

Metatranscriptomic Insights into Aerobic Biotransformation of 6:2 Fluorotelomer Sulfonate by an Enrichment Culture under Sulfur-Limiting Conditions

Md G. Kabir,^{a,b} Zhiwen Tang,^a Xiqian Zhang,^a Frederic Coulon,^c Andreas Tiehm,^d Xin Song^{a,b*}

^a State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 211135, China.

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK

^dDepartment of Water Microbiology, TZW: DVGW-Technologiezentrum Wasser, Karlsruher Straße 84, 76139, Karlsruhe, Germany

*Corresponding author:

Xin Song, Email: xsong@issas.ac.cn, Tel: +86-25-86881543, Fax: +86-25-86881126,

Postal address: 298 Chuangyou Road, Nanjing 211135, China

ABSTRACT

6:2 fluorotelomer sulfonate (6:2 FTS), an alternative for PFOS, has become an environmental concern due to its toxicity. This study investigated the aerobic biotransformation of 6:2 FTS under sulfur-limiting conditions using an enrichment culture, SXC01. The enriched culture achieved $\geq 99.5\%$ degradation of 6:2 FTS at initial concentrations of 0.5, 2.5, and 5 mg/L within 14 days, with notable corresponding defluorination ratios of 77.2%, 28%, and 16.8%, respectively. Eleven transformation products were identified and quantified over time, and the accumulation of intermediate product 6:2 FTUCA suggested that its further degradation may represent a rate-limiting step. Furthermore, the production of PFHxA is more significant than that of PFBA, suggesting the transformation of 6:2 FTUCA via 5:2 sFTOH pathway is more dominant than the other proposed alternative pathway. Metatranscriptomic analysis revealed the upregulation of key genes associated with desulfonation (e.g., *ssuEADCB* cluster, *cysI*, *sbp*, *iscS*) and defluorination (e.g., *ladA*, *dehH*, *crcB*, *dhaA*), indicating a synergistic metabolic network driving 6:2 FTS biotransformation. Active genera identified included *Brucella*, *Rhodococcus*, and *Pseudoclavibacter*. Moreover, the predominant *Brucella anthropi* SX009 was successfully isolated and shown to completely degrade 6:2 FTS within 14 days. This study provides novel insights into the mechanisms of 6:2 FTS biotransformation.

KEYWORDS: PFAS, 6:2 FTS, Biodegradation, Biotransformation pathway, Defluorination, Upregulated gene, Metatranscriptomic analysis

Synopsis

This study applied an integrated approach using an enrichment culture and metatranscriptomic analysis to investigate the 6:2 FTS biotransformation mechanism, revealing key pathways and potential functional genes involved in desulfonation and defluorination.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic organofluoride chemicals where all (per-) or nearly all (poly-) carbon atoms are fluorinated, except the terminal functional group.¹ 6:2 Fluorotelomer sulfonate (6:2 FTS) is a poly-fluoroalkyl substance composed of a six-carbon fluorotelomer chain in which alpha and beta carbons are hydrogenated. It is increasingly used as a replacement for traditional PFAS compounds, such as perfluorooctanoic acid (PFOA), Perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS), particularly in aqueous film-forming foam (AFFF) products.² 6:2 FTS is a significant component of AFFF products,³ frequently applied for controlling hydrocarbon-based fires in airports and military training bases.⁴ In addition, 6:2 FTS serves as a repellent and surfactant in paints, coatings, adhesives, waxes, and polishes that are used on surfaces such as paper, metal, wood, textiles, and leather.⁵ Consequently, 6:2 FTS has been extensively found at AFFF and industry-impacted sites, as well as in adjacent terrestrial and aquatic ecosystems.⁶ For example, 6:2 FTS concentration of 2,101 µg/kg was reported in soil near Flesland Airport's fire-drill training area,⁷ and 14,600 µg/L in groundwater was detected at Wurtsmith Air Force Base.⁸

For the effective removal of 6:2 FTS from aqueous solutions, different physical and chemical approaches have been demonstrated, such as nanofiltration,⁹ UV irradiation,¹⁰ electrochemical oxidation,^{11,12} sonolysis¹³ and other advanced oxidation processes.^{14,15} However, these approaches are expensive and may be challenging to apply under field conditions. In contrast, bioremediation, which proved to be cost-effective, environmentally friendly, and sustainable, has been explored for the degradation of 6:2 FTS. Previous studies have investigated the biodegradation of 6:2 FTS using pure bacterial strains (e.g., *Dietzia aurantiaca* J3, *Rhodococcus jostii* RHA1, and *Gordonia*

sp. NB4-1Y),^{16–18} and mixed microbial communities from activated sludge,¹⁹ river sediment,²⁰ and landfill leachate.²¹ Major stable biotransformation products of 6:2 FTS, polyfluorinated compounds such as 5:3 fluorotelomer carboxylic acid (5:3 FTCA), 6:2 fluorotelomer carboxylic acid (6:2 FTCA), and short-chain perfluoroalkyl carboxylates, such as perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), and perfluorobutanoic acid (PFBA), were reported. However, these products have different molar yields and detection frequencies.^{18–22} Yet there is still a major data gap in the 6:2 FTS biodegradation pathway, and intermediate production profiles over time may provide further insight for the exploration of 6:2 FTS biotransformation pathways.

The availability of sulfur, an essential element for cell growth,¹⁸ is crucial for bacterial growth prior to the desulfonation of 6:2 FTS breakdown, which can provide additional sulfur in the microcosm. Bacteria may induce expression of alkanesulfonate monooxygenase to obtain sulfur from organosulfur compounds for biosynthetic processes,²³ whereas its expression may be suppressed in the presence of readily assimilable alternative sulfur sources.¹⁸ It has been reported that aerobic biotransformation of 6:2 FTS by pure culture of *Gordonia* sp. NB4-1Y and *Pseudomonas* sp. D2 only occurs when these organisms are cultivated under sulfur-starvation conditions,^{17,24} highlighting the importance of sulfur-starvation conditions for initiating 6:2 FTS biotransformation.²⁵ The desulfonation of 6:2 FTS was facilitated by alkanesulfonate monooxygenase,^{16–18,25} and an FMNH₂-dependent monooxygenase enzyme was recognized for its ability to cleave the carbon-sulfur bond by alkanesulfonates.²⁶ To date, the enzymes responsible for the desulfonation of 6:2 FTS remain unknown and unidentified.

Moreover, microbial defluorination of 6:2 FTS requires the cleavage of carbon-fluorine bond, a process that can be initiated by the defluorination enzymes. Dehalogenases and oxygenases are capable of removing halogens from halogenated aliphatic compounds. For instance, haloacid dehalogenase can degrade a broad spectrum of chlorinated, brominated, and fluorinated compounds,^{27–29} and cytochrome P450 monooxygenase can transform fluorinated drugs.^{30,31} Previous studies have demonstrated that butane monooxygenase and alkane monooxygenase, expressed by *Pseudomonas oleovorans* and *P. butanovora*, can defluorinate 6:2 polyfluoroalkyl phosphates (6:2 PAPs) as well as 4:2, 6:2, and 8:2 fluorotelomer alcohol (FTOHs).^{32,33} Further research is required to understand the role of dehalogenases and oxygenases in the biotransformation of 6:2 FTS.

