Supporting Information

Biotransformation of AFFF Component 6:2 Fluorotelomer Thioether Amido Sulfonate Generates 6:2 Fluorotelomer Thioether Carboxylate under Sulfate-Reducing Conditions

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Supplemental Materials and Methods

Chemicals.

Unlabeled and stable isotope-labeled standards for PFCAs and 6:2 fluorotelomer sulfonate (FtS) were purchased from Wellington Laboratories (Guelph, ON, Canada). The AFFF used in this study was manufactured by Ansul with an estimated 2008 manufacture date and obtained from a U.S. military base.(1) The formulation contains predominantly 6:2 FtTAoS at approximately 20 mM and dissolved organic carbon at 11.5 M. Zonyl FSA was obtained from the Field laboratory. Zonyl FSA is a proprietary PFAS mixture containing n:2 (n = 6, 8, 10) fluorotelomer thioether propionate (FtTP) in unknown concentrations. HPLC-grade water and methanol were purchased from Fisher Scientific. All other chemicals and solvents were purchased from either Fisher Scientific or Sigma-Aldrich at the highest possible purity.

Microcosms.

Pristine solids were collected from the sediment of a creek on the UC Berkeley campus and AFFF-contaminated solids were from a firefighter training area at the Ellsworth Air Force Base (South Dakota). The samples obtained from AFFF-impacted site have no records of the exact makeup of AFFF formulations used onsite, but it is likely that AFFF manufactured by 3M, National Foam, and Ansul accounted for most of the materials used.(2) All microcosms were mixed by gentle swirling to avoid the formation of foams prior to incubation at 30 °C in the dark without shaking. Sodium sulfate (50 mM) and DGBE (1.5 or 3 mM) were periodically amended to ensure that sufficient electron acceptor, electron donor, and carbon source were present in the live microcosms during the incubation period (Table S1). Autoclaved control microcosms were prepared by treating the solids with three autoclave-freeze-thaw cycles to ensure the deactivation of microbial activity.

Active sulfate-reduction was confirmed in the live pristine microcosms when sulfate concentrations decreased and the organic carbon amended was consumed (Figure S2 A, C). In microcosms constructed with contaminated solids, the observed sulfate concentration did not decrease (Figure S2 B, D). One potential explanation for this is that anaerobic microorganisms in the contaminated microcosms used other terminal electron acceptors for their metabolism, such as other sulfur species (sulfite, organosulfonates, and sulfur) that may have resulted from the historical application of AFFFs at the site. Black precipitates, presumably iron sulfides, were observed in the live contaminated microcosms, but not in the autoclaved controls, indicating the presence of sulfate-reducing microbial activity (Figure S2E). The presence of sulfate-reducing bacteria in the contaminated solid microcosms was also confirmed at the end of the experiments using Biological Activity Reaction Tests (BARTs) (Hach, Loveland, Co). A black slime growth at the bottom of the test tube was visualized on the second day in all tubes inoculated with live culture but absent in the control tubes (Figure S2F). The approximate population of sulfate-reducing bacteria estimated in this test was around 2.5×10^{7} cfu/mL.

Analytical methods.

A quantification range of 0.1 to 10 μg/L was used for all PFCAs and 6:2 FtS, and 2.3 – 23.4 μg/L for 6:2 FtTAoS.(2) A semi-quantitative method for 6:2 FtTP was developed by applying the TOP assay to Zonyl FSA, the only known commercially available standard reference containing mainly 6:2 and 8:2 FtTP in unknown quantities. The 6:2 FtTP is likely the only 6:2 fluorotelomer compound in the Zonyl FSA. Its molar concentration in the Zonyl FSA

standard was estimated using the TOP assay by summing the molar concentrations of all PFCAs that were expected to be generated from an equivalent concentration of a 6:2 fluorotelomer compound after the application of the assay. (25) The semi-quantitative method of 6:2 FtTP results in a quantification range of 1.3 to 12.7 μg/L. For the other newly identified transformation products, a semi-quantitative analysis was performed. The concentrations of the compounds were determined using the LC-MS/MS instrumental responses relative to an internal standard, isotope-labelled 6:2 FtS, and the calibration curve of the parent compound, 6:2 FtTAoS.

Dissolved organic carbon (DOC) was measured in individual microcosms using a TOC analyzer (TOC-V, Shimadzu, Japan) and sulfate was measured using an ion chromatograph (Dionex ICS 1100, Thermo Fisher, Sunnyvale, CA).(3, 4) Standards of known concentrations ranging from 0.042-4.2 mM (0.5-50 mg C/L) and 0.02 to 2 mM were used to quantify DOC and sulfate, respectively. Dry weight of solids was analyzed in accordance with the standard methods.(5)

TOP assay was used for quantification of total PFASs. Because quantitative standards were not available for many hypothesized biotransformation products, TOP served as a surrogate method for indirectly quantifying these compounds. Briefly, the stored 100 μL sample-methanol mixtures were flushed with nitrogen gas until dry. The dried samples were then reconstituted in a seven mL solution containing 116 mM sodium hydroxide and 51 mM potassium persulfate and incubated for 12 hours at 85 °C (water bath). The reacted solutions were then diluted and analyzed for total PFCA concentrations using LC-MS/MS. The resulting total molar concentrations of combined PFCAs represented the total molar concentration of PFASs in the samples.

The activity of sulfate-reducing bacteria in the contaminated solid microcosms was confirmed at the end of the experiments using BARTs (Hach, Loveland, CO) following the manufacturer's instructions. Briefly, pooled slurries from the triplicate live microcosms were diluted 10 or 50 folds with sterile reduced mineral salt medium to minimize the interference of the black precipitates. Duplicates of 15 mL of diluted culture were added to the test vials and incubated at room temperature in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) with a nitrogen and hydrogen (2-3%) atmosphere (O_2 < 2 ppm). Duplicate test vials with autoclaved live culture at the same dilutions were used as negative controls. One vial for each 10- or 50-fold diluted autoclaved microcosms was also included in the test for comparison.

Statistical analysis.

Statistical significance for concentration changes between Day 0 and the end of experiments were tested using a student t test. P values less than 0.05 were considered statistically different for the two groups compared.(6)

Prediction library establishment.

The predicted biotransformation products of 6:2 FtTAoS were generated by the EAWAG-BBD Pathway Prediction System (EAWAG-BBD-PPS, http://eawag-bbd.ethz.ch/predict/, updated May 2016) using the reaction rules that take place under either aerobic or anaerobic conditions. One limitation related to the EAWAG-BBD-PPS is that this database cannot accurately predict transformation products of highly fluorinated compounds containing carbons that are bonded with more fluorines than non-fluorine atoms.(7) For example, the EAWAG-BBD-PPS prediction stops at the formation of PFHpA for 6:2 FtTAoS without taking into account the recently reported oxidation pathways that can lead to the partial

defluorination reactions that form products such as 6:2 FtUCA, 5:3 FtCA, PFPeA and PFBA. In order to obtain a more comprehensive and up-to-date library, we compensated for this limitation by including perfluoroalkyl sulfonates and carboxylates and polyfluorinated biotransformation products of 6:2 FtS and 6:2 fluorotelomer alcohol (FtOH) and 6:2 FtTAoS (aerobic pathways) compiled from the experimental observations of recent studies.(3, 8-14)

Verification of the automated suspect screening of HR-ESI-MS data.

The automated suspect screening process was examined using triplicate solutions containing 32 PFAS standard compounds, including 18 perfluoroalkyl carboxylates and sulfonates with carbon chain lengths ranging from four to 18, 11 fluorotelomers and three perfluorosulfonamides (Table S4). The concentration of each of the 32 standard compounds in the solution was around 17 µg/L, which accounted for approximately 0.6-3% (mol) mass of initially amended 6:2 FtTAoS concentration used in the HR-ESI-MS analysis. A blank control consisting of only water and methanol was included along with the triplicate solutions during SPE treatments and mass spectrometry analysis to remove background ions before matching the measured ion accurate masses (*m/z*) to the library masses during the verification experiments.

Two libraries of PFASs were used to assess the efficacy and accuracy of the suspect screening method for positive and false positive and negative identifications. The library consisted of all 32 standard compounds, referred as the "standard library". The other library was the prediction library as described above. Less than five ppm mass deviation between measured and library masses indicated a positive identification. False positives were defined as masses that were not included in the 32 listed standard compounds, but were recognized by the analysis using the prediction library. False negatives were defined as compounds that were present in the mass spectra of 32 PFAS solution but failed to match either library by the automated screening. Our tests indicate that 31 of the 32 standard compounds at the tested concentrations can be detected by HR-ESI-MS and identified by our automated analysis. Most of these 31 compounds were detected in all three replicated mixed solutions, except for four compounds, namely, PFBA, PFPeA, 8:2FtCA, and 8:2 Zonyl FSA, which were only found in two of the triplicated mixtures. Since these compounds could be quantitatively analyzed by LC-MS/MS, the inability to detect them using HR-ESI-MS did not affect our analysis. The analysis using the prediction library of 78 compounds showed only one false positive (*m*/*z* 468.9791). These analyses indicated the effectiveness of our automated matching method with HR-ESI-MS data.

Mass spectrometry analysis and workflow for identifying transformation products.

For suspect screening, we developed a Python script (version 2.7) to perform an automated analysis (Figure S1). Briefly, the mass spectra were first filtered with a mass-defect analysis in order to focus on masses that are potential PFASs.(1) A band ranging from -0.15 to 0.15 Da was used as the mass defect filter as previously reported.(15) The coverage efficiency of this filter was tested against the potential transformation products (predicted from EAWAG-BBD-PPS) of which all mass defect values were found in the range. Matching between measured and suspect library masses was performed on all samples and controls (Figure S1).

HR-ESI-MS and HR-naonESI-MS/MS for identifying transformation products.

