# Supporting Information: Aerobic Biotransformation of Fluorotelomer Thioether Amido Sulfonate (Lodyne) in AFFF-Amended Microcosms

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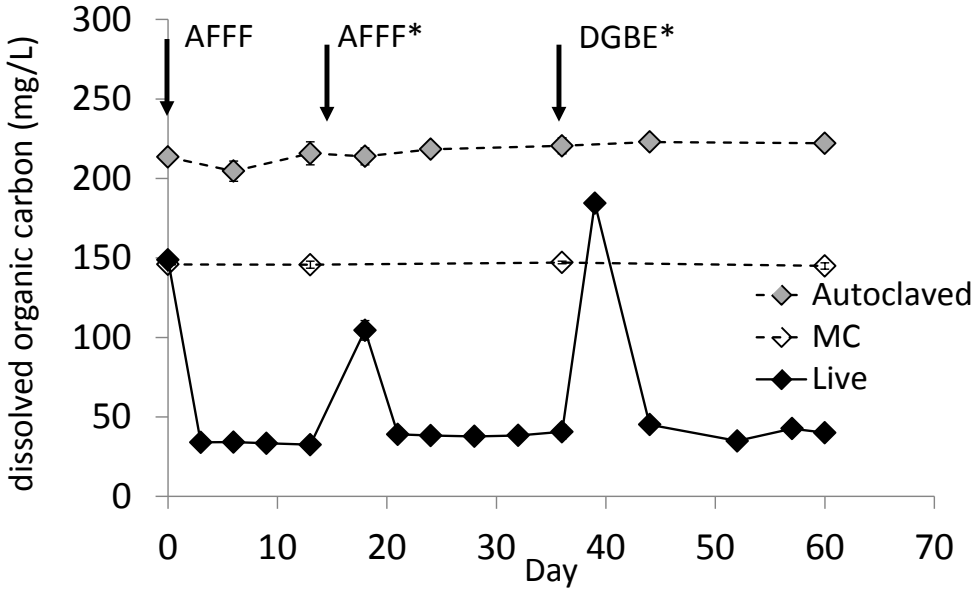


**Figure S1.** Dissolved organic carbon concentrations in microcosms. AFFF amendment on day zero was made to live, autoclaved, and medium control bottles, while the AFFF and DGBE amendments denoted with asterisks were made to live microcosms only. Error bars represent the standard deviation of triplicate microcosm bottles. MC is medium control.

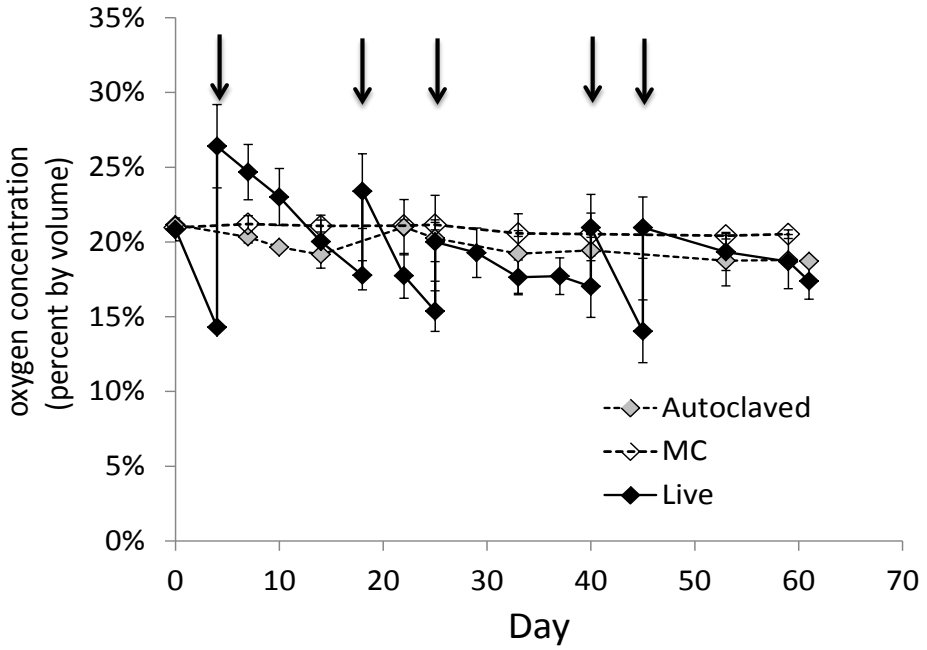


**Figure S2**. Headspace oxygen concentrations in microcosms. Oxygen amendment is denoted by an arrow and occurred in the live microcosms only. Error bars represent the standard deviation of triplicate microcosm bottles. MC is medium control.