In this study, we hypothesized that an acclimatized aerobic microbial community can perform effective biotransformation of 6:2 FTS under sulfur-limiting conditions. The objectives of this study are to (1) evaluate the 6:2 FTS biodefluorination efficiency, (2) elucidate the biodegradation pathways and identify the rate-limiting step in the metabolic processes, and (3) identify potential functional genes responsible for 6:2 FTS desulfonation and defluorination through metatranscriptomics analysis. This study represents the first investigation of 6:2 FTS biotransformation by an acclimatized enrichment culture under sulfur-limiting conditions, incorporating a metatranscriptomic approach, which may provide a potential pathway for mitigating PFAS-contaminated environments in the future.

2. Materials and Methods

2.1. Soil samples and chemicals

Soil samples were collected from a pesticide manufacturing site with a production history of over 16 years, at the depth of 1–5 m below ground surface (bgs) using a drilling rig (SM200A, Shangming, China). The total PFASs concentrations of 13 PFASs compounds ($\Sigma 13$ PFASs) range from 11.21 to 81.86 ng/g dw, and the detected 6:2 FTS concentration was in the range of 0.07 to 0.135 ng/g dw in soil. It was assumed that the microbial consortia at the site would evolve with the potential for 6:2 FTS biodegradation due to long-term exposure to PFASs. The collected soil sample were stored at 4°C before conducting the biodegradation study.

The standard chemical of 1H, 1H, 2H, 2H-Tridecafluorooctane-1-sulfonic acid (6:2 FTS) ($C_8H_5F_{13}O_3S$, CAS: 27619-97-2, 98%, Macklin) were purchased from Sigma-Aldrich (St. Louis, USA) and prepared as a 1000 mg/L stock solution using ultrapure water (18.2 M Ω ·cm) from the Milli-Q synthesis A10 system (Millipore, USA). Sulfur-free acetate (SFA) medium^{16,17} consists of KH_2PO_4 , K_2HPO_4 , NH_4Cl , $NaCH_3COO$, $MgCl_2$, $CaCl_2$, $2H_2O$, the Wolin vitamin mix, and sulfur-free trace elements.^{16,17} Detailed media compositions are provided in Supplementary Information (SI), Text S1. All chemicals were analytical grade or higher and were purchased from Aladdin (China).

2.2. Acclimatization of enrichment culture

Five grams of soil sample were enriched in 100-mL Erlenmeyer flasks containing 50 mL of SFA medium, initially spiked with 1 mg/L of 6:2 FTS as the sole sulfur source. To maintain aerobic conditions and minimize volatilization, flasks were covered with a hydrophobic PTFE membrane film. Enrichments were incubated at 30°C and shaking

at 160 rpm, and 2% transfer (v/v) of the culture was conducted every 14 days. Enrichment culture were successively transferred to freshly prepared SFA medium with stepwise increases in 6:2 FTS concentrations at 3, 5, 10, to 25 mg/L, where 6:2 FTS served as the sole sulfur source. Transfers were made once at each concentration prior to being increased to the next level.

The degradation of spiked 6:2 FTS was monitored using LC-MS/MS, while fluoride release was measured at regular intervals using an ion-selective electrode. Details of the enrichment process and consortium stability are provided in Text S2 and Fig. S1. The resulting enrichment culture, designated as SXC01, utilized 6:2 FTS as the sole sulfur source, but it did not support the growth when 6:2 FTS was used as the sole carbon source (Fig. S2). We further evaluated the consortium growth in SFA medium supplemented with 6:2 FTS (5 and 25 mg/L), with or without MgSO₄ (0.165 g/L, replacing MgCl₂ in the original medium). The results demonstrated that 6:2 FTS could serve as a sulfur source (Fig. S3). Notably, the addition of MgSO₄ enhanced growth at both concentrations of 6:2 FTS, suggesting that the culture growth remained under sulfur-limited conditions and 6:2 FTS did not exert inhibitory effects on culture growth.

To prepare SXC01 inoculum, the consortium was cultivated in SFA medium supplemented with MgSO₄ as the sulfur source. After cultivation, 50 mL cultures were centrifuged at 11,000 rpm for 14 minutes. The resulting cell pellets were rinsed three times with 50 mL phosphate-buffered saline (PBS), then resuspended in PBS to an optical density at 600 nm (OD₆₀₀) of 1.0 ± 0.1 , and used as inoculum for subsequent experiments. Growth of the enrichment culture was monitored by measuring OD₆₀₀, as presented in Fig. S4.

2.3. Biotransformation experiments of 6:2 FTS under sulfur-limiting conditions

Aerobic biotransformation experiments were conducted in 150-mL Erlenmeyer flasks filled with 80 mL of SFA medium. Inoculation of the consortium with different concentrations of 6:2 FTS as the sole sulfur source was labelled as biodegradation, whereas without 6:2 FTS, it was labeled as blank. Corresponding sterile controls were prepared with 6:2 FTS in the same manner but not inoculated with the consortium. All treatments were set up in triplicate. Details of experimental conditions are presented in Table S1. Four different concentrations of 6:2 FTS 0.5 mg/L (1.17 μ M), 2.5 mg/L (5.84 μ M), 5 mg/L (11.68 μ M), and 25 mg/L (58.39 μ M) were used in this study. Corresponding earlier research on PFAS biotransformation, e.g., 0.1 mg/L, 1.0 mg/L, 10 mg/L, 100 mg/L, and 200 mg/L of PFOA/PFOS by *Acidimicrobium* sp. A6,³⁴ 15.2 mg/L of 6:2 FtTAoS, 0.04 mg/L of 8:2 FtTAoS, and 0.003 mg/L of 4:2 FtTAoS in AFFF at Ellsworth Air Force Base,³⁵ 25.69 mg/L of 6:2 FTS by *Gordonia* NB4-1Y,²⁵ 20 mg/L of 6:2 FTS by *Rhodococcus jostii* RHA1,¹⁸ and 1.3 mg/L of 6:2 FTS in Brandywine river sediment at Pennsylvania,²⁰ supporting the relevance of the concentration in our study. The experimental flasks were incubated in an orbital shaker at 30 °C and 160 rpm for 28 days. Aliquots of 3.0 mL samples were collected at 0, 3, 7, 14, 21, and 28 days of incubation, centrifuged, filtered, and stored at -20 °C for further analysis.