Accurate mass data were obtained at QB3/Mass spectrometry Facility at the University of California, Berkeley. Samples were analyzed on Finnigan LTQ FT mass spectrometer (Thermo Fischer Scientific) equipped with electrospray ionization (ESI) source in negative ion mode in

the *m/z* range 100-1000. Samples were dissolved in methanol (0.1% ammonium hydroxide, v/v) with a final concentration of 1-3 µM and were directly infused via syringe pump at the rate 5 µL/min. Xcalibur™ software (version 2.0.7, Thermo) was used for both data acquisition and data analysis. For suspect screening, we used an in-house automated analysis to match accurate mass measurements to a list of exact masses of potential biotransformation products (Figure S1). The masses that had no match in the prediction library were selected for the non-targeted analysis only when they were present in higher relative abundance in live cultures than in the controls and with a relative ion abundance greater than 1% (relative to the most abundant ion; Figure S1). The molecular formula fit was manually performed using Xcalibur™ software.

Compound identification was considered tentatively positive when (1) the error of the HR-ESI-MS accurate mass to the exact mass of potential transformation product was no more than 5 ppm; and (2) significant differences in HR-ESI-MS ion abundance between live and control microcosms were observed.

The majority of fragment ions of proposed structures were analyzed using High-resolution (HR) nanoESI MS/MS (HR-nanoESI-MS/MS) in the negative ion mode (Synapt G2-S*i*, Waters, Milford, MA) over the *m*/*z* range of 50-1000. (Figure S3). The in-silico fragmentation interpretation was performed with the software, ACD/MS fragmenter 2015 (Advanced Chemical Development, Toronto, ON, Canada), with a setting that considered all possible fragmentation reactions. The structures of fragment ions were proposed using ACD/MS fragmenter software with mass errors less than 5 mDa or 15 ppm between the observed accurate mass and the theoretical exact mass of proposed fragments.(15) Since the structure of 6:2 FtTP was confirmed by the standard reference, Zonyl FSA, its fragmentation spectrum was used as a reference for other proposed structures that shared similar structure moiety with 6:2 FtTP.

Supplemental Figures and Tables

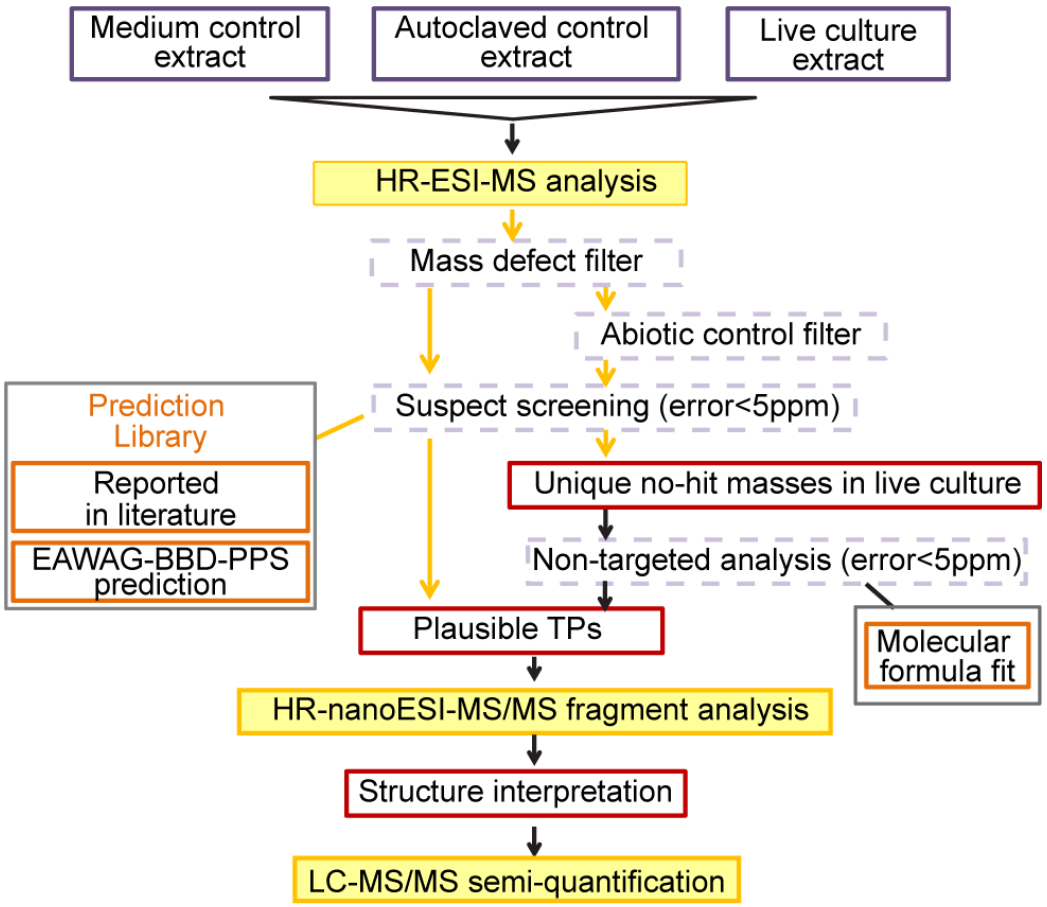


Figure S1. Workflow that utilizes automated suspect screening in conjunction with manual non-targeted analysis to identify novel biotransformation products in AFFF-amended microcosms. A library of predicted biotransformation products was established with previously reported PFASs and predicted products of 6:2 FtTAoS from EAWAG-BBD-PPS. Automated suspect screening of HR-ESI-MS data was performed using this library on all live and control microcosm samples. The masses that were not matched during the suspect screening then underwent a non-targeted analysis that compares the full-scan HR-ESI-MS data among samples to focus on the candidate masses either uniquely associated with live microcosms or present at higher abundance in live microcosms than controls for determining the molecular formulae. All plausible products were then analyzed for proposed structures using HR-nanoESI-MS/MS and quantified (or semi-quantified) using LC-MS/MS. Yellow arrows indicate the automated screening that was performed using a Python script (version 2.7). Black arrows indicate manual analyses. "TPs" stands for transformation products.

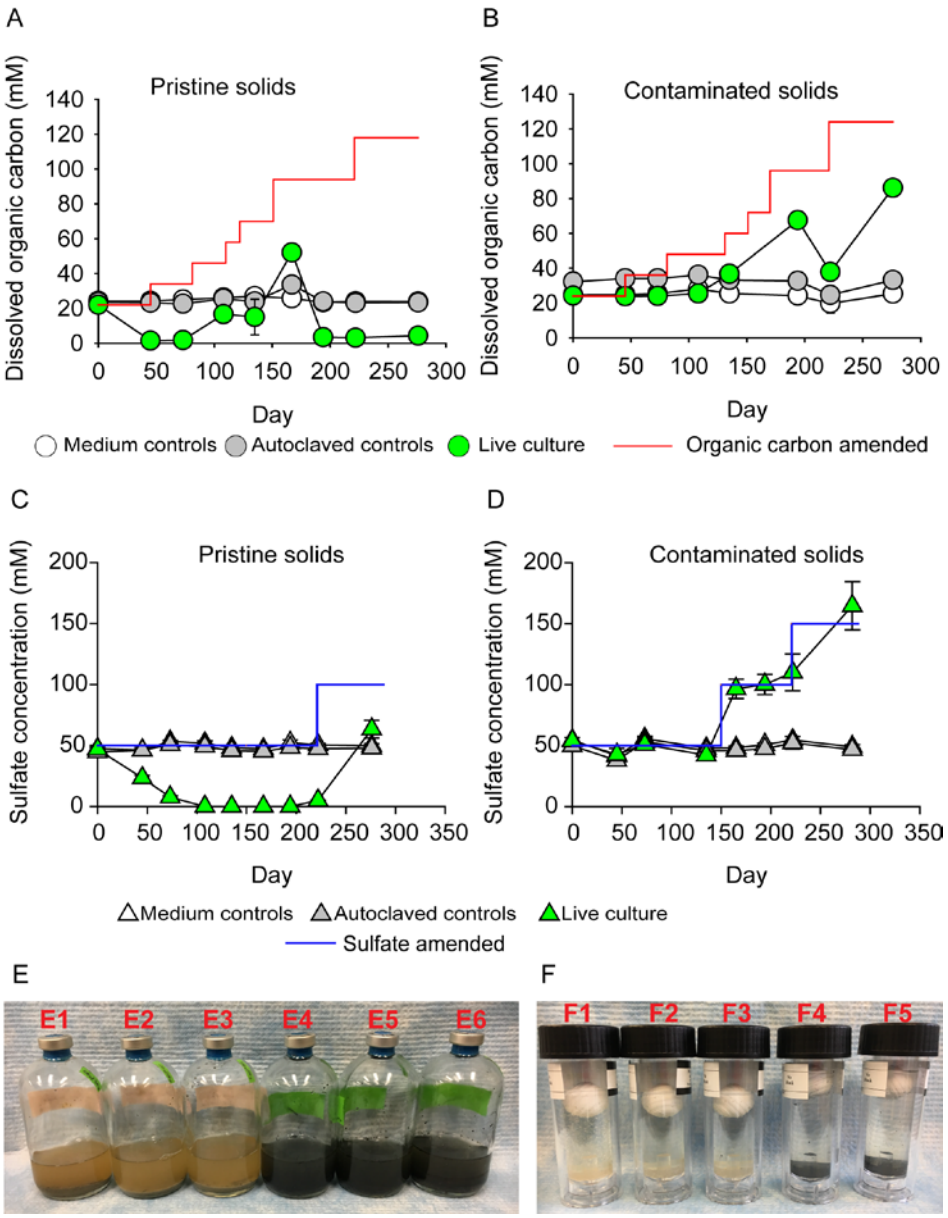
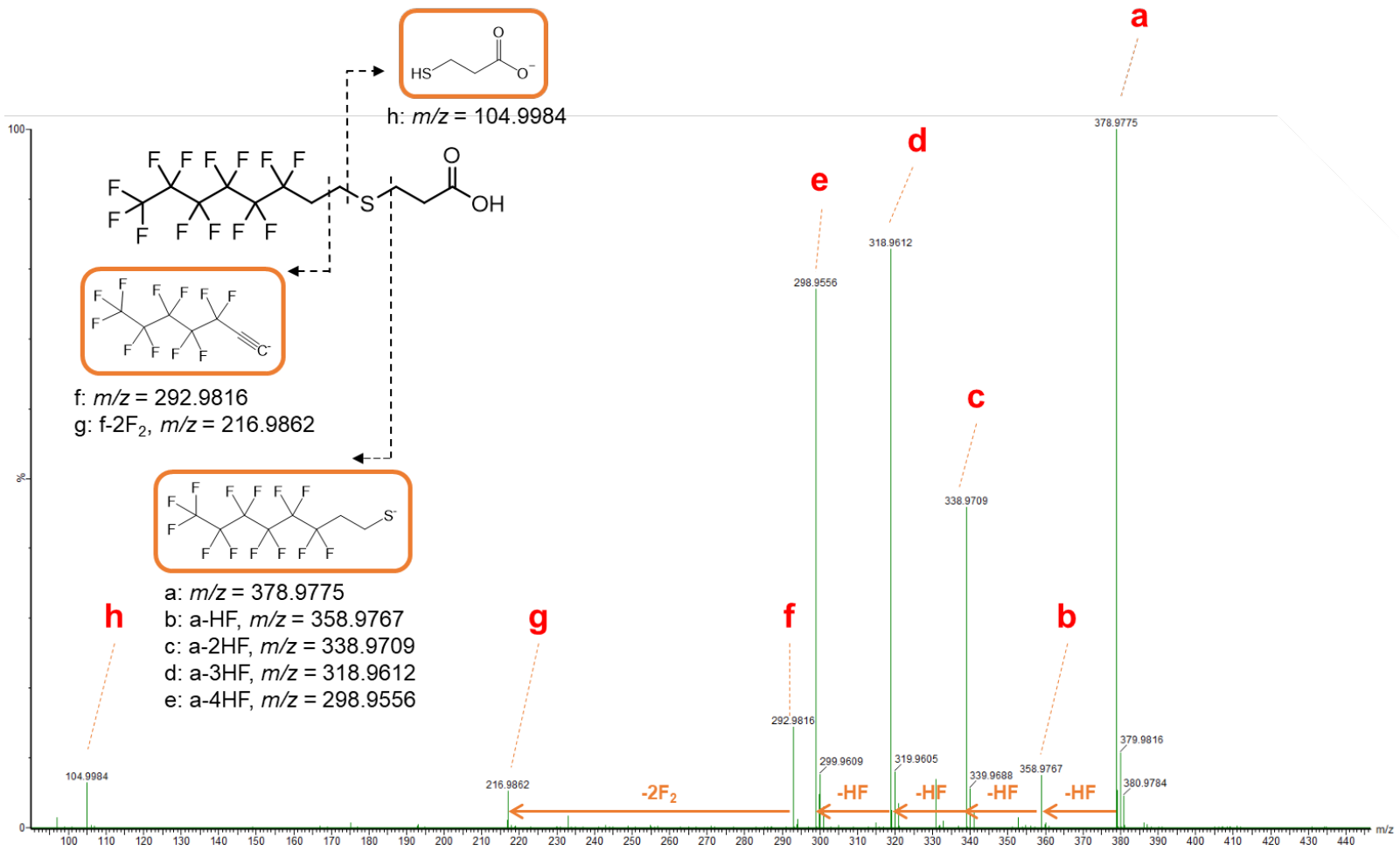


