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

SUPPLEMENTARY INFORMATION

Aerobic biotransformation of a novel highly functionalized polyfluoroether-based surfactant using activated sludge from a wastewater treatment plant

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MATERIALS AND METHODS

Chemicals. Ethanol (HPLC grade), methanol (HPLC grade), ethyl acetate (HPLC grade), ammonium acetate (HPLC grade) and perfluoropropanoic acid (PFPrA, 97%) were obtained from Sigma Aldrich (Oakville, ON). Perfluoropentanoic acid (PFPeA), perfluorohexane sulfonic acid (PFHxS), and 6:2 fluorotelomer acid (6:2 FTUCA, or FHUEA) all >99.9%, were obtained from Wellington Laboratories (Guelph, ON). All water used was 18.2 MΩ·cm.

Defined media. A sulfate-free medium was preparation followed a previous protocol,[1] who used an approach first reported by Van Hamme et al.[2] Into 1 L of water, the following was added: 1.60 g/L of K_2HPO_4, 0.40 g/L of KH_2PO_4, 5 g/L of sodium acetate, 1.55 g/L of NH_4Cl, 0.09 g/L of $CaCl_2{\cdot}2H_2O$, 0.165 g/L of $MgCl_2{\cdot}6H_2O$, and 4 g/L of glucose. The medium and all biotransformation bottles were autoclaved for 30 minutes at 121°C. For the flow through set-up using round-bottom flasks, the flasks were washed with 70% ethanol three times instead of autoclaving. Wolfe's minerals were prepared by adding into 1 L of water, the following: 50 mg of $AlCl_3$, 67 mg/L of $CaCl_2{\cdot}2H_2O$, 4.0 mg/L of $CuCl_2$, 1.0 g/L of $FeCl_3{\cdot}6H_2O$, 5.1 g/L of $MgCl_2{\cdot}6H_2O$, 0.808 g/L of $MnCl_2{\cdot}4H_2O$, 40 mg/L of $Na_2MoO_4{\cdot}2H_2O$, 1.0 g/L of NaCl, 1.5 g/L of trisodium nitrilo(tri)acetate monohydrate, and 80 mg/L of $ZnCl_2$. Pfenning's vitamins were prepared by adding into 1 L of water, the following: 10 mg of biotin, 50 mg/L of p-aminobenzoic acid, 100 mg/L of thiamine hydrochloride, and 50 mg/L of vitamin B12. Wolfe's minerals were added to the experiments at 5.0 mL/L and Pfenning's vitamins were added at 1.0 mL/L. Both were added through a 0.2 μm nylon syringe filter (Chromatographic Specialties, Brockville, Canada).

Table S1. Preparing chemical doses

Chemical tested	Molar dose	Molecular Mass	Parts per billion dose (ng/mL)	Concentration of dose stock solution required (to add 0.5 mL to 150 mL total)
diFESOS	0.6 µM	849	509.5 ppb	152.85 ppm
FESOH	1.2 µM	344	412.8 ppb	123.84 ppm
FESCA	1.2 µM	358	429.6 ppb	128.88 ppm

Table S2. Composition of each experimental bottle or flask

Experiment Type	Chemical in EtOH (0.6 µM diFESOS, 1.2 µM FESOH & FESCA)*	Live sludge, 10% by volume	Autoclaved sludge, 10% by volume	Wolfe's Minerals	Pfennig's Vitamins	Hg_2Cl_2, 0.75 mg/mL	Defined media (total volume 150 mL)
Live	0.5 mL	15 mL	0	0.75 mL	0.15 mL	0	133.6 mL
Autoclaved	0.5 mL	0	15 mL	0.75 mL	0.15 mL	112.5 mg	133.6 mL
Media Only	0.5 mL	0	0	0.75 mL	0.15 mL	0	148.6 mL
Blank Sludge	0.5 mL EtOH only	0	15 mL	0.75 mL	0.15 mL	0	133.6 mL
Raw, undiluted mixed liquor	0.5 mL (only diFESOS experiment)	149.5 mL (unwashed)	0	0	0	0	0

* Amount of chemical per bottle

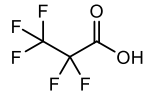
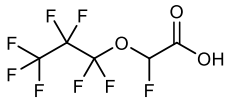
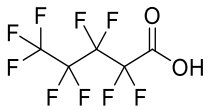
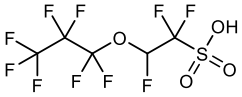
Spike and recovery test. Experimental concentrations of diFESOS, FESOH and FESCA were spiked into autoclaved sludge using the same composition as the experimental controls into closed bottles. After 2 days, the samples were extracted according to the methods described in the main text, and recovery was 94.6 ± 0.3 for diFESOS, 109.2 ± 2.0 for FESOH, and 96.3 ± 0.2 for FESCA.

Measuring headspace oxygen in closed bottles. Oxygen content was monitored using a Perkin Elmer Clarus 500 gas chromatograph with a thermal conductivity detector (GC-TCD outfitted with a Supelco 60/80 Carboxen 1000 column (15 ft x 1/8 in x 2.1 mm). The injector was set to 150 °C, the detector was set to 230 °C, and the carrier gas and reference gasses were set to 30 mL/min. The oven was set to 60 °C for an isocratic run for 10 minutes. A volume of 200 µL was sampled from each microcosm bottle through the Mininert™ valves using a gas-tight syringe and was injected for analysis. onto a GC-TCD. When oxygen levels were below 5%, pure oxygen was injected to bring the concentration back up to about 21%, similar to what was done by Mejia-Avendaño *et al*.[3] Ultimately, this resulted in the addition of 10 mL of pure oxygen into the live bottles every 7 days, as the bottles were often at 4% oxygen before addition up to 21%. Based on our experimental results, the cycle of high and low oxygen levels did not appear to create more viable days with increased biotransformation compared to some lower days.