**Table S1**. Chemical composition of Ansul AFFF, as specified in a representative MSDS sheet (left) and measured in the stock solution used in this study (right).

| **Ansul AFFF MSDS[1]** | | **Determined in this study** | |
|---|---|---|---|
| **Listed constituent** | **Proportion of ingredients** | **Detected Constituent[2]** | **Concentration in neat Ansul AFFF stock** |
| Diethylene glycol monobutyl ether | 17% | Diethylene glycol monobutyl ether | 220 g/L |
| "Proprietary mixture of hydrocarbon surfactants, fluorosurfactants, and inorganic salts not otherwise specified" | 5-10% | 4:2 FtTAoS | 0.0024 g/L (0.005 mM) |
| | | 6:2 FtTAoS | 15.2 g/L (25.9 mM) |
| | | 8:2 FtTAoS | 0.038 g/L (0.056 mM) |
| | | 6:2 FtSOAoS | *Not quantified* |
| 1-Propanol | 0.4% | *Not analyzed* | |
| Hexylene glycol | 0.5% | *Not analyzed* | |
| Water | 75-80% | *Not analyzed* | |

[1]A representative MSDS sheet was used to provide the information shown: Ansulite 3 % AFFF AFC-5-A Foam Concentrate MSDS Prepared by Wormald/Tyco International, Rydalmere, Australia, April 2008.

[2]All other fluorinated surfactants described in the text or in Houtz *et al.* 2013 were not detected in the Ansul AFFF formulation used for this study, including fluorotelomer thiohydroxy ammonium (6:2 $FtTHN^{+}$).

**Table S2**. Monitored ion transitions, MS conditions, and internal standard for 4:2, 6:2, and 8:2 FtS on an Agilent LC-MS/MS.

| Compound | Internal Standard | Molecular Ion | Fragmentor Voltage (V) | Quantifier Ion (m/z) | Collision Energy (V) | Qualifier Ion (m/z) | Collision Energy (V) | Polarity |
|---|---|---|---|---|---|---|---|---|
| 4:2 FtS | [$^{13}C_2$] 6:2 FtS | 327 | 135 | 307 | 20 | 81 | 20 | Negative |
| 6:2 FtS | [$^{13}C_2$] 6:2 FtS | 427 | 140 | 407 | 25 | 81 | 35 | Negative |
| 6:2 FtS | [$^{13}C_2$] 6:2 FtS | 527 | 140 | 507 | 30 | 81 | 40 | Negative |

**Table S3**. Solvent gradient program used for FtCA quantification on Simadzu Nexera X2 UHPLC / ABSciEX 5500 Triple Quad MS system.

| Time (min) | % MeOH |
|---|---|
| 0.1 | 30 |
| 1 | 50 |
| 3.5 | 80 |
| 4 | 90 |
| 5 | 90 |
| 6 | 30 |
| 7 | 30 |

**Table S4**. Monitored ion transitions, MS conditions, and internal standards for Shimadzu Nexera X2 UHPLC / ABSciEX 5500 Triple Quad MS system.

| Compound | Internal Standard | Molecular Ion | Decluster Potential (V) | Quantifier Ion (m/z) | Collision Energy (V) | Collision Cell Exit Potential (V) | Qualifier Ion (m/z) | Collision Energy (V) | Collision Cell Exit Potential (V) | Polarity |
|---|---|---|---|---|---|---|---|---|---|---|
| 5:3 FtCA (FPePA) | [$^{13}C_2$] 6:2 FtS | 341 | 50 | 237 | 20 | 15 | | | | Negative |
| 7:3 FtCA (FHpPA) | [$^{13}C_4$] PFOA | 441 | 55 | 337 | 30 | 25 | 317 | 18 | 13 | Negative |
| 6:2 FtCA (FHEA) | [$^{13}C_2$] 6:2 FtS | 377 | 35 | 293 | 22 | 25 | 137 | 12 | 15 | Negative |
| 8:2 FtCA (FOEA) | [$^{13}C_5$] PFNA | 477 | 60 | 393 | 20 | 15 | 217 | 28 | 15 | Negative |
| 6:2 FtUCA (FHUEA) | [$^{13}C_2$] 6:2 FtS | 357 | 40 | 293 | 16 | 11 | 121 | 50 | 13 | Negative |
| 8:2 FtUCA (FOUEA) | [$^{13}C_5$] PFNA | 457 | 40 | 393 | 20 | 15 | 343 | 52 | 31 | Negative |

**Quantification of 4:2 and 8:2 FtTAoS for which authentic standards were not available:**

For the quantification of 6:2 FtTAoS, a commercial source material was available. To quantify 4:2 and 8:2 FtTAoS, the raw instrument responses for the compounds were first normalized to the instrument response of the mass labeled-6:2 FtS internal standard. This response ratio was then applied to the calibration curve obtained for 6:2 FtTAoS. This is delineated in Table S5.