Figure S2. Dissolved organic carbon concentrations and sulfate concentrations in pristine (A, C) or contaminated (B, D) microcosms. The red and blue lines represent the total amended organic carbon and sulfate concentrations that were calculated from amended concentrations, respectively (Table S1). Error bars represent the standard deviation of averages from triplicate microcosms. The autoclaved (E1-3) and live (E4-6) microcosms that were inoculated with contaminated solids (E). The Biological Activity Reaction Tests (BARTs; Hach, Loveland, CO) for sulfate-reducing bacteria (F). F1, autoclaved microcosms (50× dilution); F2-3, autoclaved live microcosms (50× dilution); F4-5, live microcosms (50× dilution).

A


m/z 451
h: m/z = 104.9984
f: m/z = 292.9816
g: f-2F2, m/z = 216.9862
a: m/z = 378.9775
b: a-HF, m/z = 358.9767
c: a-2HF, m/z = 338.9709
d: a-3HF, m/z = 318.9612
e: a-4HF, m/z = 298.9556
-2F2
-HF
-HF
-HF
-HF
m/z
%

218

219

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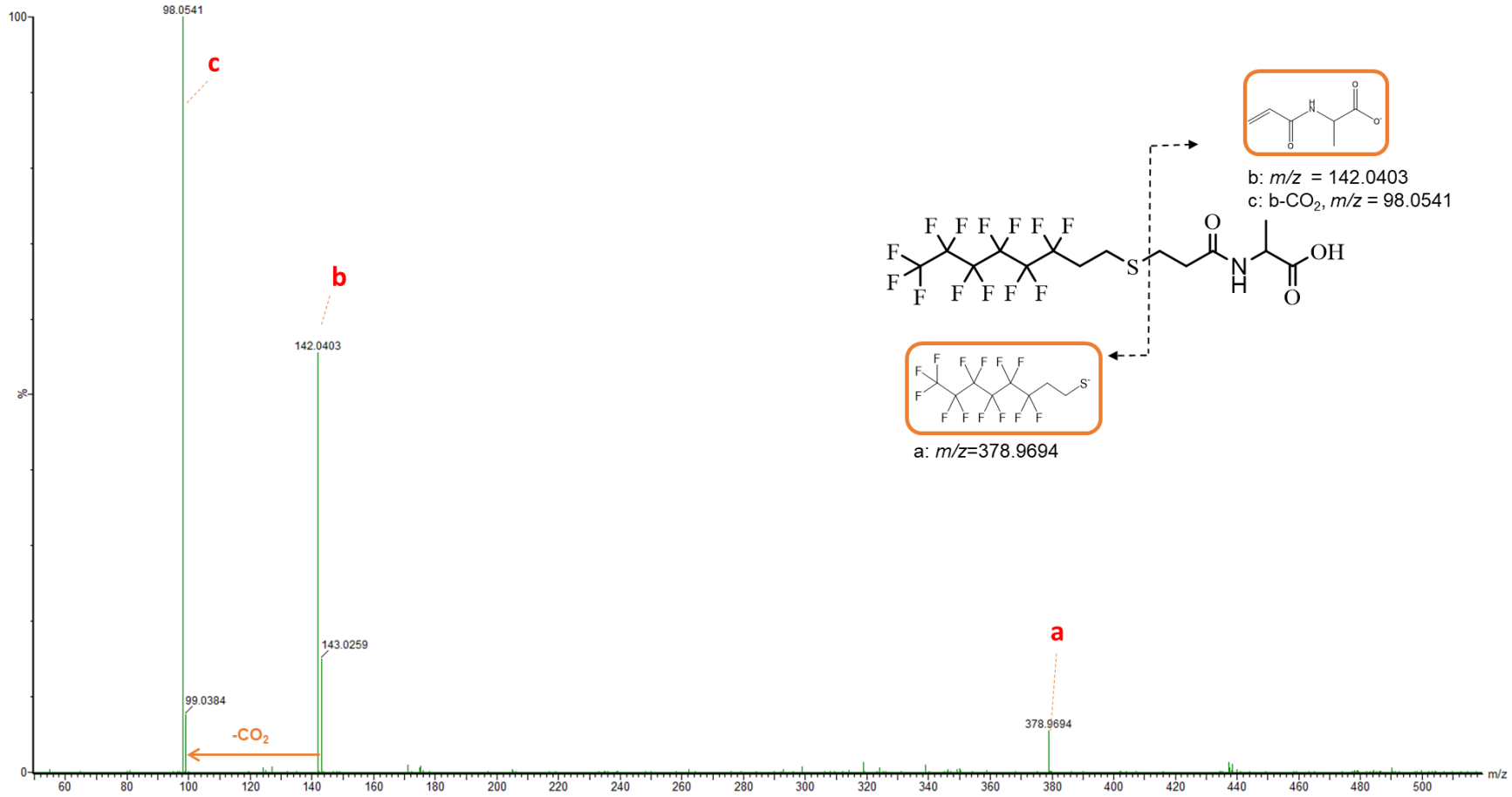
B


m/z 522
98.0541
c
b
142.0403
143.0259
99.0384
-CO2
a
378.9694
100
%
0
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
b: m/z = 142.0403
c: b-CO2, m/z = 98.0541
a: m/z=378.9694