Details of flow-through microcosms. The microcosms for the longer FESCA experiments had the same composition as the closed bottles used for diFESOS and FESOH, plus the addition of a positive control 6:2 FTUCA.

Figure S1. Flow-through microcosm set-up for FESCA experiment showing the air inflow and condensers.

Table S3. Mass spectrometry parameters for targeted analytes.

Name, Chemical formula $[M-H]^-$	**Structure**	**MRM transition (q = qualifier)**	**Cone voltage**	**Collision voltage**	**Standard?**
PFPrA, $C_3O_2F_5^-$		163>119	20	10	Yes
2H-3:2 PFECA, $C_5HO_3F_8^-$		261>185 261>119 (q)	10 10	20 20	No. PFPeA surrogate
PFPeA, $C_4O_2F_7^-$		263>219	24	8	Yes
$^{13}C_5$ **PFPeA**		268>223	8	8	IS for 2H-3:2 PFECA
2H-3:2 PFESA, $C_5HO_3F_{10}^-$		347>185 347>99 (q)	10	20	No. PFHxS surrogate
PFHxS, $C_6O_3SF_{13}^-$		399>80	45	33	Yes, surrogate for $C_5HO_3F_{10}^-$

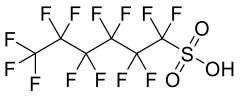
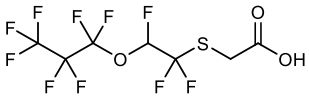
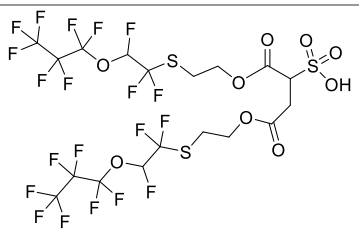
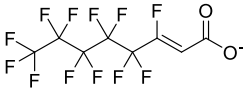
$^{18}O_2$ PFHxS		403>103	45	30	IS for 2H-3:2 PFESA
FESCA, **$C_7H_3O_3SF_{10}^-$**		357>185 357>119 (q)	2 2	34 16	Yes
diFESOS, **$C_{18}H_{13}F_{20}O_9S_3^-$**		849>185 849>441 (q)	60 60	48 36	Yes
6:2 FTUCA **$C_8HO_2F_{12}^-$**		357>293	10	10	Yes
$^{13}C_2$ 6:2 FTUCA		359>294	10	10	IS for 6:2 FTUCA

Table S4. Suspect list of potential metabolites of diFESOS, FESOH and FESCA for high-resolution mass spectrometry data

Molecular formula, neutral	**Molecular formula, negative [M-H]$^-$**	**Name**	**Theoretical m/z**	**Observed m/z (sample)**	**ppm**	**Peak area**
$C_7H_4O_3SF_{10}$	$C_7H_3O_3SF_{10}$	FESCA*	356.96487	356.9647 (FESCA Live, day 42)	-0.48	3.86E+08
$C_7H_4O_4SF_{10}$	$C_7H_3O_4SF_{10}$	FESCA+O	372.95978			
$C_7H_4O_5SF_{10}$	$C_7H_3O_5SF_{10}$	FESCA+O_2	388.95470			
$C_7H_3O_3SF_9$	$C_7H_2O_3SF_9$	FESCA-HF	336.95864			
$C_5H_2O_4SF_{10}$	$C_5HO_4SF_{10}$	2H-3:2 PFESA	346.94413			
$C_5H_2O_2F_{10}$	$C_5HO_2F_{10}$	2H-3:2 alcohol	282.98223	282.9832 (FESCA Live, day 42)	3.43	1.69E+05
$C_5H_2O_3F_8$	$C_5HO_3F_8$	2H-3:2 PFECA*	260.97925	260.9779 (FESCA Live, day 42)	2.40	1.85E+06
$C_3HO_2F_5$	$C_3O_2F_5$	PFPrA*	162.98239	162.9811 (FESCA Live, day 42)	-7.91	1.85E+06
C_2HOF_3	C_2OF_3	trifluoroethanol	96.99067			
$C_2HO_2F_3$	$C_2O_2F_3$	TFA	112.98559			
$C_7H_6O_2SF_{10}$	$C_7H_5O_2SF_{10}$	FESOH	342.98561	Not sensitive on LC-MS, only GC-MS		
$C_7H_6O_3SF_{10}$	$C_7H_5O_3SF_{10}$	FESOH+O	358.98052			
$C_7H_6O_4SF_{10}$	$C_7H_5O_4SF_{10}$	FESOH+O_2	374.97544			
$C_7H_5O_2SF_9$	$C_7H_4O_2SF_9$	FESOH-HF	322.97938			
$C_{18}H_{14}O_9S_3F_{20}$	$C_{18}H_{13}O_9S_3F_{20}$	diFESOS*	848.94078	848.9406 (diFESOS Live, day 38)	-0.21	8.60E+06
$C_{11}H_{10}O_8S_2F_{10}$	$C_{11}H_9O_8S_2F_{10}$	TP 524	522.95846			
$C_2H_4O_3S$	$C_2H_3O_3S$	Sulfinate/carboxylate loss	106.98084			
$C_2H_4O_5S$	$C_2H_3O_5S$	Sulfonate/carboxylate loss	138.97067			
$C_2H_6O_2S$	$C_2H_5O_2S$	Sulfinate/alcohol loss	93.00157			
$C_2H_6O_4S$	$C_2H_5O_4S$	Sulfonate/alcohol loss	124.99140			
$C_4H_6O_7S$	$C_4H_5O_7S$	diFESOS headgroup	196.97615			