**Table S5**. Calibration parameters and quantification of FtTAoS on Agilent LC-MS/MS.

| Analyte | Calibration Range µg/L | Number of Calibration Points | $R^2$ | Internal Standard |
|---|---|---|---|---|
| 4:2 FtTAoS | *calculated using 6:2 FtTAoS calibration curve* | | | [$^{13}C_2$] 6:2 FtS |
| 6:2 FtTAoS | 1 – 24 | 5 | >0.95 | [$^{13}C_2$] 6:2 FtS |
| 8:2 FtTAoS | *calculated using 6:2 FtTAoS calibration curve* | | | [$^{13}C_2$] 6:2 FtS |

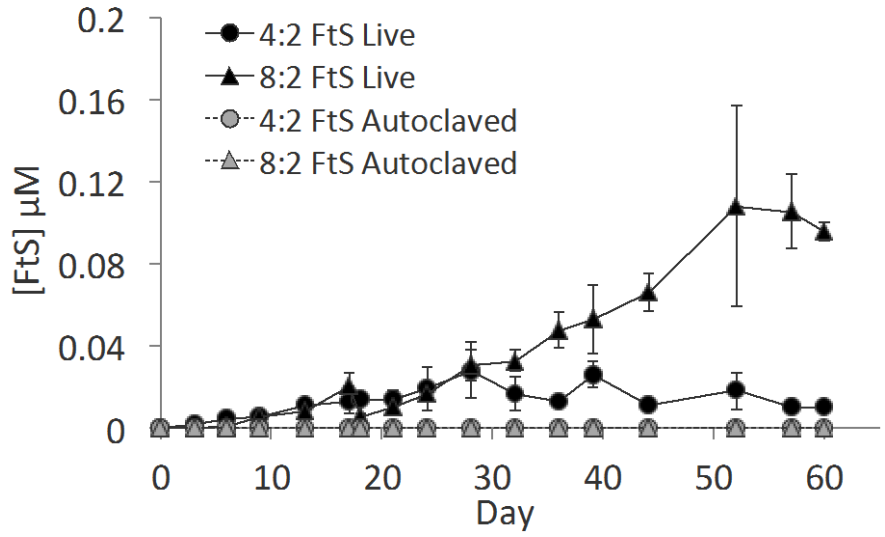


**Figure S3.** Concentrations of 4:2 and 8:2 FtS in microcosms. Error bars represent the standard deviation of triplicate microcosm bottles.

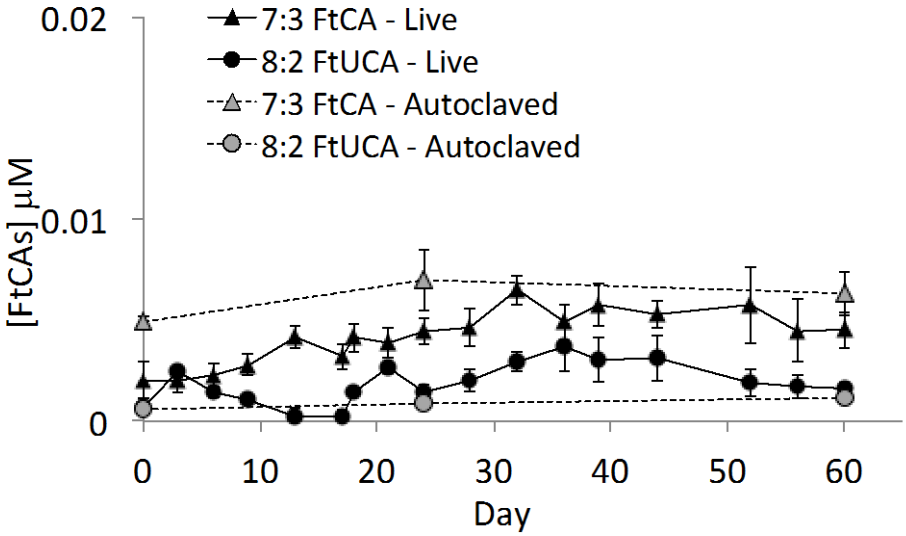


**Figure S4.** Concentrations of 7:3 FtCA and 8:2 FtUCA in microcosms. Error bars represent the standard deviation of triplicate microcosm bottles.