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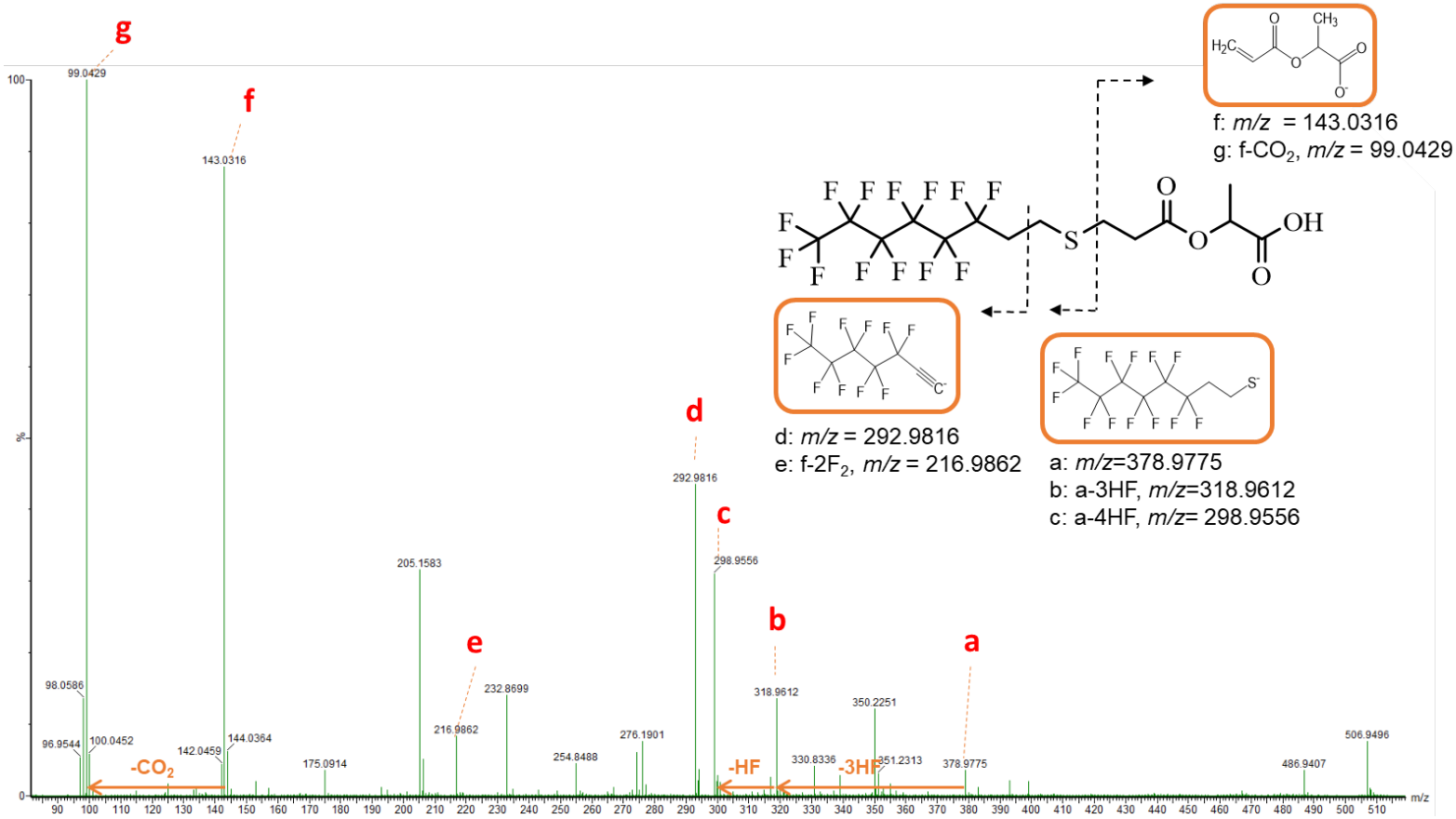
C



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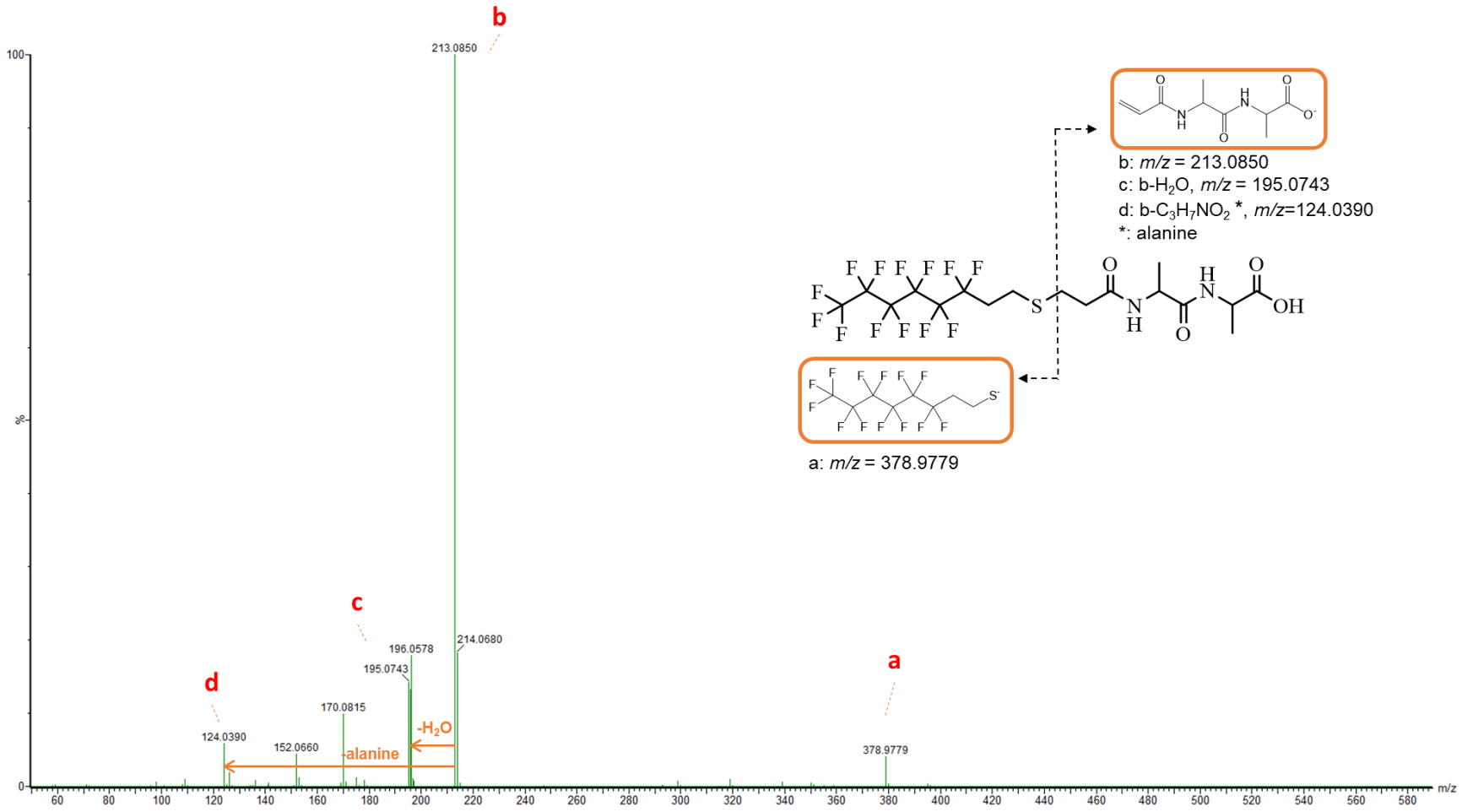
D


m/z 593
b
213.0850
100
%
0
c
196.0578
195.0743
214.0680
d
170.0815
124.0390
152.0660
-alanine
-H2O
a
378.9779
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580
m/z
b: m/z = 213.0850
c: b-H2O, m/z = 195.0743
d: b-C3H7NO2 *, m/z=124.0390
*: alanine
a: m/z = 378.9779

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235

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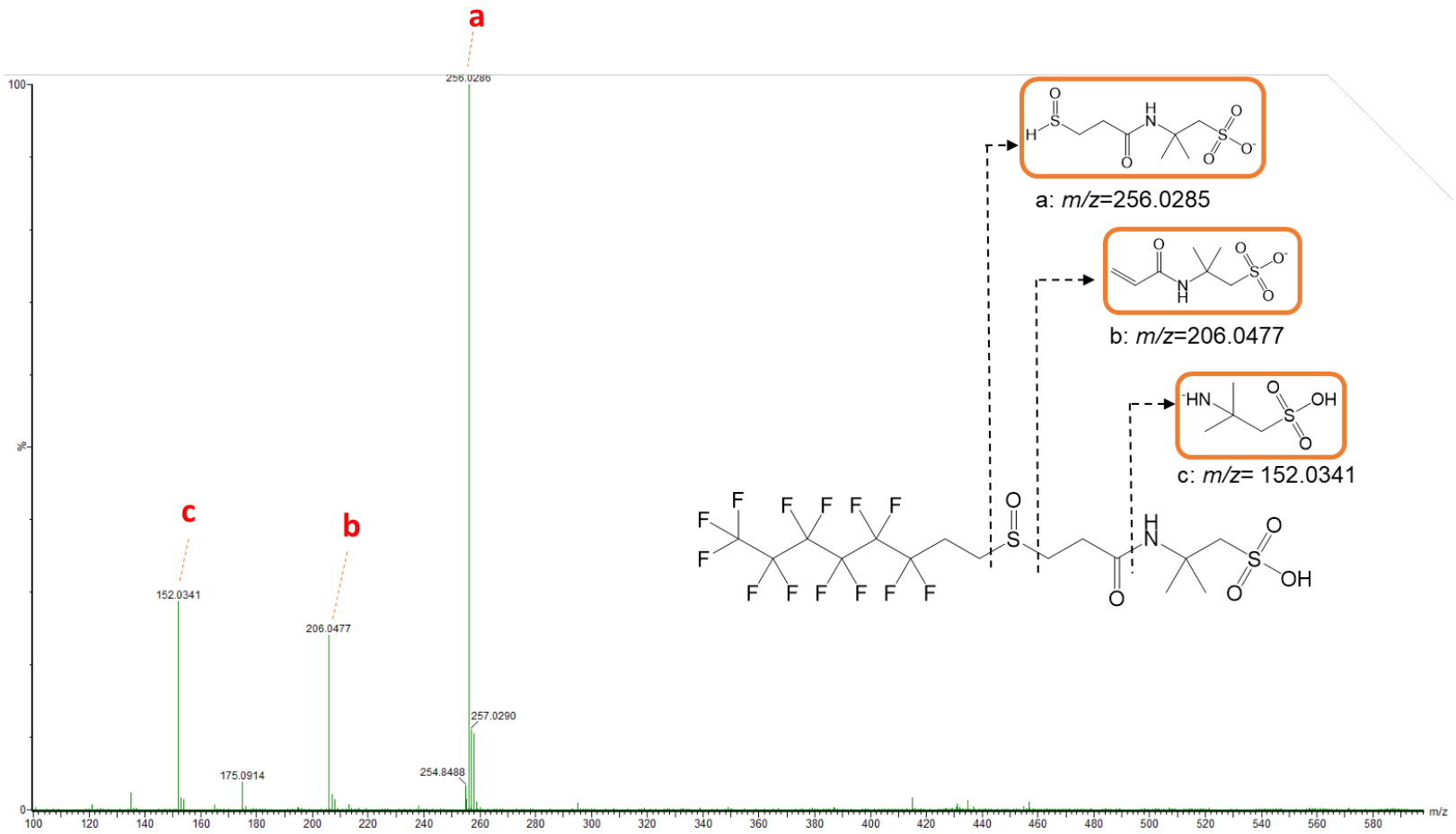
E