*quantified using LC-MS/MS triple quadrupole

RESULTS

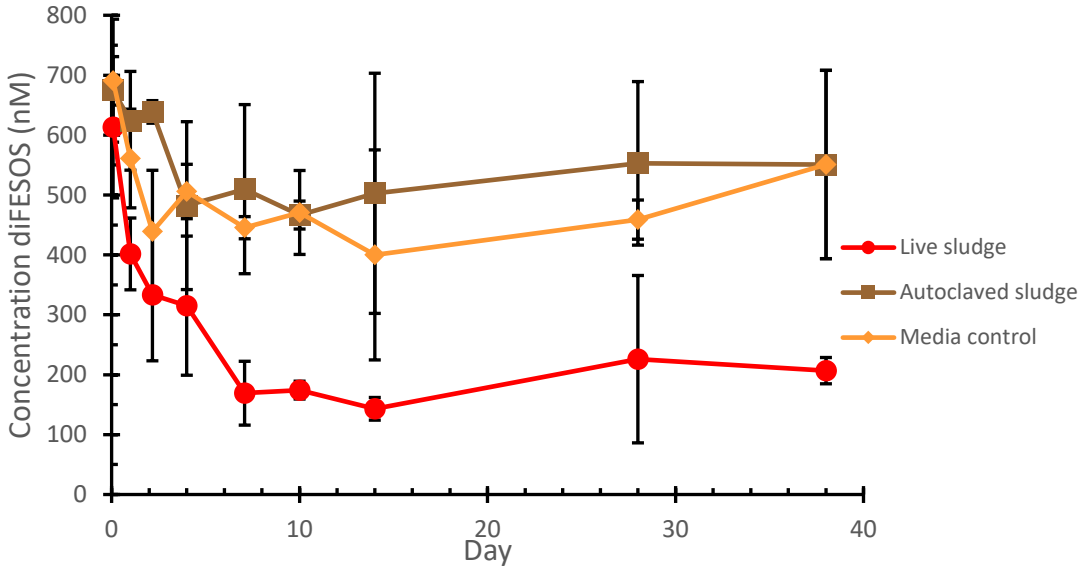


Figure S1. diFESOS incubated with autoclaved sludge or in media controls in the closed bottle experiments did not decrease greatly over the 38 day time period once settling after the first 4 days.

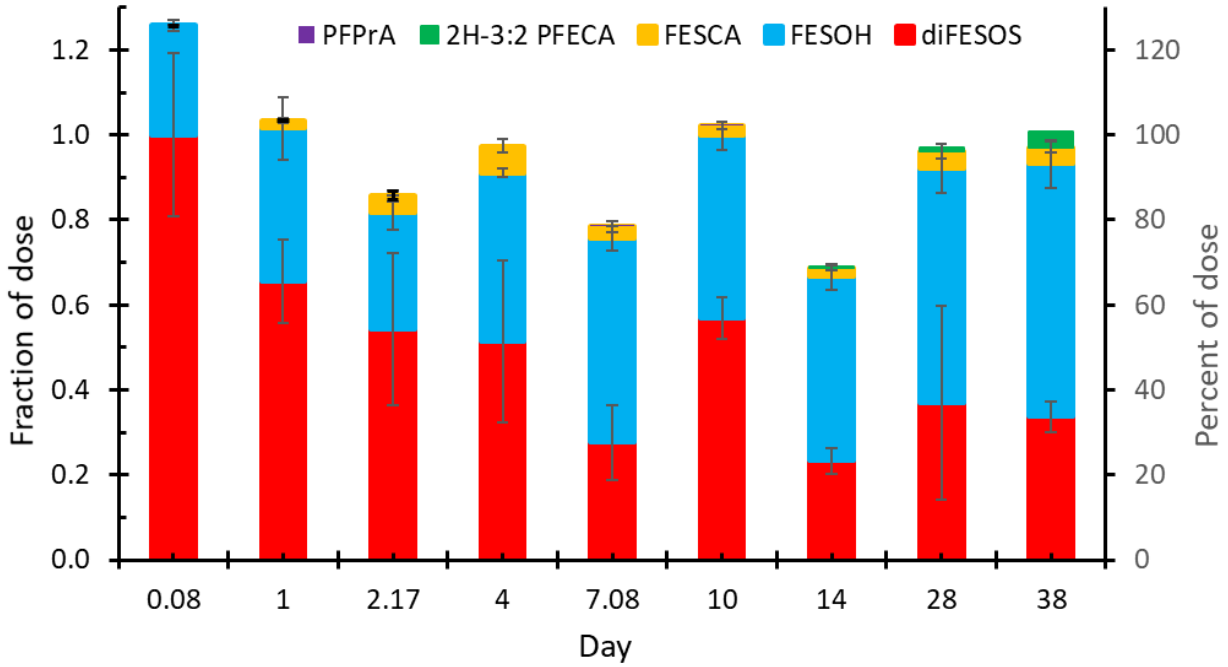


Figure S2. Mass balance of live diFESOS in the closed bottle experiments (n=3).