2.4. Analytical methods

2.4.1. Analysis of 6:2 FTS and its biotransformation products

Samples were extracted using the method described by MacKay et al.³⁶ with minor modifications. Briefly, the samples were processed using a solvent mixture comprising 50% methanol, 30% acetonitrile, and 20% water and centrifuged at 16,000 $\times$ g for 14

minutes at 4 °C. The resulting supernatants were filtered through 0.22 µm polypropylene sterilized syringe membranes, transferred to polypropylene (PP) HPLC tubes, and stored at -20 °C until LC-MS analysis.

The concentrations of 6:2 FTS were quantified using ultra-high-performance liquid chromatography coupled with a triple-quadrupole mass spectrometer (LC-MS/MS AB 5500 Sciex, Framingham, MA, USA). The target biotransformation products of 6:2 FTS analytes are summarized in Table S2. The target and non-target intermediate products were identified using LC-MS/MS AB 5500 and liquid chromatography quadrupole time-of-flight mass spectrometer LC-QTOF-MS, X500R (Sciex, Framingham, MA, USA). The instruments were operated in a multiple reaction monitoring mode (MRM) using negative electrospray ionization (ESI). The transformation products were quantified using LC-MS/MS AB 5500. For the analysis, an ACQUITY UPLC BEH C18 analytical column (100 mm × 3.0 mm, 1.7 µm, Waters, MA, USA) was employed to separate 3 µL of the injected diluted sample. Gradients of mobile phase A (2 mM of ammonium acetate) and mobile phase B (acetonitrile) were used for sample separation. The solvent gradient, MRM conditions, and instrumental parameters used in the targeted method are detailed in Tables S3 and S4. Limits of detection (LOD) for the targeted PFASs were determined based on a signal-to-noise (S/N) ratio of 3 and are provided in Table S5.

Data acquisition and processing for targeted and non-targeted analysis were performed using SCIEX OS software (version 1.5). The extracted ion chromatogram (XIC) library was used for non-target product screening. The following parameters were set, such as mass error (≤ 5 ppm), mass range (100-400 m/z), retention time (RT) (0-12 min), and peak area ($\geq 10^4$) during the non-target analysis.

2.4.2. Fluoride determination

Concentrations of fluoride ions in the liquid sample were determined by an ion-selective electrode (ISE, Sanxin). The ISE was calibrated before each measurement following the manufacturer's instructions. For the sample determination, samples were diluted with an equal volume of total ionic strength adjustment buffer (TISAB) to ensure constant ionic strength throughout the measurements. The detection limit of fluoride ions was 0.025 mg/L. The defluorination ratio of 6:2 FTS was calculated as follows:

Defluorination ratio (%)

$$= \frac{\text{Maximum fluoride formation}}{6:2 \text{ FTS conc. in control} \times \text{number of F in one molecule}} \times 100 \%$$

2.5. Metatranscriptomic analysis

Metatranscriptomic sequencing analysis was employed to investigate the active microbial community and functional gene expression profiles during 6:2 FTS biotransformation. Microbial samples were collected from a duplicate biodegradation experiment using 5 mg/L of 6:2 FTS, where 6:2 FTS biodegradation and fluoride release closely mirrored those observed in the main study. 10 mL of samples were collected in triplicate on Days 0, 7, and 14. Samples from Days 7 and 14 represented the active transformation phase of 6:2 FTS, while Day 0 was used as the control. However, the Day 14 sample did not pass the RNA quality control and was therefore excluded from sequencing.

Collected samples were filtered through 0.22 μm sterilized membrane filters and immediately transported to MajorBio (Shanghai, China) for total RNA extraction, rRNA removal, and library preparation. RNA was extracted and purified using a Soil RNA Extraction Kit (Majorbio, Shanghai, China), following the manufacturer's

protocol. RNA integrity and concentration were assessed using an Agilent 5300 Bioanalyzer and a Qubit 2.0 Fluorometer. Library construction was performed using the Illumina Stranded mRNA Prep Kit, with rRNA depletion conducted using the RiboCop Kit, followed by sequencing on an Illumina NovaSeq Xplus platform.

Raw reads were quality-filtered using fastp,³⁷ and rRNA sequences were removed by aligning reads to the SILVA database via SortMeRNA.³⁸ De novo transcriptome assembly was performed with Trinity,³⁹ and redundant sequences were clustered with CD-HIT⁴⁰ to generate a non-redundant Unigene set. Open reading frames (ORFs) were predicted using TransGeneScan.⁴¹ Gene expression levels were quantified using RSEM. Differentially expressed genes (DEGs) were identified using DESeq2⁴² (log2-fold change ≥ 2 , P-adjust < 0.05). Taxonomic annotation was performed against the NCBI NR database using DIAMOND,⁴³ while functional annotation was conducted using the KEGG database. Further details of the metatranscriptomic analysis are provided in Text S3. The raw sequencing dataset has been deposited in the NCBI database using the BioProject accession number PRJNA1176362. This integrative approach provided a robust framework for elucidating the functional potential of microbial communities, particularly regarding their roles in biotransformation, desulfonation, and defluorination pathways. It is also worth noting that the analysis focused on the bacterial community only, and other domains were not considered in the current study.

2.6. Identification and evaluation of 6:2 FTS degradation potential of pure bacterial cultures

Post the metatranscriptomic sequencing analysis, single bacterial strains were isolated from SXC01 using the dilution plate technique. The enrichment culture was diluted and plated out on LB agar media, and individual bacterial colonies showing distinct

morphological characteristics on agar media were isolated and purified by performing three successive streaks on the same medium. A pure bacterial strain of *Brucella anthropi* SX009 was isolated through 16S rRNA gene sequencing by the Sanger method. DNA extraction and sequencing were performed by MajorBio (Shanghai, China). Genomic DNA was extracted using the Fast DNA Spin Kit (MP Biomedicals, USA) following the manufacturer's protocol. The 16S rRNA gene was amplified using bacterial primers 27F: AGAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACTT. The purified PCR products were sequenced, analyzed through BLAST on NCBI, and deposited in the NCBI database under GenBank accession number PQ357575.1. A phylogenetic tree was constructed using MEGA 11 to determine the sequence similarity of SX009 with closely related species. The sequences were aligned using Muscle, and the tree was constructed with 1,000 bootstrap replicates using the Neighbor-Joining method.

6:2 FTS biodegradation by pure bacterial strains was tested using the same process described in 2.3. The inoculation of pure bacterial strain with 6:2 FTS (5 mg/L) as the sole sulfur source was marked as biodegradation, whereas without 6:2 FTS, it was labeled as blank. The sterile controls were prepared with 6:2 FTS in the same manner, but not inoculated with the bacteria. All treatments were set up in triplicate. The experimental flasks were incubated in an orbital shaker at 30 °C and 160 rpm for 28 days. Aliquots of 2.0 mL samples were collected at 0, 3, 7, 14, 21, and 28 days of incubation, centrifuged, filtered (0.22 µm PP sterilized syringe membrane), and tested for degradation and defluorination. The growth of the strain was monitored via OD₆₀₀. The growth curve analysis of *Brucella anthropi* SX009 in the SFA medium (Fig. S5) showed doubling times of 51 hours with 6:2 FTS and 141 hours without 6:2 FTS, likely due to the absence of an accessible sulfur source, which might limit the growth.

