated only 84.9755, 334.9593, 426.9904, and 466.9987. Sciex OS 1.7 Explorer was used for data analysis.

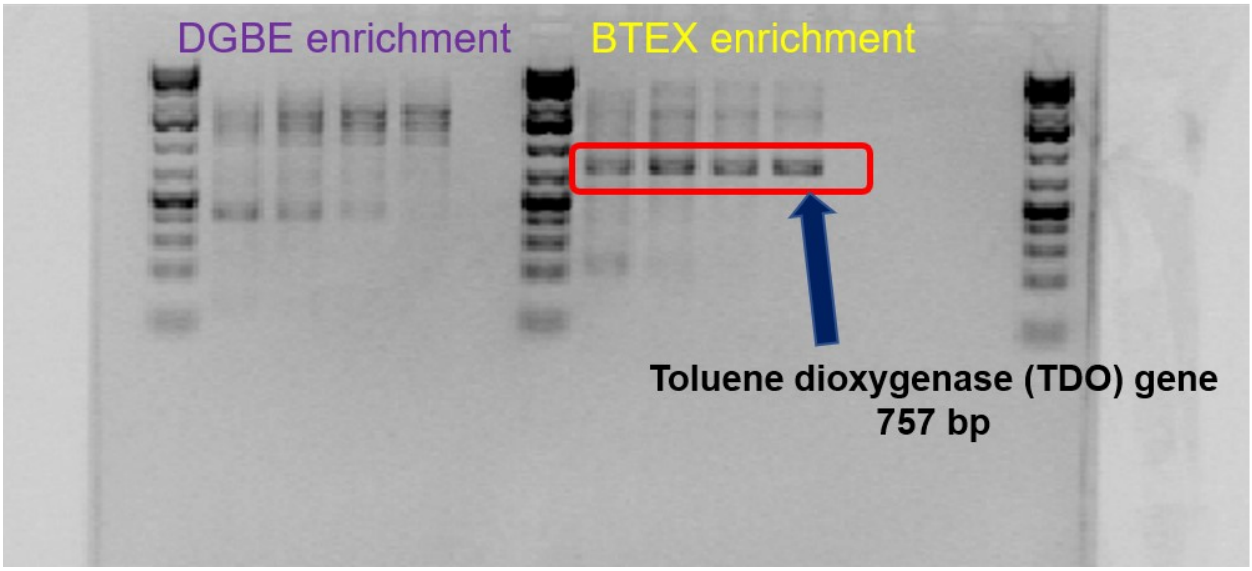

Figure S4. Gel electrophoresis image of AFFF-impacted solids enrichments selecting for DGBE biodegradation (left bands), and BTEX biodegradation (right bands). Four biological replicates each.

Table S1. First-order 6:2 FtTAoS biotransformation rate constants and R-squared values. Wilcoxon Rank Sums test performed in comparison with 6:2 FtTAoS only (no BTEX) treatment.

	6:2 FtTAoS only		**6:2 FtTAoS + DGBE**		**Ansul AFFF**	
	No BTEX	**BTEX**	**No BTEX**	**BTEX**	**No BTEX**	**BTEX**
Rate (d^{-1})	0.11±0.02	0.11±0.02	0.18±0.02	0.23±0.02	0.19±0.06	0.17±0.03
R^2	0.9572	0.8795	0.95	0.8849	0.9051	0.95587
Range	days 0-18	days 0-10	days 0-14	days 4-10*	days 0-12	days 0-18
Wilcoxon Rank Sum Test (*p* value)	N/A	0.7125	0.3777	0.3404	0.2189	0.1978

***** For this treatment, there was a lag phase (days 0-4) that was not considered to calculate the rate

Table S2. Additional targeted analysis transformation products detected with high-resolution mass spectrometry collected in ESI- mode at day 60. Average concentration and t-test between BTEX and DGBE enrichment microcosms (biological triplicates, n=3). Compounds in red were more abundant in BTEX enrichment. Compounds in blue were more abundant in the DGBE enrichments. Minimum Reporting Level (MRL) = 0.1 µg/L

Compound	DGBE	BTEX	*T-test*	Confidence level

name	Enrichment (µg/L)	Enrichment (µg/L)	p ≤0.05(*)	based on Shymanski et al.[1]
5:3 FTCA	< MRL	0.52±0.14	*	1
6:2 FTUCA	0.97±0.06	0.22±0.13	0.06	1

References:

(1) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097–2098.