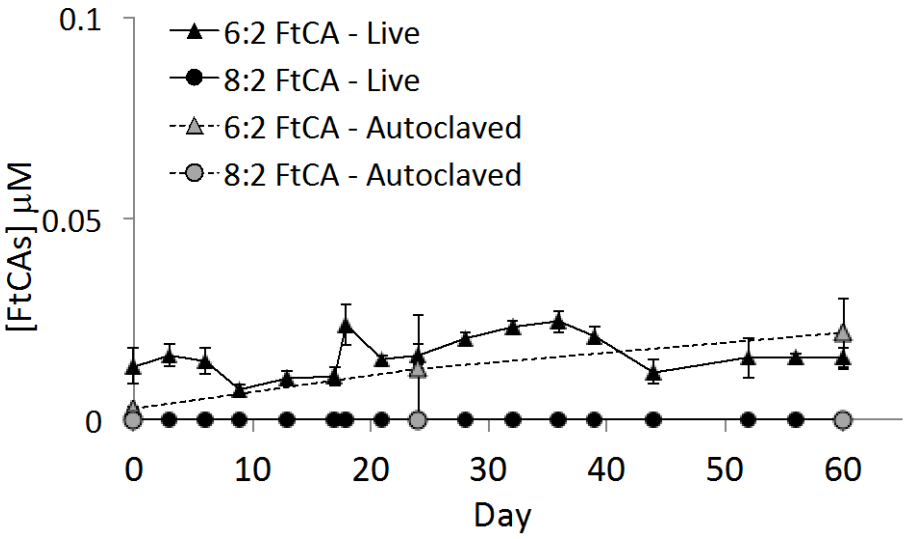


**Figure S5.** Concentrations of 6:2 FtCA and 8:2 FtCA in microcosms. Error bars represent the standard deviation of triplicate microcosm bottles.

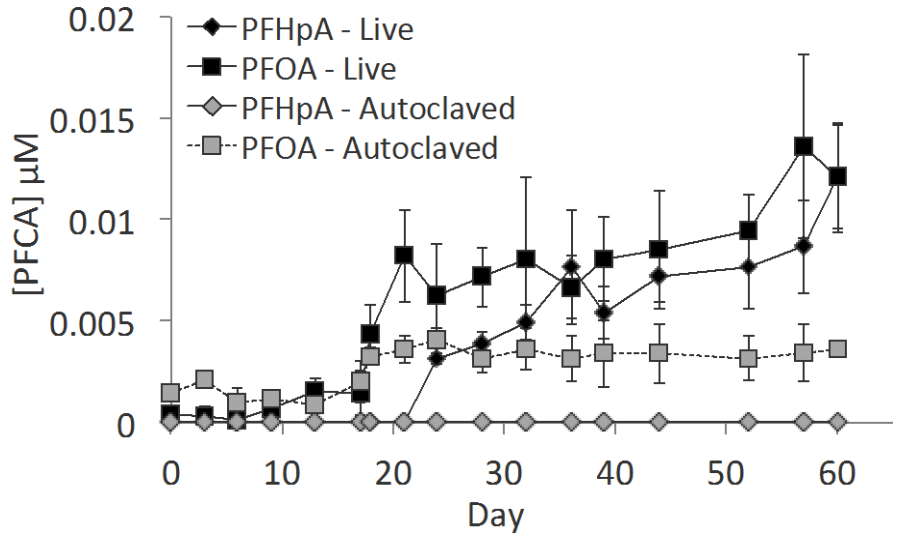


**Figure S6.** Concentrations of PFHpA and PFOA in microcosms. Error bars represent the standard deviation of triplicate microcosm bottles.

**Table S6**. Measured mass, theoretical mass, and mass accuracy of intermediate products identified by high resolution mass spectrometry. Retention time refers to the compound's LC-MS/MS elution time.

| Compound | m/z | Retention Time, minutes | Composition | Measured Mass | Theoretical Mass | Mass Accuracy, ppm |
|---|---|---|---|---|---|---|
| 6:2 $FtSO_2AoS$ | 618 | 8.1 | $C_{15}H_{17}O_6NF_{13}S_2$ | 618.0276 | 618.0295 | -3.11 |
| 6:2 FtSOAoS | 602 | 8.0 | $C_{15}H_{17}O_5NF_{13}S_2$ | 602.0329 | 602.0346 | -2.84 |
| 8:2 $FtSO_2AoS$ | 718 | 8.6 | *NA* | | | |
| 8:2 FtSOAoS | 702 | 8.5 | *NA* | | | |

*NA*: Masses were not confirmed with HRMS.