2.7. Data analysis

All samples were analyzed in triplicate, and results are presented as the mean $\pm$ standard error. Experimental data were processed using Microsoft Office 2019 (Microsoft Co., USA), and plotting was performed using OriginPro 2024 (OriginLab, MA, USA).

3. Results and Discussion

3.1. Degradation and defluorination of 6:2 FTS under sulfur-limiting conditions

The decay in 6:2 FTS concentrations and the corresponding defluorination ratio by the consortium under sulfur-limiting conditions were evaluated across four different initial concentrations (0.5, 2.5, 5, and 25 mg/L) over 28 days (Fig. 1 and Fig. S6). The degradation efficiencies of 6:2 FTS observed were 97.2%, 97.1%, and 75.5%, respectively, at the initial concentrations of 0.5, 2.5, and 5 mg/L within the first 7 days. The degradation efficiencies increased significantly, exceeding 99.5% across all three concentrations by day 14. The 6:2 FTS degradation efficiency was 31.5% at the initial concentration of 25 mg/L, much lower than those observed at lower initial concentrations. The slower degradation observed at the highest concentration may be attributed to the potential toxicity of fluoride ions released during C–F bond cleavage. Fluoride can inhibit microbial activity by interfering with essential enzymatic functions, thereby limiting further biodegradation of PFAS and other organofluoride compounds.^{44–46} In contrast, almost no degradation occurred in the control where consortium was absent (Fig. S6).

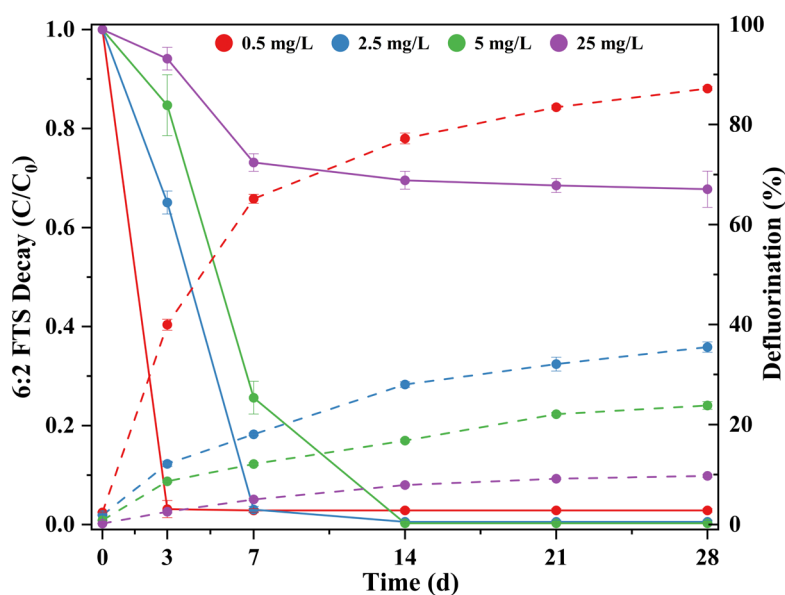


Fig. 1. Degradation efficiency and defluorination ratio of 6:2 FTS at different concentrations in the microcosm by consortium SXC01. The degradation efficiency of 6:2 FTS is represented with solid lines, and the percent defluorination data are represented with dashed lines. Error bars represent the standard error of triplicate.

The half-lives were 1.5, 4.0, and 5.1 days for the near-complete degradation of 6:2 FTS observed at initial concentrations of 0.5, 2.5, and 5.0 mg/L after 3, 7, and 14 days of incubation. The degradation rates observed in the current study are much higher than those reported for microbial consortium cultures in previous studies, where several months were required to degrade 6:2 FTS (Table S6). For example, a consortium obtained from AFFF-impacted soil could completely degrade 6:2 FTS at 1.7 mg/L after 224 days.⁴⁷ Examples of consortia enriched from landfill leachate achieved 21% of 6:2 FTS degradation at 0.65 mg/L after 60 days,⁵ 98.1% of 6:2 FTS at 1.3 mg/L after 90 days,²⁰ and 36.3% of 6:2 FTS at 2.6 mg/L after 90 days.¹⁹ It has been reported that the availability of alternative sulfur sources, such as inorganic sulfur rather than organosulfur in sludge, sediment, or soil may affect the degradation rates of 6:2 FTS in mixed consortia.²⁵ The metabolism of 6:2 FTS may be biologically favored, when

readily assimilated inorganic sulfur sources are absent in the medium.⁴⁸ Microbes preferentially utilize organosulfur compounds in the absence of alternative sulfur sources, as demonstrated in *Gordonia* sp. NB4-1Y when grown with 6:2 FTS.⁴⁹ We speculate in the current study that the absence of readily available inorganic sulfur sources has contributed to the higher 6:2 FTS degradation rates observed compared to those reported in previous studies. Bacteria may have evolved degradation activity as an adaptive response to prolonged contaminant exposure and nutrient-limiting conditions, where the contaminant may serve as essential elements and energy.⁵⁰ Such adaptation likely conferred a selective advantage by enabling access to essential nutrients.

The generation of fluoride ions is one of the most important evidence of the biodegradation of PFAS compounds.^{34,51} In consortium-treated biodegradation groups, a noticeable increase in fluoride ion generation was observed over time, reflecting effective defluorination of 6:2 FTS. At lower initial concentrations of 0.5, 2.5, and 5 mg/L, the defluorination ratios at day 14 were 77.2%, 28% and 16.8%, respectively, increasing to 87.2%, 35.5%, and 23.8% by day 28. In contrast, at 25 mg/L, the defluorination ratio was 7.9% at day 14, which slightly increased to 9.7% by day 28 (Fig. 1). No F⁻ generation was detected in the corresponding sterile control, where consortium was not inoculated (data not shown).^{33,48} It was reported that *Dietzia aurantiaca* J3 pure strain degraded 6:2 FTS with a defluorination efficiency of 22% in aerobic conditions.¹⁶ The much higher defluorination ratios observed in our study (Fig. 1) provide another line of evidence for the much higher biodegradation efficiency of 6:2 FTS. The increasing fluoride release and loss of 6:2 FTS over time in the biodegradation group, compared to abiotic controls, support that defluorination was

biologically mediated. Therefore, our findings provide further evidence supporting the biodefluorination of 6:2 FTS.