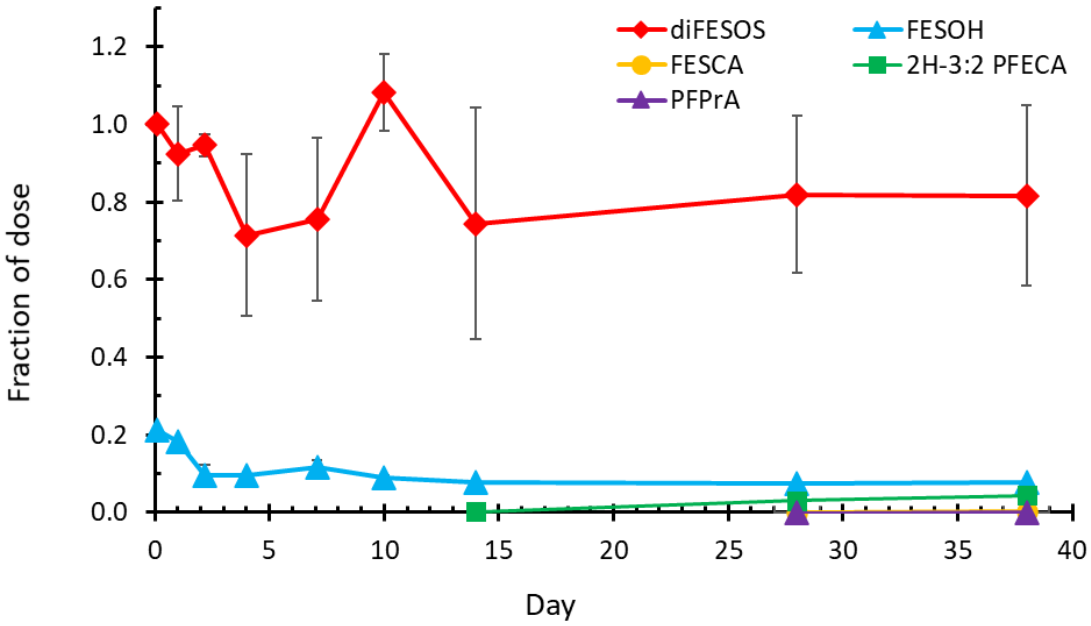


Figure S3. diFESOS incubated with 10% autoclaved sludge in closed bottles. Data points are normalized to the first measurement of diFESOS, correction for the 1:2 moles of polyfluoroalkyl chain present in diFESOS vs its transformation products. Error bars represent standard deviation of the replicate experiments (n=3).

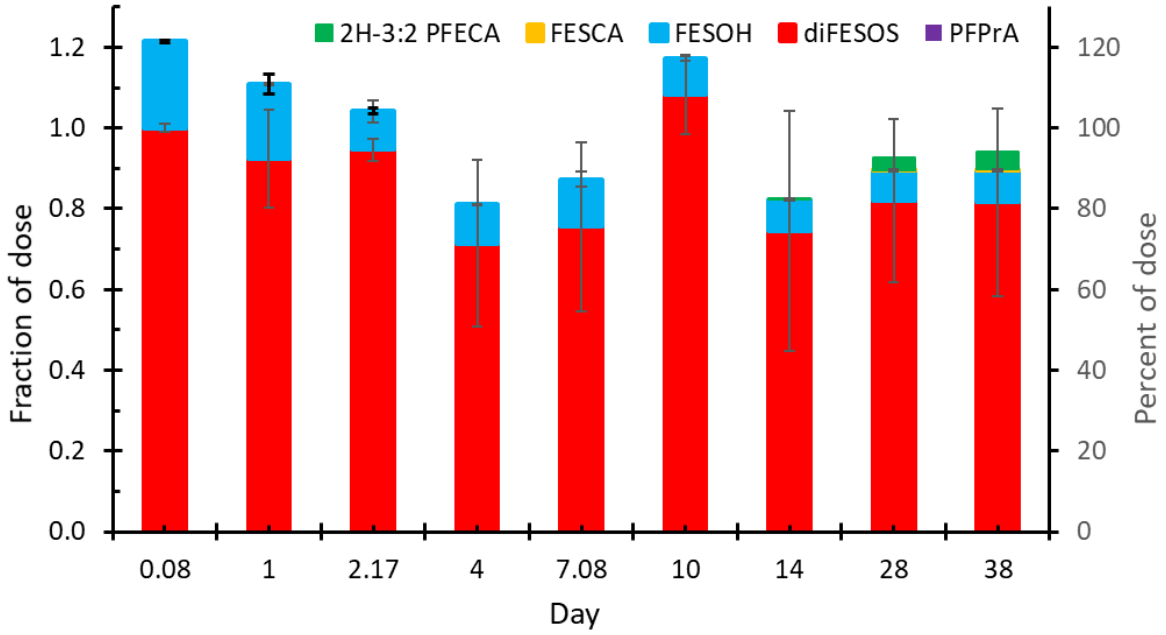


Figure S4. Mass balance of live diFESOS in the closed bottle experiments (n=3).

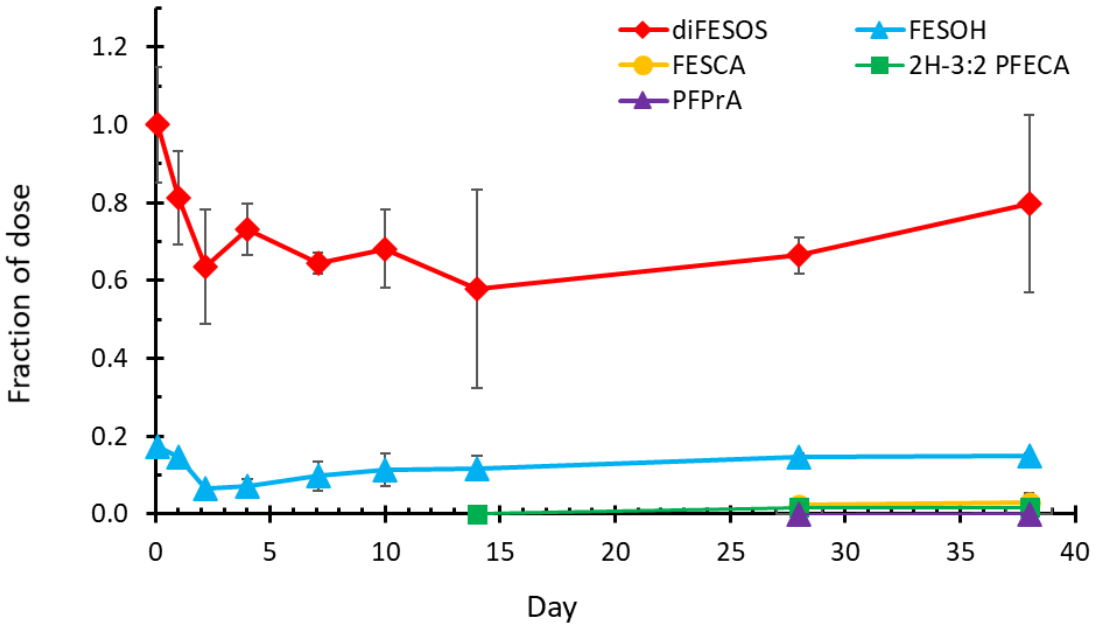


Figure S5. diFESOS media controls in the closed bottle experiments. Data points are normalized to the first measurement of diFESOS, correction for the 1:2 moles of polyfluoroalkyl chain present in diFESOS vs its transformation products. Error bars represent standard deviation of the replicate experiments (n=3).