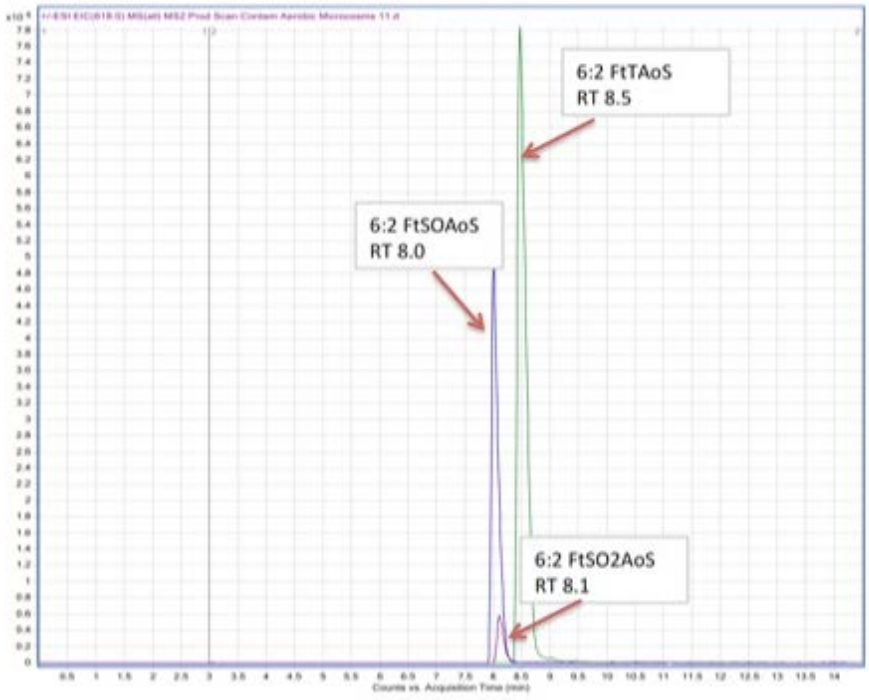


**Figure S7.** Chromatograph of 6:2 FtTAoS (m/z 586), 6:2 FtSOAoS (m/z 602), and 6:2 $FtSO_2AoS$ (m/z 618) molecular ions in a live microcosm.

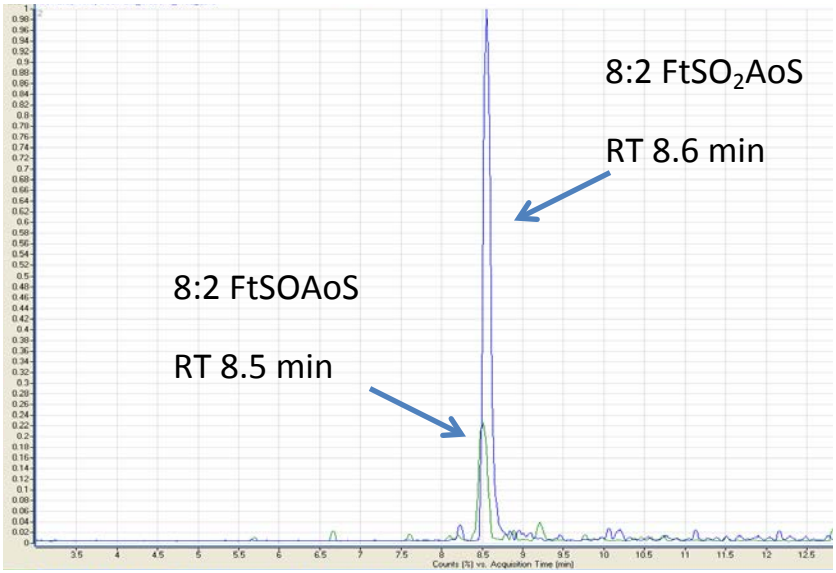


**Figure S8.** Chromatograph of 8:2 FtSOAoS (m/z 702), and 8:2 $FtSO_2AoS$ (m/z 718) molecular ions in a live microcosm on day 60 of the incubation. 8:2 FtTAoS (m/z 686) was not detected on day 60.