3.2. Intermediate product identification, proposed pathway and formation profiles of intermediate products

3.2.1. Intermediate product identification

Nine biotransformation products from the 6:2 FTS biodegradation group were identified and quantified, including five polyfluorinated compounds, namely 5:3 FTCA, 5:3 fluorotelomer unsaturated carboxylic acid (5:3 FTUCA), 6:2 FTCA, 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), 6:2 FTOH, as well as four end-products comprising PFBA, PFPeA, PFHxA, and PFHpA (Fig. S7-S8). Moreover, two transformation products, 6:2 fluorotelomer aldehyde (6:2 FTAL) and 4:2 fluorotelomer carboxylic acid (4:2 FTCA), were putatively identified through target analysis using LC-QTOF (Table S7 and Fig. S9), as a biotransformation product of 6:2 FTS by the consortium. However, structural confirmation via a reference standard or derivatization⁵² was not possible due to the unavailability of commercial standards and the known instability of FTALs in an aqueous solution. The consistent detection of a signal corresponding to the expected $[M-H]^-$ ion in the biodegradation group and absent in the control supports its tentative identification. For the non-targeted analysis of 6:2 FTS, ten (10) additional potential biotransformation metabolites were screened and tentatively identified using LC-QTOF (Table S8). None of these biotransformation compounds were detected in the control group (Fig. S8), confirming that the transformation products observed were a result of the aerobic biotransformation of the consortium. These findings provide additional evidence supporting the aerobic

degradation pathway of 6:2 FTS and the diverse transformation products formed in this process.

3.2.2. Proposed 6:2 FTS biodegradation pathway

Based on the identified biotransformation products using LC-MS/MS and QTOF analyses, also taking into consideration of the pathways previously reported,^{18,20,25,47} the biotransformation pathway of 6:2 FTS was proposed under sulfur-limiting aerobic conditions (Fig. 2A). The initial biotransformation step involves microbial desulfonation, where the sulfonate group is removed from 6:2 FTS. This reaction is likely catalyzed by monooxygenases, leading to the formation of 6:2 FTOH. Subsequently, 6:2 FTOH undergoes oxidation to 6:2 FTAL via alcohol dehydrogenase, which is further oxidized to 6:2 FTCA by aldehyde dehydrogenase.^{17,21,25,47,53} In this study, the formation of PFHpA was observed, which may result from the microbial α -oxidation of 6:2 FTCA, consistent with findings in earlier studies.^{18,20,47}

It was proposed that a dehydrohalogenation reaction occurs, involving the elimination of a hydrogen fluoride bond from 6:2 FTCA, which results in the formation of 6:2 FTUCA. Similar patterns of 6:2 FTS biotransformation have been reported previously, where 6:2 FTOH is converted to 6:2 FTCA and subsequently to 6:2 FTUCA.^{18,47,54} It was proposed that FTUCA is the key branching point for further biotransformation. In the transformation pathway, the defluorination of 6:2 FTUCA occurs, likely catalyzed by reductive dehalogenase, and produces 5:3 FTUCA, which subsequently transforms into 5:3 FTCA. Similar reductive defluorination processes have been reported in previous studies.^{47,55} 5:3 FTCA then undergoes further transformation into 4:2 FTCA and short-chain PFAS, eventually leading to PFBA formation through one-carbon removal pathways.⁵⁶

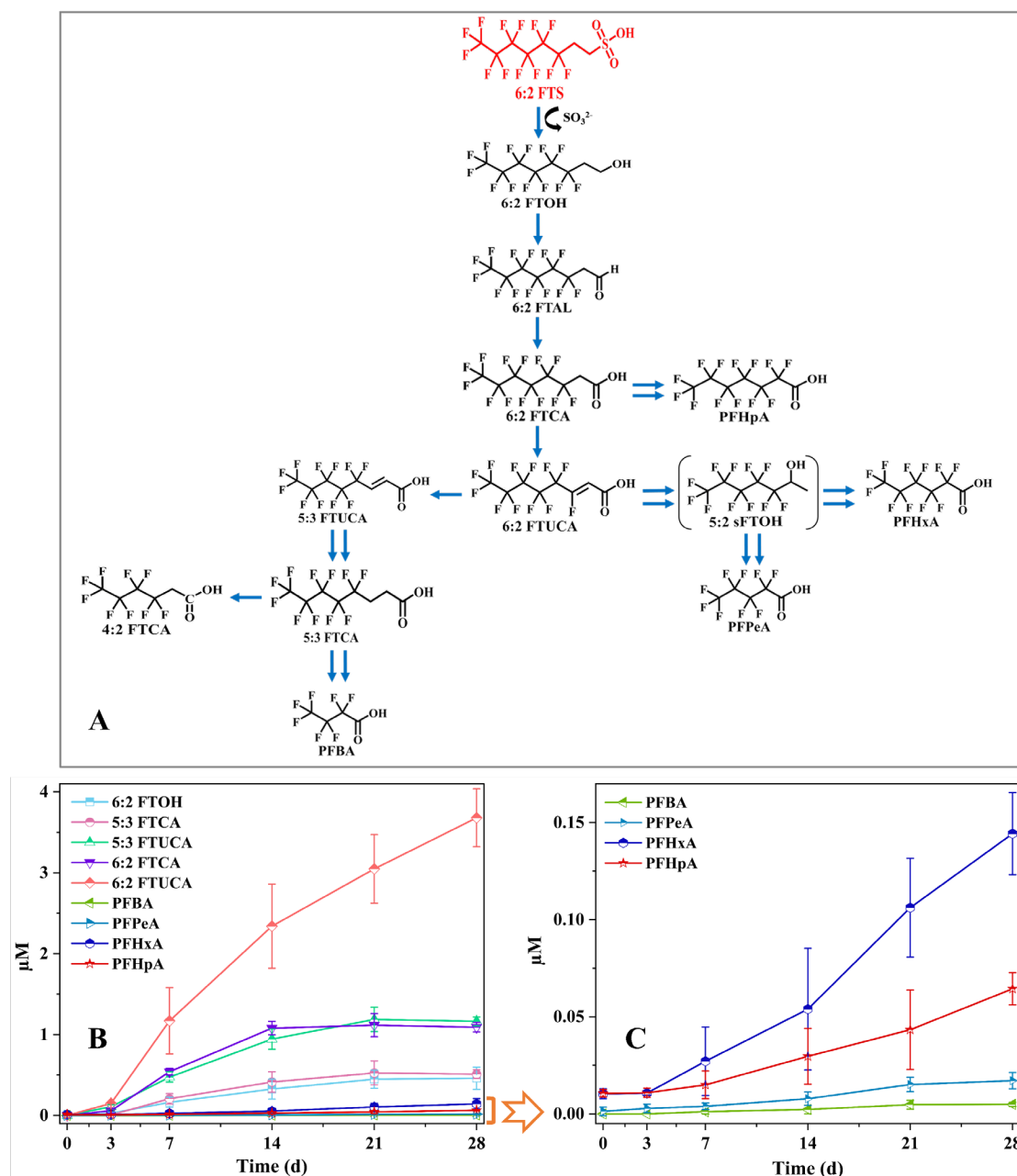


Fig. 2. Proposed aerobic biotransformation pathways of 6:2 FTS under sulfur-limiting conditions (A). The black PFAS structure represents those identified, while the bracket is a proposed transformation product (not detected) based on the present findings and previous studies.^{20,25,47} Double arrows represent multiple transformation steps. Concentrations of polyfluorinated and perfluorinated products (B); and perfluorinated end products enlarged (C) were quantified during the biotransformation of 6:2 FTS by the consortium. Error bars represent the standard error of triplicate samples.