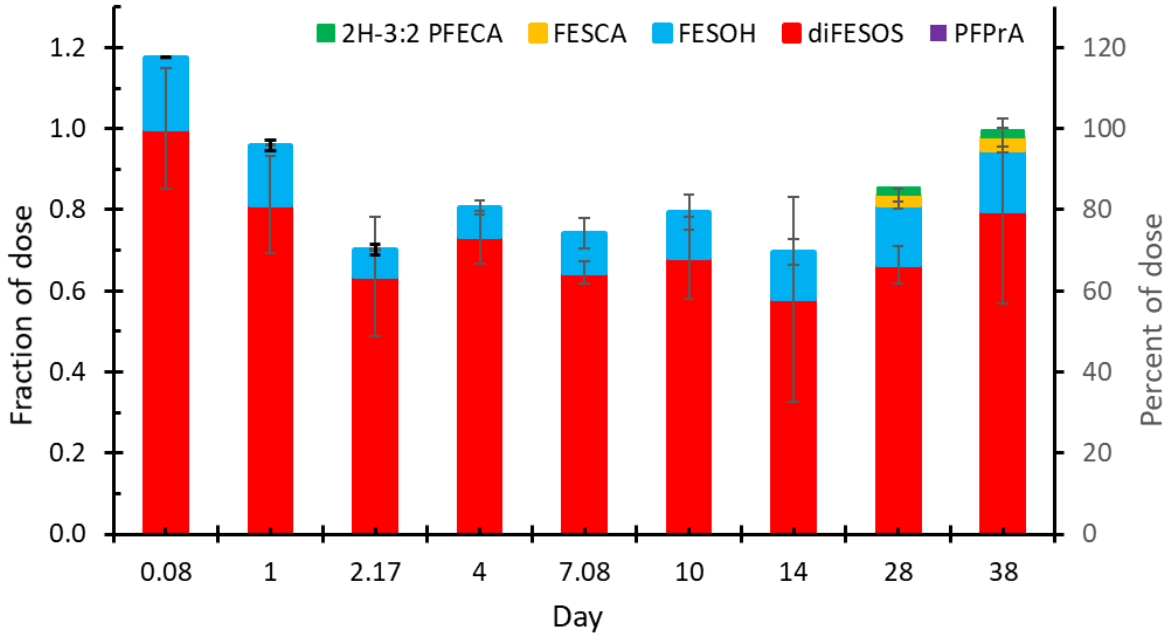


Figure S6. Mass balance of diFESOS media controls in the closed bottle experiments (n=3).

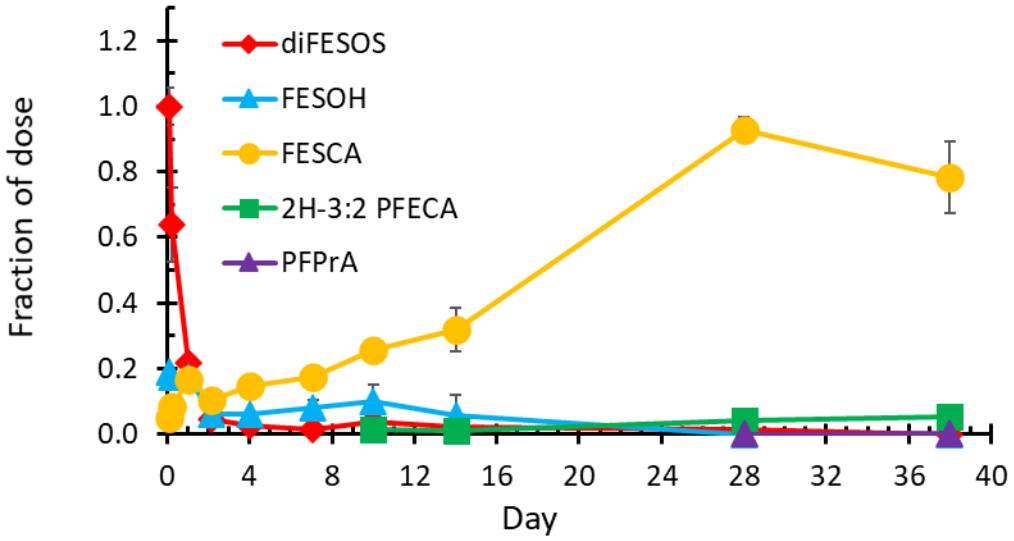


Figure S7. diFESOS incubated in undiluted, raw, mixed liquor in closed bottles. Data points are normalized to the first measurement of diFESOS, correction for the 1:2 moles of polyfluoroalkyl chain present in diFESOS vs its transformation products. Error bars represent standard deviation of the replicate experiments (n=3).