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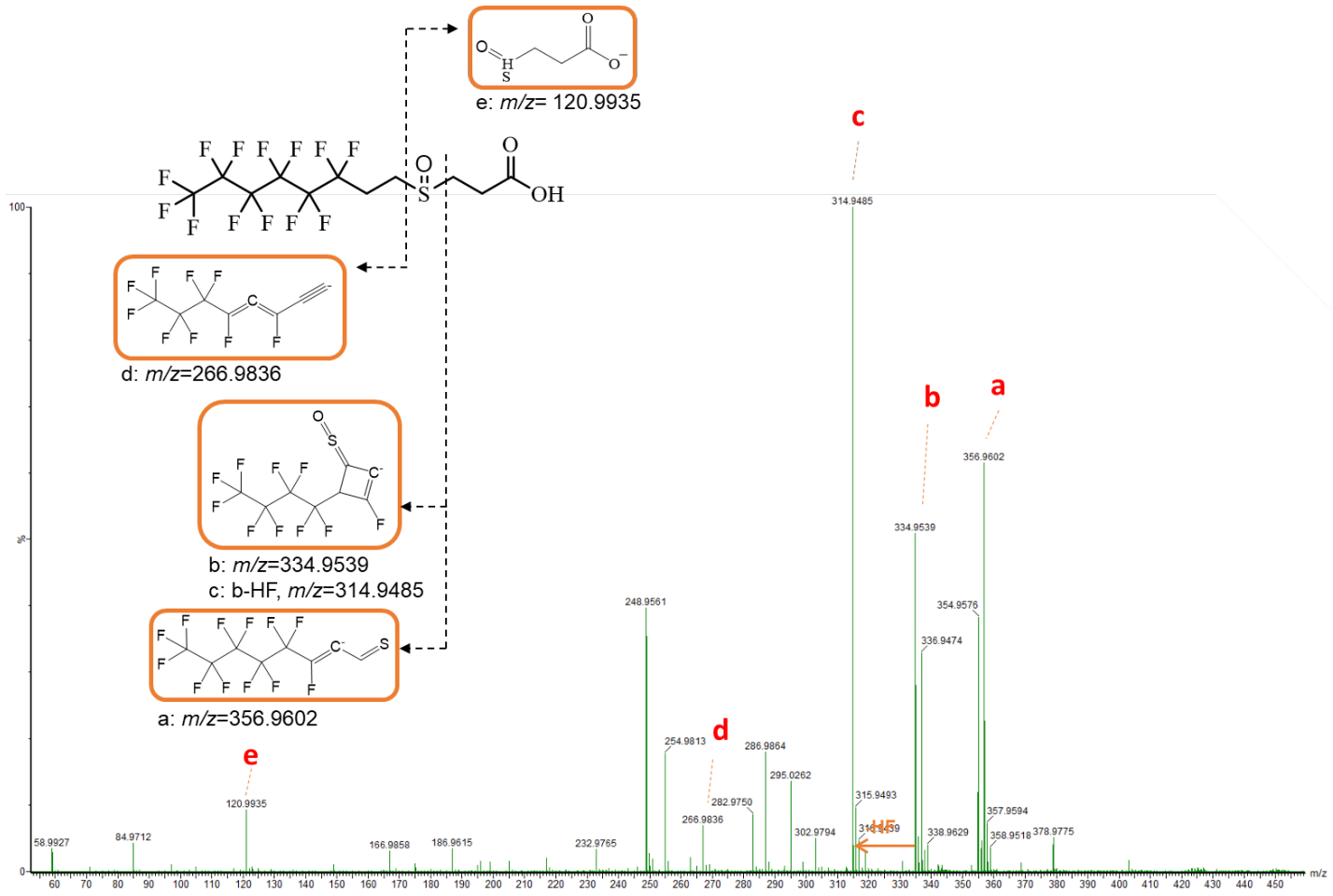
F *m/z* 467



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247 **Figure S3.** High resolution nanoESI MS/MS spectra and *in silico* fragment interpretation. The structure shown was proposed by the
248 software, ACD/MS fragmenter 2015 (Advanced Chemical Development, Toronto, ON, Canada) with mass errors less than 5 mDa or
249 15 ppm relative to the theoretical exact mass of proposed fragments. Exact mass and error of proposed fragments is shown in Table S5.
250 A, *m/z* 415; B, *m/z* 522; C, *m/z* 523; D, *m/z* 593; E, *m/z* 602; F, *m/z* 467.

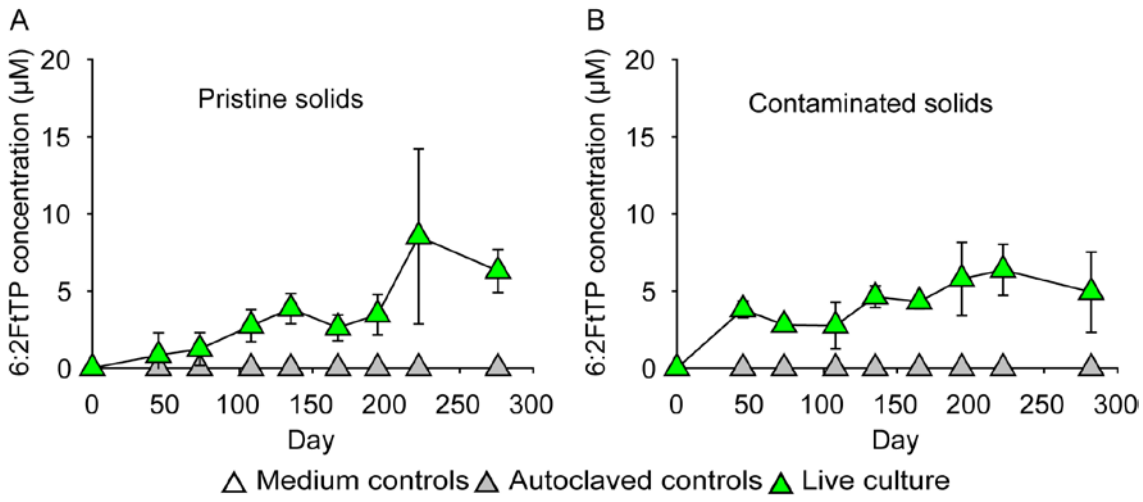


Figure S4. Production of 6:2 FtTP (*m*/*z* 451) during 6:2 FtTAoS biotransformation was confirmed and semi-quantified using a commercially available standard reference, Zonyl FSA. Error bars represent the standard deviation of averages from triplicate microcosms. A, pristine solids; B, contaminated solids.