In an alternative pathway, 6:2 FTUA may convert to 5:2 sFTOH through a series of enzymatic decarboxylation and defluorination reactions.^{19,47} Subsequently, 5:2 sFTOH could undergo further fluorine and carbon eliminations through multiple unknown reactions, ultimately transforming into PFHxA and PFPeA.^{16,19,25} It is worth noting that 5:2 FTOH was not detected in this study, suggesting that its degradation to the next transformation product may be fast, resulting in concentrations below the LOD.

3.2.3. Formation profiles of intermediate products

We further quantified the intermediate byproducts and end products generated during the biodegradation of 6:2 FTS at an initial concentration of 5 mg/L. The concentrations of polyfluorinated and perfluorinated byproducts at different time intervals during the biotransformation of 6:2 FTS by the consortium are presented in Fig. 2B-C and Table S9. Among polyfluorinated byproducts, 6:2 FTUCA was the most abundant, followed by 5:3 FTUCA, 6:2 FTCA, 5:3 FTCA, and 6:2 FTOH (Fig. 2B). 6:2 FTOH, the direct intermediate product of 6:2 FTS (Fig. 2A), was quantified with the lowest molar yield, reaching $0.46 \pm 0.14 \mu\text{M}$ on day 28, probably due to its fast degradation by the consortium.^{20,47} We could not quantify 6:2 FTAL, an intermediate product, because no authentic standard was available. The molar concentrations of 6:2 FTCA reached $1.12 \pm 0.14 \mu\text{M}$ by day 21, followed by a minor decrease to $1.09 \pm 0.06 \mu\text{M}$, suggesting further biotransformation into a lower metabolite, 6:2 FTUCA. The molar concentration of 6:2 FTUCA continuously increased, reaching a peak molar yield of $3.68 \pm 0.36 \mu\text{M}$ by day 28 (Fig. 2B and Table S9), suggesting the accumulation of 6:2 FTUCA in the system. A comparable trend was reported during the biotransformation of 6:2 FTS by *Gordonia sp.* NB4-1Y, where a molar yield of 13.7 mol% was observed for 6:2 FTUCA.²⁵ The accumulation of 6:2 FTUCA implies that further 6:2 FTUCA

transformation might be the rate-limiting step in the 6:2 FTS biodegradation pathway, which warrants further exploration.

The molar concentrations of 5:3 FTUCA exhibited similar trends to that of 6:2 FTCA, reaching $1.19 \pm 0.15 \mu\text{M}$ by day 21, followed by a minor decrease to $1.16 \pm 0.05 \mu\text{M}$. In this study, 5:3 FTUCA was quantified using 5:3 FTCA standards due to their close retention times in LC-MS/MS analysis. Notably, 5:3 FTUCA has not been previously reported in studies on the biotransformation of 6:2 FTS by *Dietzia aurantiaca* J3,¹⁶ *Rhodococcus jostii* RHA1,¹⁸ activated sludge,¹⁹ river sediment,²⁰ wetland slurry,⁵⁷ or aerobic soil.⁴⁷ While one study on *Gordonia* sp. NB4-1Y reported 5:3 FTUCA as a transformation product of 6:2 FTS,²⁵ another study did not detect it.¹⁷ The peak molar yield of 5:3 FTCA, an intermediate product of 5:3 FTUCA, was $0.53 \pm 0.15 \mu\text{M}$ on day 21, which decreased slightly to $0.51 \pm 0.05 \mu\text{M}$ by day 28. 4:2 FTCA, the intermediate product of 4:2 FTCA, could not be quantified either due to the unavailability of standard.

The end products of perfluorinated compounds, including PFBA, PFPeA, PFHxA, and PFHpA, were also quantified over time (Fig. 2C). Their concentrations gradually increased over the incubation period, reaching peak molar yield of 0.005 ± 0.001 , 0.017 ± 0.005 , 0.14 ± 0.06 , and $0.064 \pm 0.009 \mu\text{M}$, respectively, by day 28 (Fig. 2C). It is noteworthy that trace concentrations of PFPeA, PFHxA, and PFHpA were detected in the initial concentrations at the beginning of the incubation (day 0) in both of the control and biodegradation groups (Table S9), and the presented concentrations in Fig. 2C were corrected, excluding the inference of those in the control. Therefore, the production of end products is a solid line of evidence for the biodegradation of polyfluorinated compounds. Furthermore, the production of PFHxA is much more significant than that of PFBA, suggesting the alternative branching transformation of 6:2 FTUCA via the

5:2 sFTOH pathway is more significant than the other proposed pathway, one of the key discoveries in the current study.

Furthermore, a fluorine mass balance was conducted by quantifying 6:2 FTS, its known transformation products, and released fluoride (F^-) over 28 days (Table 1). In the control group, total fluorine remained stable throughout the experiment, indicating negligible 6:2 FTS transformation. In contrast, the biodegradation group exhibited a significant decrease in 6:2 FTS levels, accompanied by the accumulation of multiple transformation products and a continuous increase in F^- levels. Total fluorine recovery, which was 96.98% on Day 0, dropped to 53.54% by Day 14 and gradually rose to 74.42% by Day 28 in the biodegradation group. The incomplete fluorine balance can be attributed to the formation of unidentified or unquantified fluorinated compounds.⁵⁵ As previously reported,⁵⁸ highlighting the need for further non-targeted analysis to fully elucidate the fate of fluorine during 6:2 FTS biotransformation. The increase in the fluorine recovery observed in the later phase was mainly due to the continuous release of F^- and the accumulation of intermediate products (e.g., 6:2 FTUCA), as discussed above. The fluorine mass balance provides additional supporting evidence for the 6:2 FTS biodefluorination.