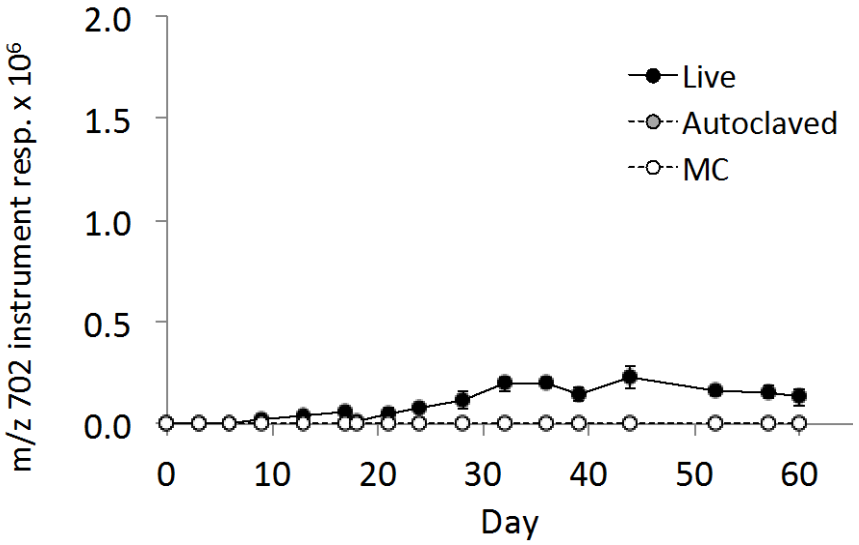


**Figure S9.** Average LC-MS/MS analyte response of molecular ion 702 at a retention time of 8.5 minutes, normalized to the response of the mass labeled-6:2 FtS internal standard. Error bars represent the standard deviation of triplicate bottles. MC is medium control.

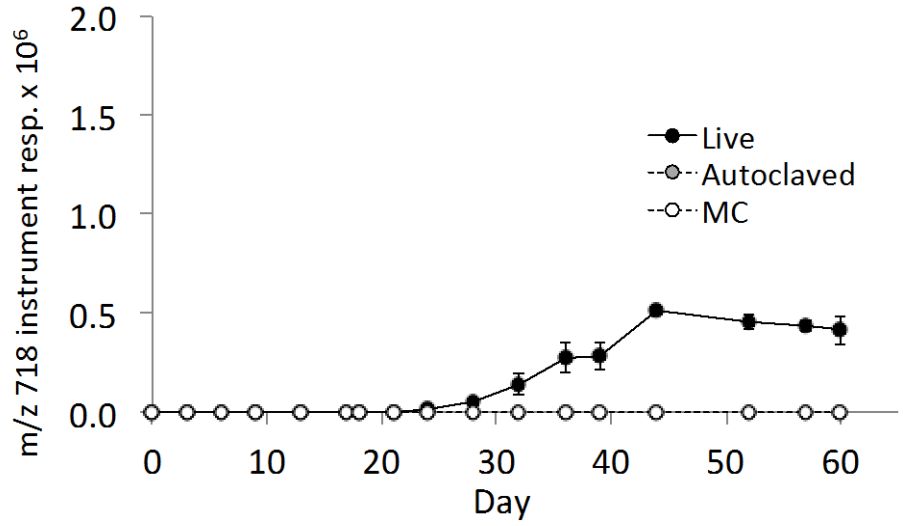


**Figure S10.** Average LC-MS/MS analyte response of molecular ion 718 at a retention time of 8.6 minutes, normalized to the response of the mass labeled-6:2 FtS internal standard. Error bars represent the standard deviation of triplicate bottles. MC is medium control.