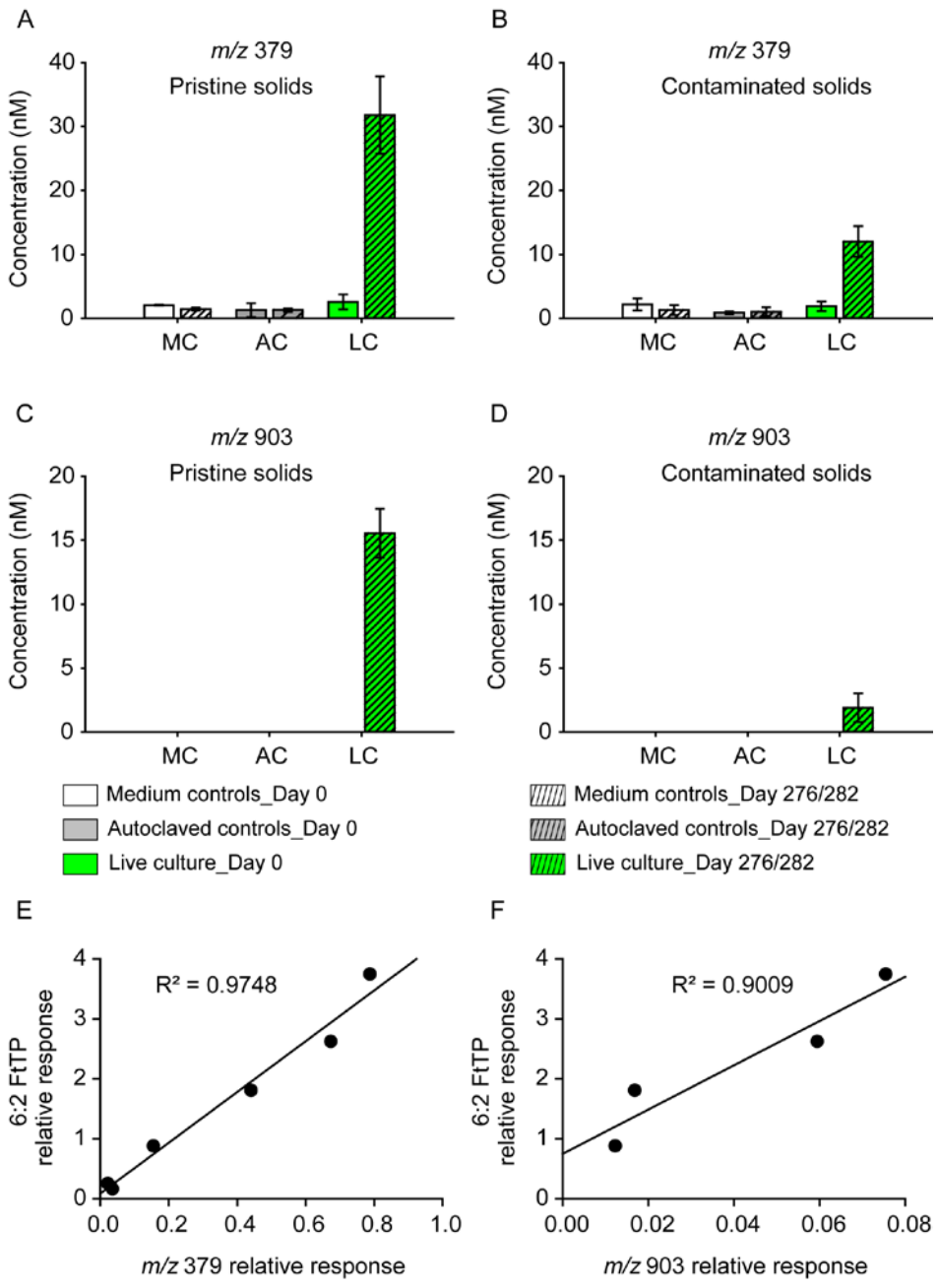


Figure S5. Semi-quantification of *m/z* 379 (A, B) and 903 (C, D) based on the estimation of molar response using the calibration of the parent compound, 6:2 FtTAoS. MC, AC, and LC stand for medium controls, autoclaved controls and live culture, respectively. Error bars show the standard deviation of the average value obtained from triplicate microcosms. The correlation of instrumental relative responses of 6:2 FtTP and *m/z* 379 (E) or *m/z* 903 (F) in the standard solutions of Zonyl FSA.

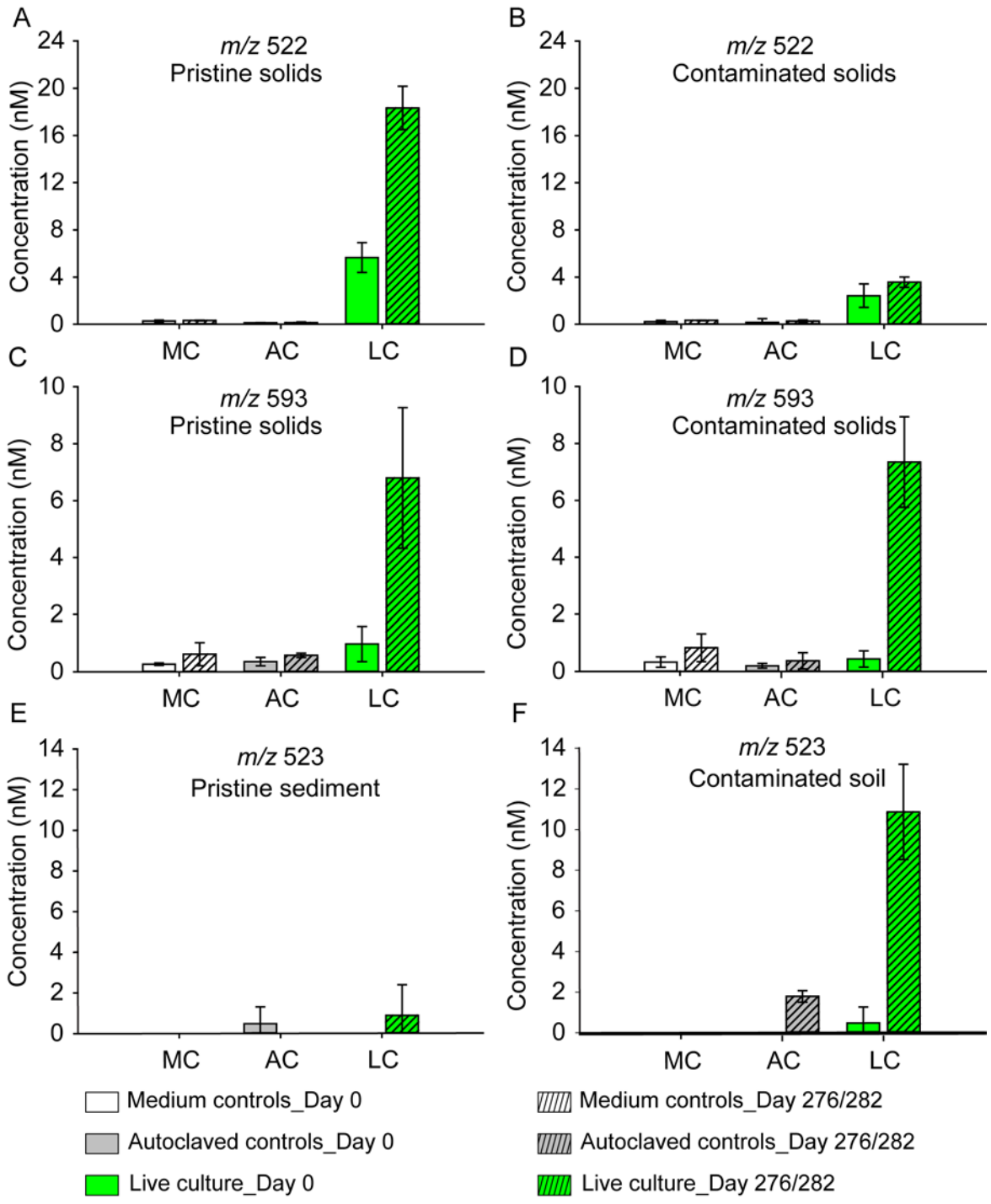


Figure S6. Concentrations of newly identified transformation products at Day 0 and at the end of experiments. These intermediates were semi-quantified based on the estimation of molar response using the calibration of the parent compound, 6:2 FtTAoS. MC, AC, and LC stand for medium control, autoclaved control and live culture, respectively. Error bars show the standard deviation of the averages from biological triplicates. *m/z* 522, A, B; *m/z* 593, C, D; *m/z* 523, E, F.

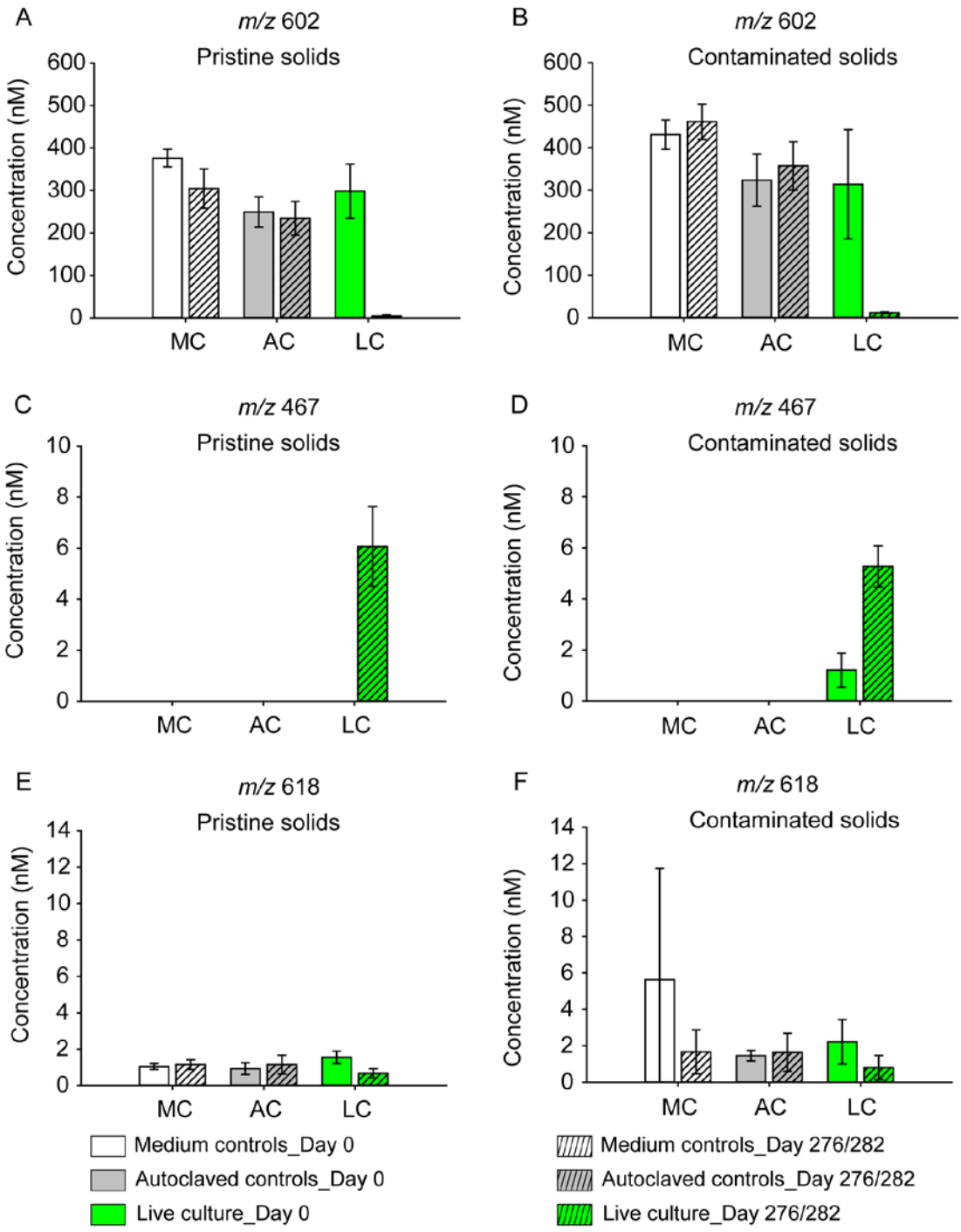


Figure S7. Semi-quantification of *m/z* 602 (A, B), 467 (C, D) and 618 (E, F) at Day 0 and at the end of experiments based on the estimation of molar response using the calibration of the parent compound, 6:2 FtTAoS. MC, AC, and LC stand for medium controls, autoclaved controls and live culture, respectively. Error bars show the standard deviation of the average value obtained from triplicate microcosms.