Table 1. Fluorine mass balance (µg/L) in control and biodegradation groups (5 mg/L) over time during 6:2 FTS degradation

D ay	F in 6:2 FTS	F in PFBA	F in PFPeA	F in PFHxA	F in PFHpA	F in 5:3 FTCA	F in 5:3 FTUCA	F in 6:2 FTCA	F in 6:2 FTUCA	F in 6:2 FTOH	F in 6:2 FTAL	F in 4:2 FTCA	F ⁻	Total F	F- balance (%)
Control group															
0	3160.86	N.D.	0.20	2.17	2.57	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3165.81	-
3	3091.65	N.D.	0.21	2.11	2.21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3096.18	
7	3183.94	N.D.	0.2	2.21	2.18	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3188.53	
14	3226.23	N.D.	0.22	2.58	2.11	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3231.14	-
21	3160.86	N.D.	0.25	2.55	2.24	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3165.90	
28	3178.17	N.D.	0.29	2.56	2.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3183.03	-
Biodegradation group															
0	3037.81	N.D.	0.22	2.56	2.61	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	27.00	3070.20	96.98
3	2572.53	N.D.	0.48	2.24	2.32	1.38	43.17	13.62	6.84	6.60	N.D.	N.D.	268.00	2917.17	94.22
7	778.68	0.15	0.67	5.66	3.68	46.27	98.59	125.57	266.81	40.38	N.D.	N.D.	385.33	1751.81	54.94
14	13.84	0.30	1.33	11.27	7.32	92.51	196.11	250.81	533.45	80.60	N.D.	N.D.	542.33	1729.88	53.54
21	8.08	0.63	2.59	22.18	10.70	117.55	246.55	259.38	694.89	110.89	N.D.	N.D.	695.33	2168.77	68.50
28	8.08	0.61	2.93	30.15	15.91	114.16	241.89	253.51	838.86	113.38	N.D.	N.D.	749.33	2368.81	74.420

3.3. Metatranscriptomic analysis

3.3.1. Gene response of SXC01 consortium to 6:2 FTS biotransformation

Metatranscriptome sequencing yielded $11,399,125,799 \pm 953,282,094$ and $13,617,701,957 \pm 1,453,050,798$ bps of raw sequence data for Day 0 and 7, respectively (detailed quality assessment is provided in Text S4 and Table S10). The analysis, including quality filtering, read assembly, and functional annotation, followed by differential gene expression analysis (e.g., DESeq2), identified 60,533 unique genes within the communities comparing Days 0 to 7, of which 36,773 differentially expressed genes (DEGs) ($\log_2$ fold change ≥ 2 , P-adjust < 0.05). Among the significant DEGs, 24,903 genes were upregulated, while 11,870 were downregulated (Fig. 3A). Downregulated genes may reflect inhibited growth and metabolism of the consortium, whereas upregulated genes suggest enhanced metabolic activities in response to 6:2 FTS exposure.⁵⁹ The presence of pollutant toxicity may impair cellular metabolism and downregulate certain genes, while upregulated genes suggest an adaptive response enabling the microbial consortium to modulate metabolic pathways and tolerate environmental stress.^{60,61} This adaptation may allow the consortium to utilize 6:2 FTS as a sulfur source, thereby supporting microbial growth and the production of metabolites.

From the upregulated DEGs, 123 functional genes potentially involved in fluorotelomer biotransformation were selected for further analysis (Table S11). However, most of these genes are associated with KEGG pathways, including carbohydrate, amino acid, energy, lipid, nucleotide metabolism, xenobiotics biodegradation, secondary metabolism biosynthesis, genetic information processing, signaling, and cellular processes, which means such processes might be involved in fluorotelomer

biotransformation or metabolic pathways.⁶² Notably, 19 key genes encoding enzymes relevant to 6:2 FTS biotransformation pathways were identified based on prior literature^{16,18,62} and predictions in this study, which are highlighted in Table S11.

The log2 FC of 19 functional gene expressions of Day 0 and Day 7 during 6:2 FTS biotransformation is illustrated in heatmap (Fig. 3B). Notably, the *ssu* gene cluster, including alkanesulfonate monooxygenase (*ssuD*, K04091), alkanesulfonate-binding protein (*ssuA*, K15553), alkanesulfonate ABC transporter (*ssuB*, K15555), alkanesulfonate permease protein (*ssuC*, K15554), and FMN reductase (*ssuE*, K00299) is implicated in the desulfonation of 6:2 FTS. The identification of the *ssuEADCB* gene cluster in consortium (Fig. S10), facilitating alkanesulfonate assimilation, aligns with prior reports of similar *ssu* proteins in *Dietzia aurantiaca* J3,¹⁶ *Rhodococcus jostii* RHA1,¹⁸ and *Gordonia* sp. NB4-1Y.¹⁷ In the consortium, this gene cluster exhibited significant upregulation, with log2 FC 3.91 to 6.37 on Day 7, compared to -3.16 to -9.75 on Day 0 (Fig. 3B), indicating their putative involvement in 6:2 FTS metabolism. The *ssuEADCB* cluster was first discovered in *E. coli*⁶³ and is conserved among bacterial isolates capable of degrading 6:2 FTS.¹⁶ The *ssuD* gene, which encodes an FMN-dependent monooxygenase with broad substrate specificity, catalyzes the cleavage of C-S bonds in sulfonated hydrocarbons, yielding sulfite and aldehydes.^{16,64–}
⁶⁶ The *ssuE* gene, which encodes a NAD(P)H-dependent FMN oxidoreductase, supplies *ssuD* with reduced flavins (FMNH₂). Additional enzymes potentially involved in desulfonation and sulfur metabolism, including sulfur dioxygenase (K17725),⁶⁷ sulfite reductase (encoded by *cysI*, K00381),⁶⁸ sulfate transport system substrate-binding protein (encoded by *sbp*, K23163),⁶⁹ cysteine desulfurase (encoded by *iscS*, K04487)⁷⁰ and sulfur-carrier protein (encoded by *moaD*, K03636).⁷¹ These enzymes likely play

complementary roles in sulfur utilization and metabolism during 6:2 FTS biotransformation.

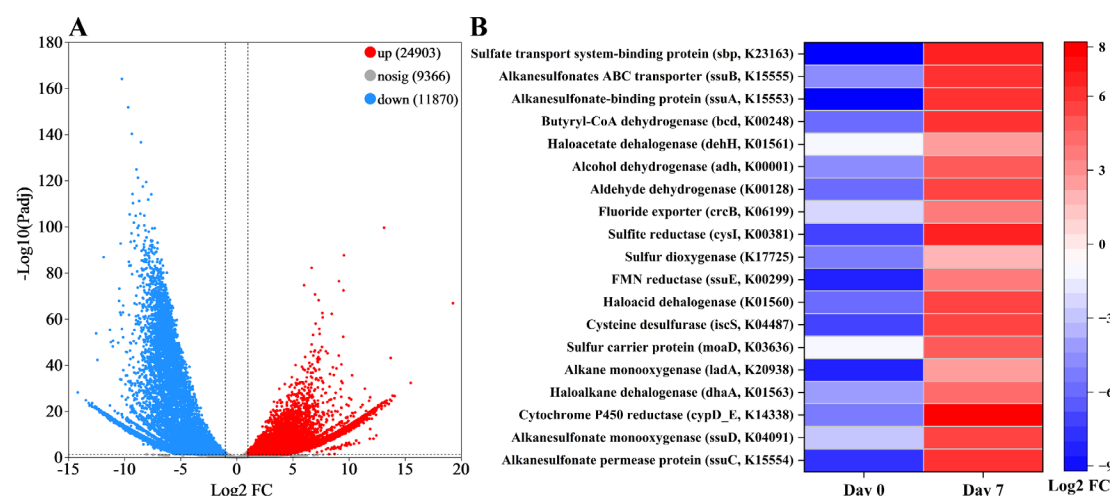


Fig. 3. Analysis of differentially expressed genes (DEGs) and functional genes encoding metabolic pathways of 6:2 FTS biotransformation by the consortium. Volcano map of DEGs during 6:2 FTS biotransformation (A); Heatmap showing the fold change of identified 19 functional genes associated with metabolic pathways between consortia on Day 0 and Day 7 (B).