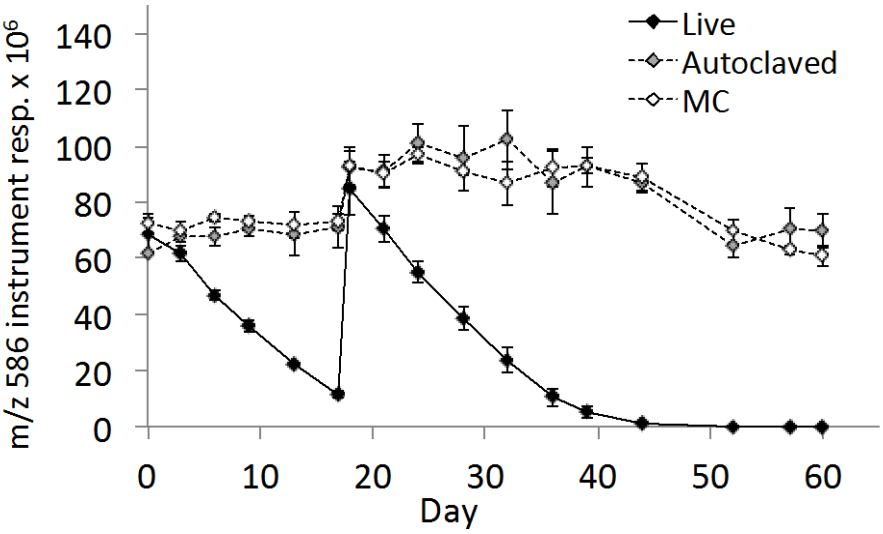


**Figure S11.** Average LC-MS/MS analyte response of molecular ion 586 (6:2 FtTAoS) at a retention time of 8.0 minutes, normalized to the response for the mass labeled-6:2 FtS internal standard. Error bars represent the standard deviation of triplicate bottles. MC is medium control.

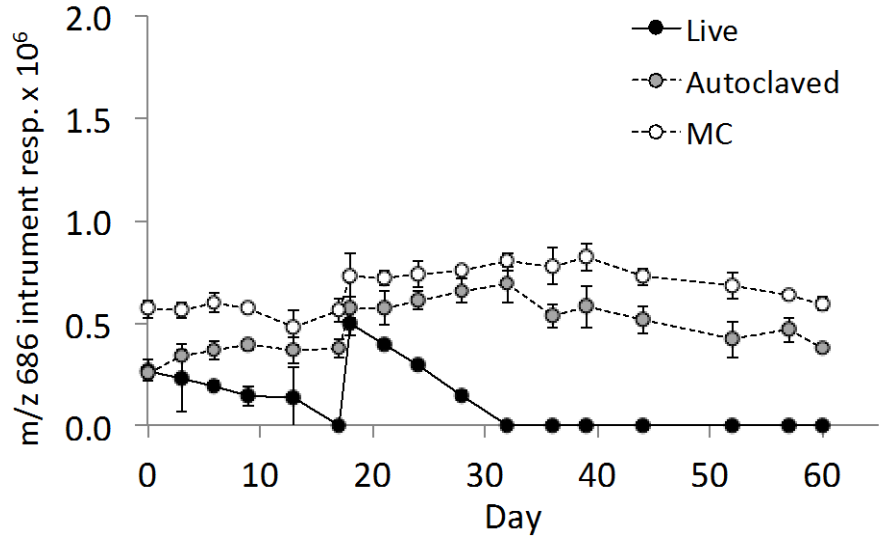


**Figure S12.** Average LC-MS/MS analyte response of molecular ion 686 (8:2 FtTAoS) at a retention time of 8.1 minutes, normalized to the response of the mass labeled-6:2 FtS internal standard. Error bars represent the standard deviation of triplicate bottles. MC is medium control.