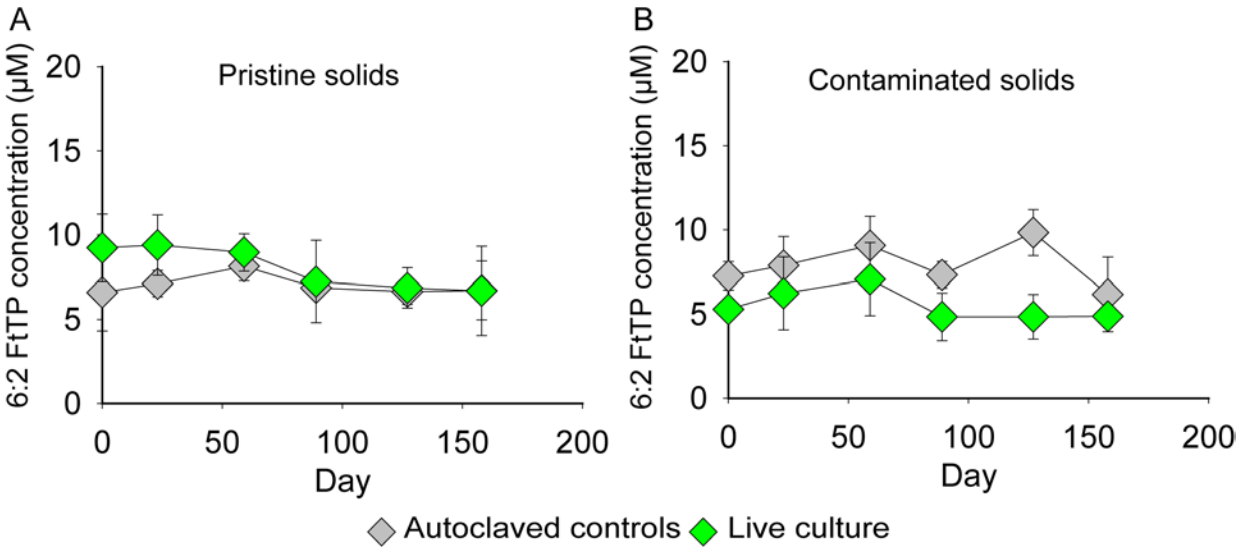


Figure S8. Concentrations of 6:2 FtTP in autoclaved and live microcosms amended with Zonyl FSA. Error bars show the standard deviation of averages of triplicate microcosms. A, pristine solids; B, contaminated solids.

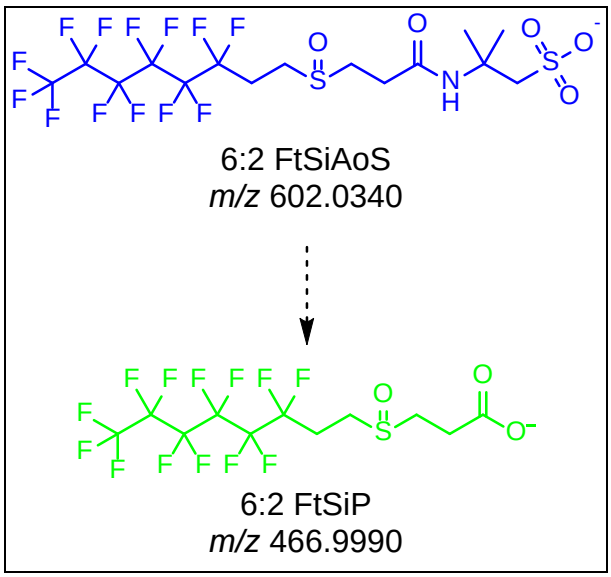


Figure S9. Hydrolysis reaction that was possibly responsible for the removal of 6:2 FtSiAoS and the generation of 6:2 FtSiP. Level of confidence in the structures identified using high-resolution mass spectrometry: blue, possible structures (Level 2); green, tentative candidates (Level 3).(16)

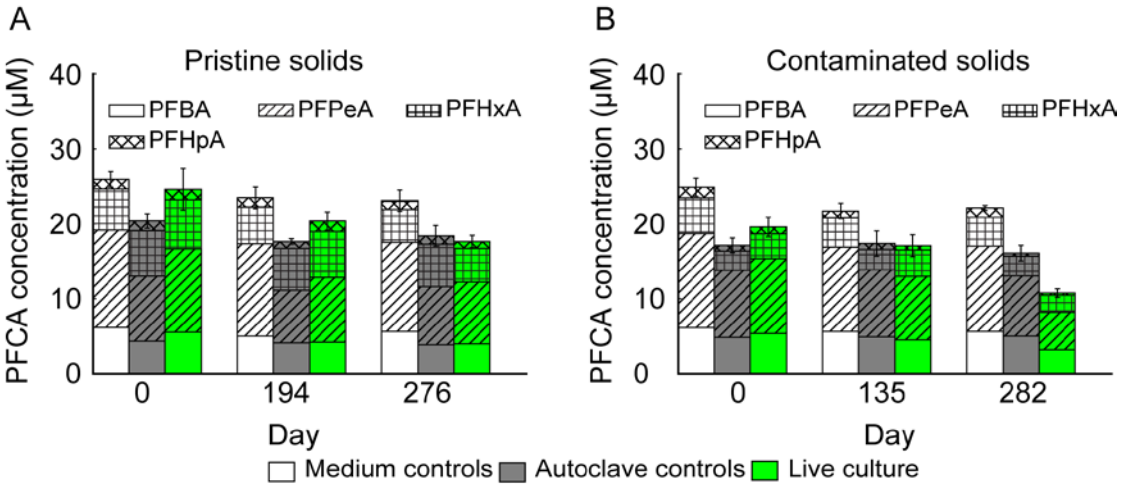


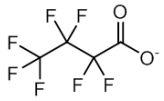
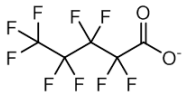
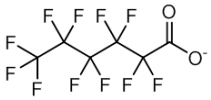
Figure S10. Total oxidative precursor (TOP) assay of microcosms constructed with pristine solids (A) or contaminated solids (B). Error bars show the standard deviation of the average value obtained from biological triplicates. PFCA, perfluoroalkyl carboxylate; The structures of PFBA, PFPeA, PFHxA, and PFHpA are displayed in Table S2.

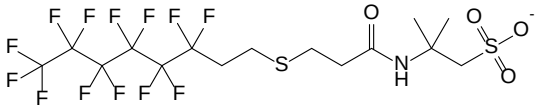
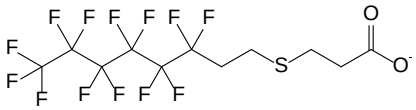
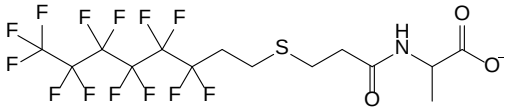
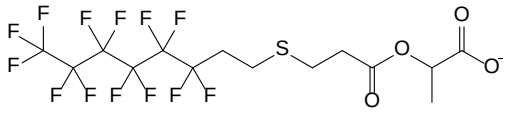
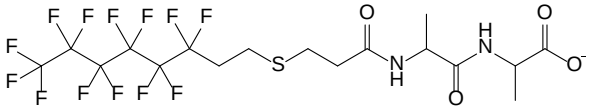
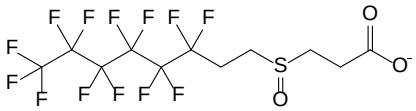
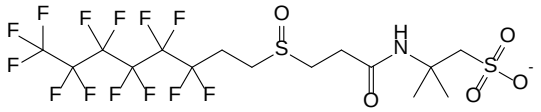
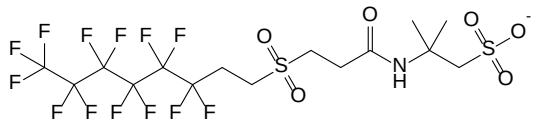
Table S1. Microcosms setup conditions. (*Conditions are the same in all live and control microcosms.)

Conditions	Replicates	Inoculum	Medium composition (50 ml)*	Carbon source & electron donor*	Electron acceptor*†	AFFF*	Initial DGBE*†
Pristine							
Medium Control	3	Nil					
Autoclaved Control	3	Autoclaved pristine solids					
Live Culture	3	Live pristine solids					
Contaminated			Mineral salt medium + Cysteine sulfide + resazurin indicator	AFFF organics +DGBE	Na_2SO_4 (50 mM)	50 µl	1.5 mM
Medium Control	3	Nil					
Autoclaved Control	3	Autoclaved contaminated solids					
Live Culture	3	Live contaminated solids					

†1.5 (or 3 mM) DGBE was provided on days 46, 82, 111, 123, 151 (3 mM), and 222 (3 mM) in pristine microcosms and on days 46, 82, 132, 151, 171 (3 mM) and 222 (3 mM) in contaminated microcosms. 50 mM Na_2SO_4 was amended on days 151 (for contaminated microcosms) and 222 (for both pristine and contaminated microcosms).

304 **Table S2.** Structures and names of PFASs present in this study.