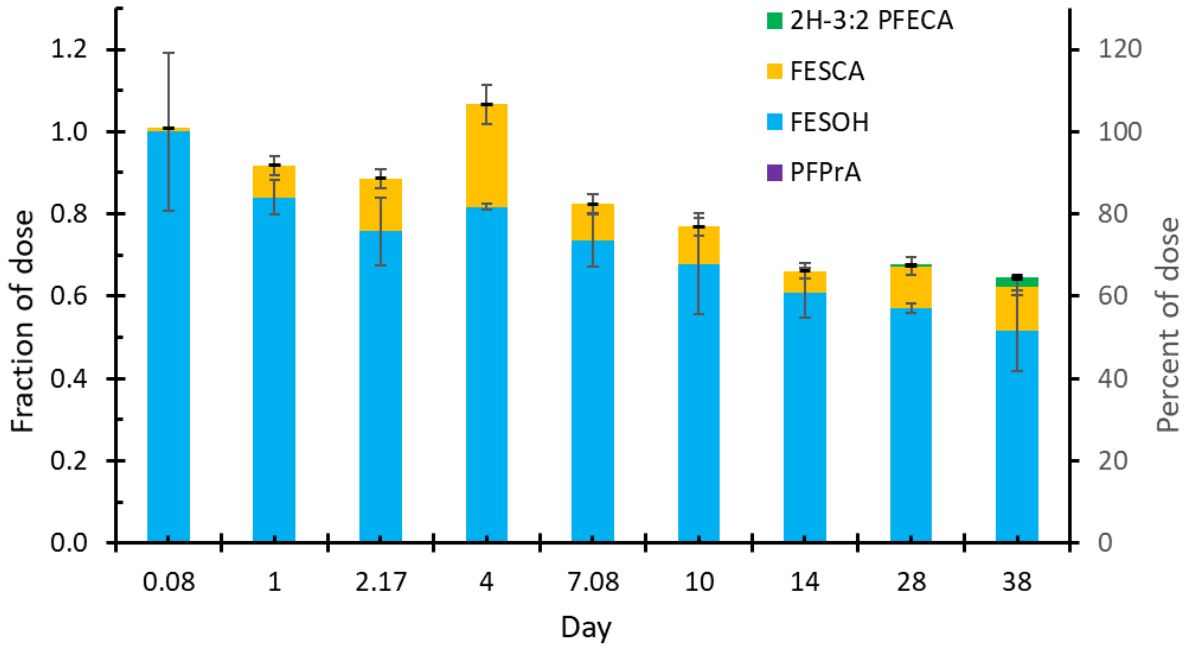


Figure S8. Mass balance of FESOH live bottles in the closed bottle experiments (n=3).

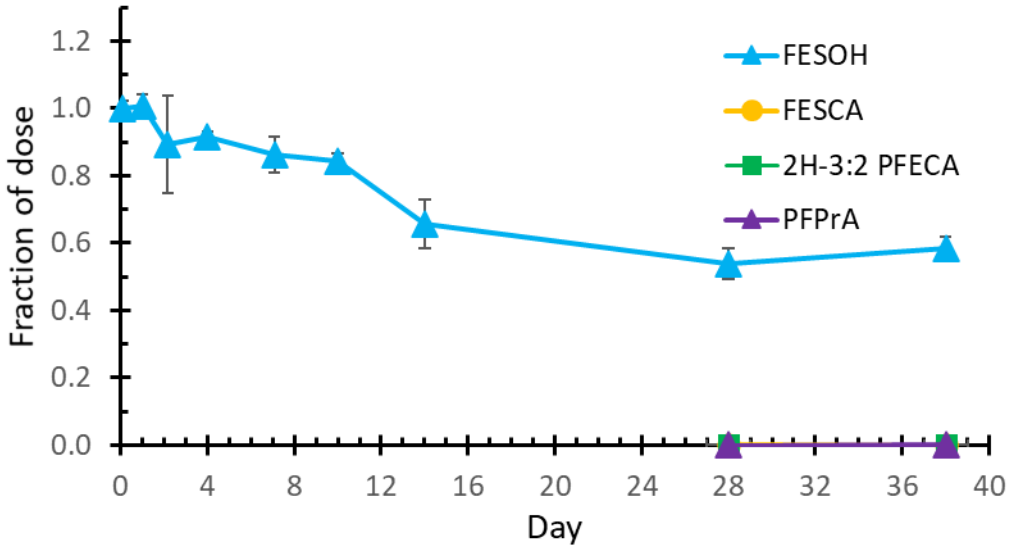


Figure S9. FESOH incubated with 10% autoclaved sludge in the closed bottle experiments. Data points are normalized to the first measurement of FESOH. Error bars represent standard deviation of the replicate experiments (n=3).

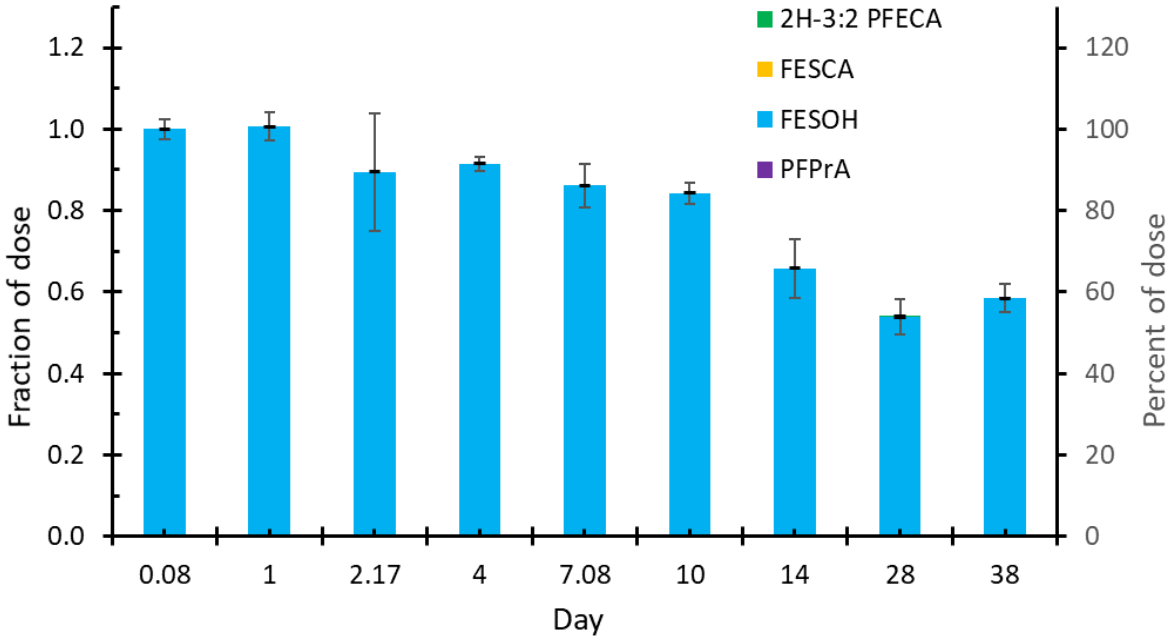


Figure S10. Mass balance of FESOH, autoclaved, in the closed bottle experiments.