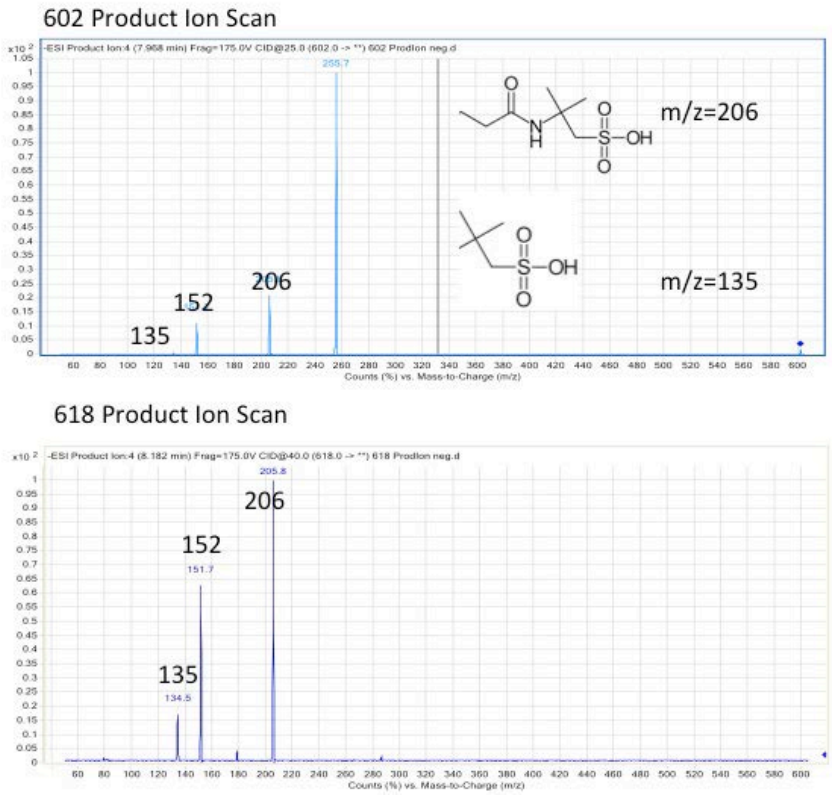


**Figure S13.** Product ion scans of m/z 602 (6:2 FtSOAoS) and 618 (6:2 $FtSO_2AoS$) and proposed structures of daughter ions.

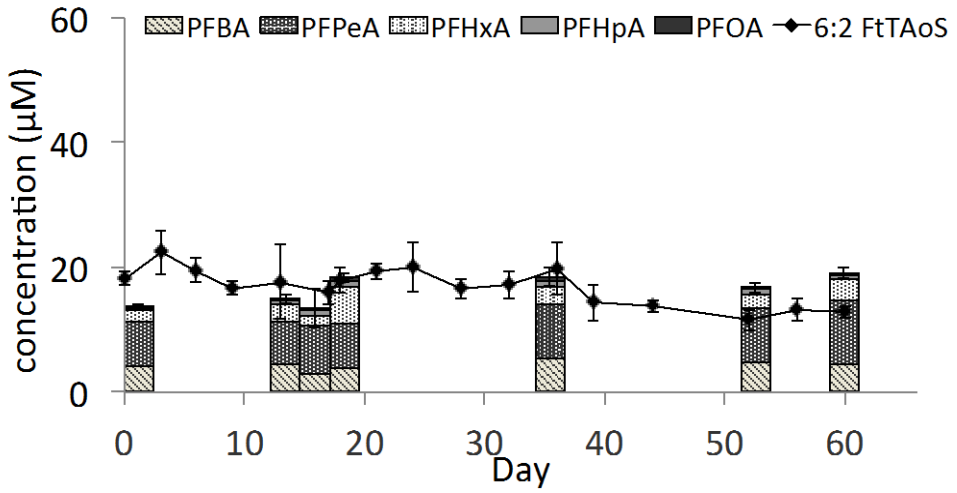


**Figure S14.** Concentration of 6:2 FtTAoS measured in the autoclaved microcosms and concentration of PFCA products measured in autoclaved microcosm samples after they were subjected to the total oxidizable precursor assay. PFNA was not detected.

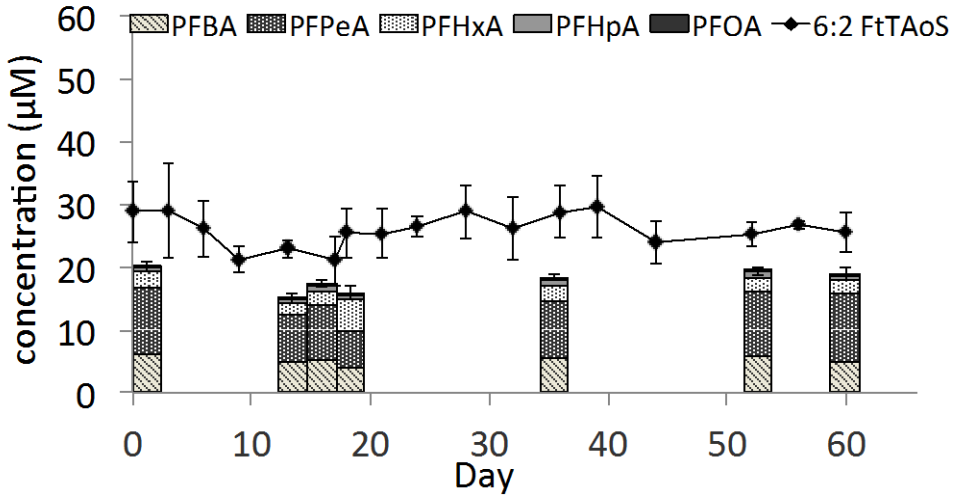


**Figure S15.** Concentration of 6:2 FtTAoS measured in the medium controls and concentration of PFCA products measured in medium control samples after they were subjected to the total oxidizable precursor assay. PFNA was not detected.