Acronym	Structure	Name used in this study (previously reported names (Reference))*
PFBA		Perfluorobutanoate
PFPeA		Perfluoropentanoate
PFHxA		Perfluorohexanoate
PFHpA		Perfluoroheptanoate
PFOA		Perfluorooctanoate
PFNA		Perfluorononanoate
PFDA		Perfluorodecanoate
PFUdA		Perfluoroundecanoate
PFDoA		Perfluorododecanoate
6:2 FtS		6:2 Fluorotelomer sulfonate

6:2 FtTAoS		6:2 Fluorotelomer thioether amido sulfonate (n:2 tridecaFluoroAlkylThia PropanoAmido-MethylPropylSulfonate, n:2 Fluorotelomer Thio Amido Sulfonate (15))
6:2 FtTP		6:2 Fluorotelomer thioether propionate (*n*:2 heptadecaFluoroDecylThia Propanic acid (15))
6:2 FtTPlA		6:2 Fluorotelomer thioether propanoyl alanine
6:2 FtTPoP		6:2 Fluorotelomer thioether propanoyl oxy propanoate (6:2 FtTPoP)
6:2 FtTPlAA		6:2 Fluorotelomer thioether propanoyl alanyl alaninate
6:2 FtSiP[3]		6:2 Fluorotelomer sulfinyl propanoate
6:2 FtSiAoS		6:2 Fluorotelomer sulfinyl amido sulfonate
6:2 FtSoAoS		6:2 Fluorotelomer sulfonyl amido sulfonate (*n*:2 tridecaFluoroAlkyl Sulfonyl(SO2) PropanoAmido-MethylPropylSulfonate (15))

* The naming strategy and acronyms of the newly identified transformation products were adapted from literature precedents of the fluorotelomer compounds. The newly identified transformation products were named using a n:2 format to represent the backbone of perfluorinated and unsubstituted carbon numbers. Additional groups were named starting from the immediate end adjacent to the backbone carbon chain. Since all the transformation products were formed in the physiological conditions (e.g., neutral pH), the ionic forms instead of acid forms were used in the names.

Table S3. Quadrupole LC-MS/MS methods for novel biotransformation products identified in this study.

Name	Internal standard	Molecular ion (*m*/*z*)	Quantifier ion (*m*/*z*)	Qualifier ion (*m*/*z*)	Fragmentor voltage (V)	Collision energy (V)	Polarity	Standard reference
6:2 FtTP (*m*/*z* 451)	[^{13}C2]6:2 FtS	451	379	105	60	10	negative	Zonyl FSA
6:2 FtSiP (*m*/*z* 467)	[^{13}C2]6:2 FtS	467	315	59	60	20	negative	6:2 FtTAoS
6:2 FtTPlA (*m*/*z* 522)	[^{13}C2]6:2 FtS	522	142	98	90	10	negative	6:2 FtTAoS
6:2 FtTPoP (*m*/*z* 523)	[^{13}C2]6:2 FtS	523	357	144	135	30	negative	6:2 FtTAoS
6:2 FtTPlAA (*m*/*z* 593)	[^{13}C2]6:2 FtS	593	213	152	90	10	negative	6:2 FtTAoS
6:2 FtSiAoS (*m*/*z* 602)	[^{13}C2]6:2 FtS	602	256	206	135	40	negative	6:2 FtTAoS
m/*z* 618	[^{13}C2]6:2 FtS	618	206	152	30	30	negative	6:2 FtTAoS

Table S4. Standard PFASs used in the verification experiments for suspect-screening analysis of HR-ESI-MS accurate mass data.

No	Standard library Name	Exact mass *m/z*	Nominal mass *m/z*	Perfluoro -chain	Conc. in mixture		Accurate mass (*m/z*) (HR-ESI-MS)					
					(µg/L)	nM	Mix1	Error (ppm)	Mix2	Error (ppm)	Mix3	Error (ppm)
1	PFBA	212.9787	213	4	18	84						
2	PFPeA	262.9754	263	5	18	68	262.9762	3.04			262.976	2.28
3	PFHxA	312.9723	313	6	18	57	312.973	2.24	312.9732	2.88	312.973	2.24
4	PFHpA	362.9691	363	7	18	49	362.9698	1.93	362.9698	1.93	362.9698	1.93
5	PFOA	412.9659	413	8	18	43	412.9667	1.94	412.9667	1.94	412.9667	1.94
6	PFNA	462.9627	463	9	18	39	462.9635	1.73	462.9635	1.73	462.9636	1.94
7	PFDA	512.9595	513	10	18	35	512.9603	1.56	512.9603	1.56	512.9604	1.75
8	PFuDA	562.9564	563	11	18	32	562.9568	0.71	562.9569	0.89	562.9569	0.89
9	PFdDA	612.9532	613	12	18	29	612.9536	0.65	612.9536	0.65	612.9536	0.65
10	PFtrDA	662.9500	663	13	18	27	662.9503	0.45	662.9504	0.60	662.9505	0.75
11	PFtDA	712.9468	713	14	18	25	712.9474	0.84	712.9474	0.84	712.9475	0.98
12	PFhDA	812.9405	813	16	18	22	812.9415	1.23	812.9415	1.23	812.9416	1.35
13	PFoDA	912.9341	913	18	18	20	912.9357	1.75	912.9357	1.75	912.9357	1.75
14	PFBS	298.9424	299	4	18	60	298.9431	2.34	298.9432	2.68	298.9432	2.68
15	PFHxS	398.9361	399	6	18	45	398.9369	2.01	398.9369	2.01	398.9369	2.01

16	PFHpS	448.933	449	7	18	40	448.9338	1.78	448.9338	1.78	448.9338	1.78
17	PFOS	498.9298	499	8	18	36	498.9304	1.20	498.9305	1.40	498.9305	1.40
18	PFDS	598.9234	599	10	18	30	598.9236	0.33	598.9236	0.33	598.9237	0.50
19	4:2 FtS	326.9737	327	4	17	51	326.9744	2.14	326.9745	2.45	326.9746	2.75
20	6:2 FtS	426.9673	427	6	16	38	426.9682	2.11	426.9682	2.11	426.9682	2.11
21	8:2 FtS	526.9609	527	8	16	31	526.9616	1.33	526.9616	1.33	526.9617	1.52
22	6:2 FtTP	451.0038	451	6	17	38	451.0047	2.00	451.0047	2.00	451.0047	2.00
23	8:2 FtTP	550.9974	551	8					550.9954	-3.63	550.9978	0.73
24	6:2 FtTAoS	586.0392	586	6	17	28	586.0397	0.85	586.0396	0.68	586.0397	0.85
25	6:2 PAP	442.9717	443	6	17	37	442.9727	2.26	442.9727	2.26	442.9731	3.16
26	8:2 PAP	542.9655	543	8	17	31	542.966	0.92	542.9659	0.74	542.9658	0.55
27	6:2 diPAP	788.9745	789	6	17	21	788.9756	1.39	788.9757	1.52	788.9758	1.65
28	8:2 diPAP	988.962	989	8	16	17	988.9629	0.91	988.9629	0.91	988.9632	1.21
29	8:2FtCA	476.9784	477	8	17	35	476.9785	0.21	476.9792	1.68		
30	FOSA	497.9457	498	8	17	33	497.9464	1.41	497.9464	1.41	497.9465	1.61
31	FOSAA	555.9512	556	8	17	30	555.9517	0.90	555.9517	0.90	555.9518	1.08
32	MeFOSAA	569.9669	570	8	17	30	569.9671	0.35	569.9672	0.53	569.9672	0.53
33	False positive										468.9791	1.28

Error: mass error (ppm); False positives were defined as masses that were not included in the 32 listed standard compounds, but were recognized by the automated matching analysis using the prediction library.

Table S5. Annotated fragments of HR-nanoESI-MS/MS analysis based on in-silico fragment analysis using the software, ACD/MS fragmenter 2015 (Advanced Chemical Development, Toronto, ON, Canada) or the reference spectrum of 6:2 FtTP. The MS/MS spectra and the proposed fragment structures are shown in Figure S2.

	Theoretical exact mass	Observed accurate mass	Error		Proposed formula
			(ppm)	(mDa)	
m/z 451					
a	378.9832	378.9775	15.0	5.7	C8H4F13S-
b	358.9769	358.9767	0.6	0.2	C8H3F12S-
c	338.9707	338.9709	0.6	0.2	C8H2F11S-
d	318.9645	318.9612	10.4	3.3	C8HF10S-
e	298.9582	298.9556	8.7	2.6	C8F9S-
f	292.9830	292.9816	4.8	1.4	C7F11-
g	216.9894	216.9862	14.8	3.2	C7F7-
h	105.0016	104.9984	30.5	3.2	C3H5O2S-
m/z 522*					
a	378.9832	378.9694	36.4	13.8	C8H4F13S-
b	142.0510	142.0403	75.3	10.7	C6H8NO3-
c	98.0611	98.0541	71.4	7.0	C5H8NO-
m/z 523					
a	378.9832	378.9775	15.0	5.7	C8H4F13S-
b	318.9645	318.9612	10.3	3.3	C8HF10S-
c	298.9583	298.9556	8.7	2.6	C8F9S-
d	292.9830	292.9816	4.7	1.4	C7F11-
e	216.9894	216.9862	14.6	3.2	C7F7-
f	143.0350	143.0316	23.6	3.4	C6H7O4-
g	99.0452	99.0429	22.7	2.3	C5H7O2-
m/z 593					
a	378.9832	378.9779	14.0	5.3	C8H4F13S-
b	213.0881	213.0850	14.5	3.1	C9H13N2O4-
c	195.0775	195.0743	16.4	3.2	C9H11N2O3-
d	124.0404	124.0390	11.3	1.4	C6H6NO2-
m/z 602					
a	256.0319	256.0286	12.9	3.3	C7H14NO5S2-
b	206.0493	206.0477	7.8	1.6	C7H12NO4S-
c	152.0387	152.0341	30.3	4.6	C4H10NO3S-
m/z 467					
a	356.9612	356.9602	2.8	1	C8HF12S-
b	334.9593	334.9539	16.1	5.4	C8HF10OS-
c	314.9532	314.9485	14.9	4.7	C8F9OS-

d	266.9862	266.9836	9.7	2.6	C8F9-
e	120.9929	120.9935	5.0	0.6	C3H5O3S-

* Standard reference of 6:2FtTP (*m/z* 451) showed the same fragment with *m/z* at 378.9694.

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