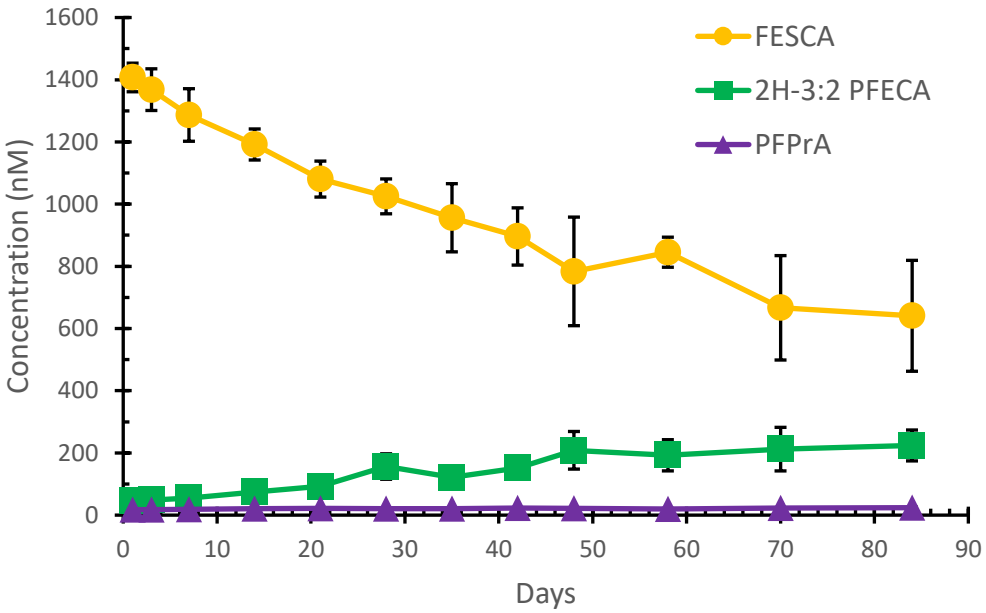


Figure S11. FESCA incubated with live sludge with continuous air flow for 84 days (n=3, except n=2 for days 58, 70, 84).

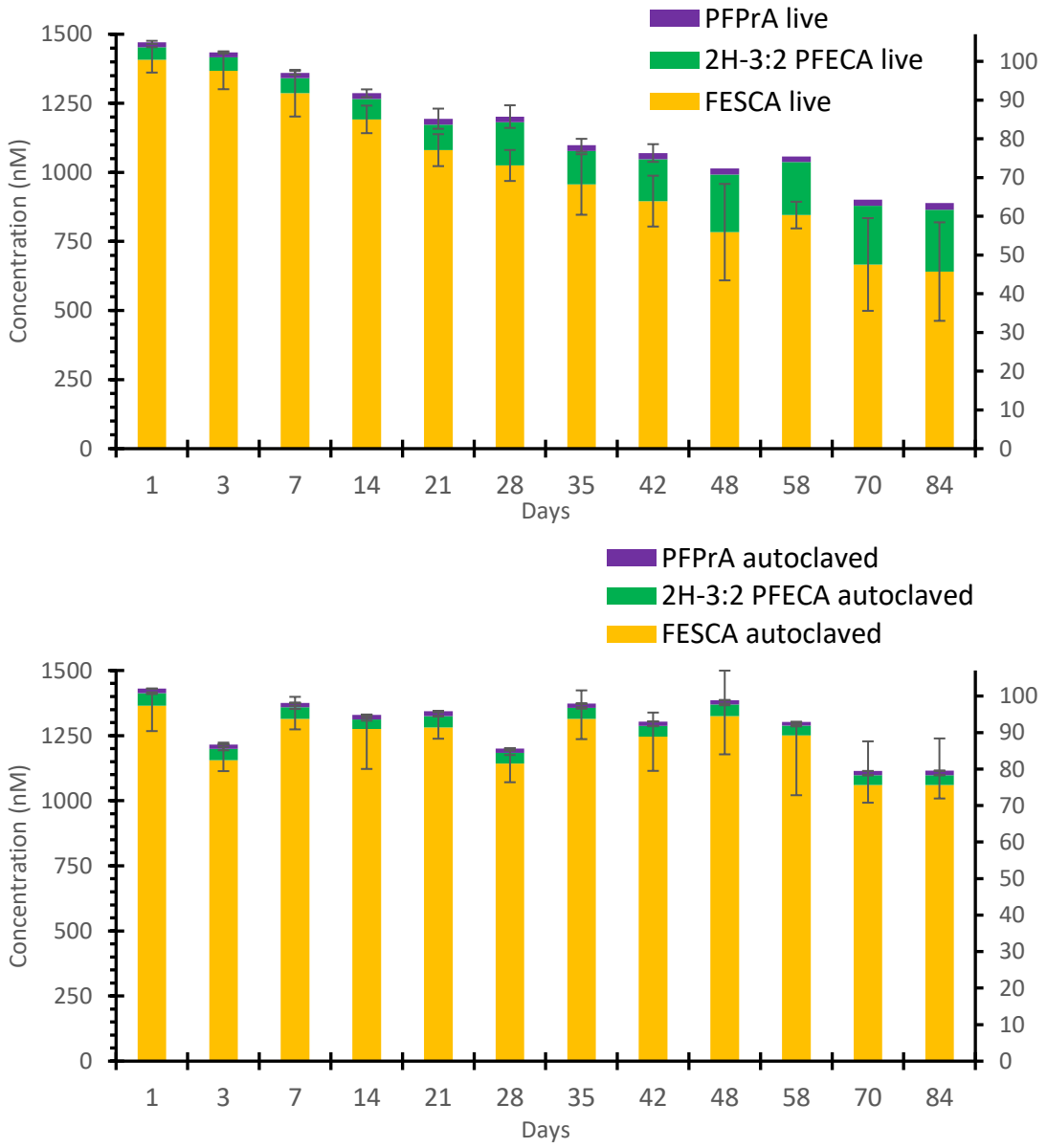


Figure S12. FESCA live (top) and autoclaved (bottom) experiments mass balance in the flow-through experiments (n=3, except n=2 for days 58, 70, 84).

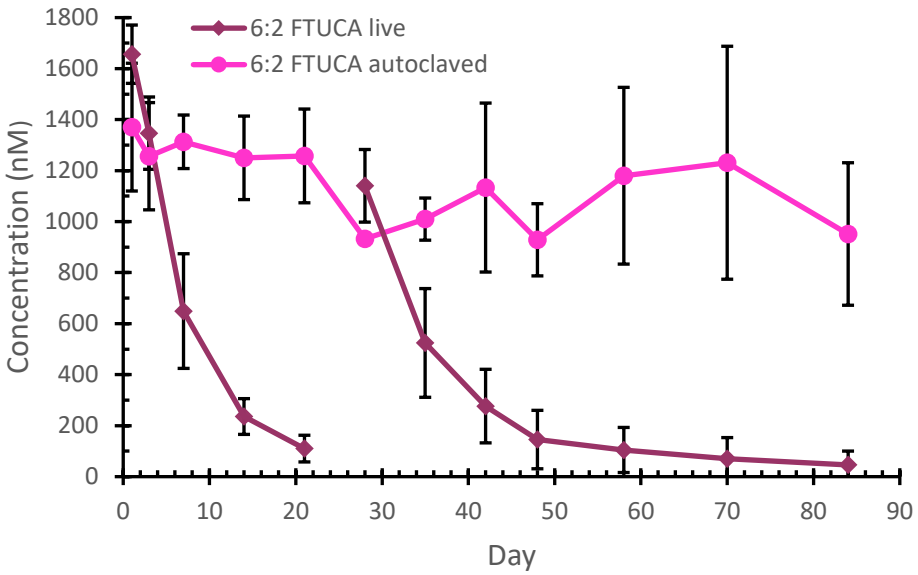


Figure S13. 6:2 FTUCA as a positive control was co-incubated in the FESCA flow-through experiments, showing the viability of the live experiment and confirming the autoclaved experiments remained inactive. A second spike was performed on day 27 (n=3, except n=2 for days 58, 70, 84).

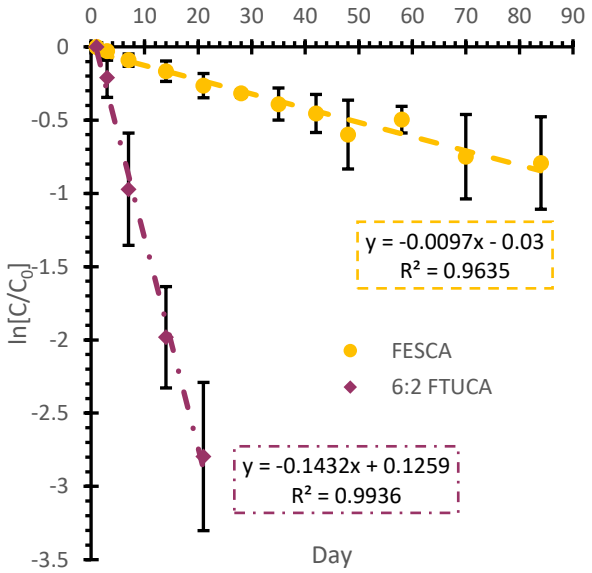
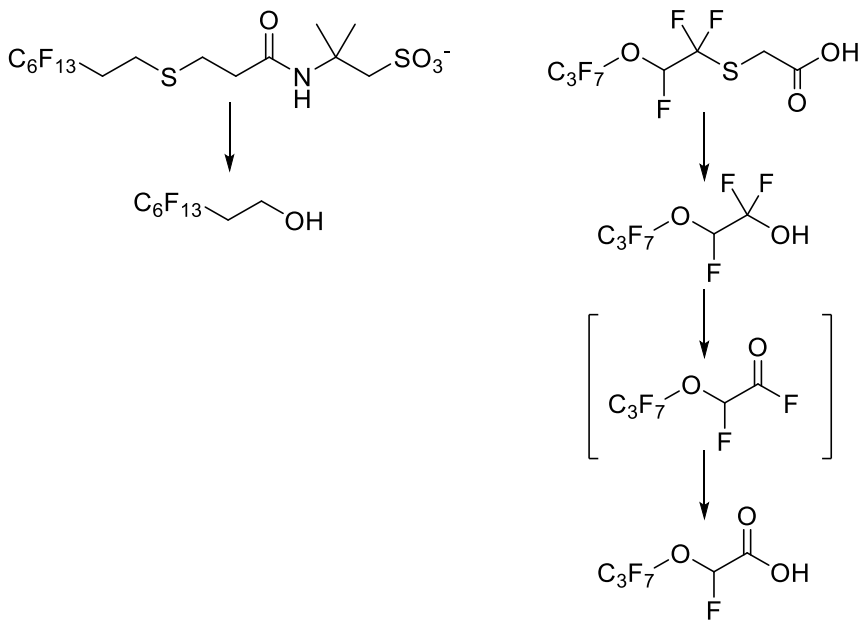


Figure S14. First order kinetics of FESCA and 6:2 FTUCA biotransformation. Only the first dose of 6:2 FTUCA was used to determine the rate in this system (n=3, except n=2 for days 58, 70, 84).

Figure S15. Oxidation at sulfur atom analogous to FTSAS biotransformation[4]



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