Previous studies have demonstrated the role of dehalogenase enzymes, such as haloacetate and haloalkane dehalogenase, in degrading halogenated compounds, including fluorinated, chlorinated, and brominated substances.^{62,72} In this study, several enzymes were upregulated in response to 6:2 FTS exposure, suggesting potential involvement in fluorotelomer biotransformation in the consortium. These include alkane monooxygenase (encoded by *ladA*, K20938), haloalkane dehalogenase (encoded by *dhaA*, K01563), haloacid dehalogenase (K01560), haloacetate dehalogenase (encoded by *dehH*, K01561), cytochrome P450 reductase (encoded by *cypD_E*, K14338), and fluoride exporter (encoded by *crcB*, K06199), which may facilitate 6:2 FTS defluorination (Fig. 3B, Table S11). However, the specific enzymatic functions of

these genes in the biotransformation of PFAS compounds remain to be experimentally validated. A recent study on *Rhodococcus jostii* RHA1 proposed that alkane monooxygenase, haloacetate dehalogenase, haloalkane dehalogenase, and cytochrome P450 are key enzymes involved in 6:2 FTS biotransformation, based on quantitative reverse transcription PCR (RT-qPCR) analysis.¹⁸ Similarly, alkane monooxygenase in *Pseudomonas oleovorans* grown on n-octane has been shown to defluorinate 6:2 FTOH.⁷³ The presence of fluoride exporters, such as *crcB*, is critical for mitigating fluoride toxicity in polyfluorinated compound-degrading organisms.⁷⁴ These findings align with and support our results on 6:2 FTS defluorination. Additionally, several dehydrogenases and reductase enzymes were predicted to participate in oxidation-reduction reactions relevant to fluorotelomer biotransformation. Alcohol dehydrogenase (*adh*, K00001) may catalyze the oxidation of 6:2 FTOH,⁶² aldehyde dehydrogenase (K00128) may facilitate the conversion of 6:2 FTCA from 6:2 FTAL⁷⁵ and butyryl-CoA dehydrogenase (*bcd*, K00248) may oxidize fluorotelomer carboxylic acids.⁷⁶ This study suggests the molecular mechanisms underlying 6:2 FTS degradation and highlights the potential involvement of specific DEGs in desulfonation and defluorination. However, further experimental validation is required to confirm their functional roles.

3.3.2. Microbial community abundance and correlation with gene expression

A total of 428 genera and 2,153 species were identified, spanning 22 phyla in the consortia. Taxonomic compositions of active microbial genera, represented by the relative abundance (%) based on transcript-level annotations, are presented in Fig. 4A. Bacterial genera *Brucella* dominated the relative abundance at 77%, followed by *Rhodococcus* at 7% prior to the incubation on Day 0. The consortium community structure shifted on day 7, with *Brucella*, *Rhodococcus*, and *Pseudoclavibacter*, which

might play key roles in 6:2 FTS biodegradation. *Brucella* remained the most relatively abundant genus at 52%, while *Rhodococcus* and *Pseudoclavibacter* increased their relative abundance from 7% and 3% to 15% and 11%, respectively. Other genera, such as *Agrobacterium* and *Achromobacter*, exhibited significant expansion from 0.1% and 2% to 6% and 5% of relative abundance, respectively. These genera could potentially contribute to metabolic processes that facilitate the breakdown of 6:2 FTS and its intermediate degradation products.

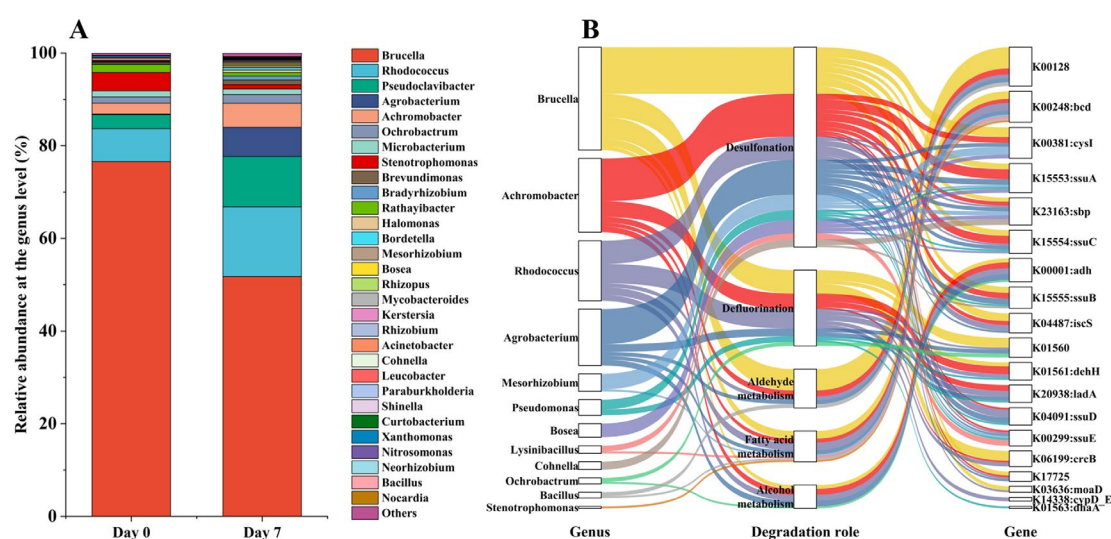


Fig. 4. Relative abundance (%) of active microbial genera and distribution of functional genes across dominant taxa involved in 6:2 FTS biotransformation. Relative abundance (%) of active microbial taxa at the genus level based on annotated transcript reads reflecting transcriptional activity (A); the Sankey diagram depicts the distribution of functional genes across abundant bacterial species involved in 6:2 FTS biotransformation (B). The left side of the Sankey diagram represents bacterial genera linked to the upregulated DEGs on the right, with color-coded flows indicating their associations. The flow width reflects the strength or prevalence of each association. The middle column highlights specific 6:2 FTS degradation pathways, providing an overview of the functional processes within the microbial community.

The observed shifts in the community composition suggest the selective pressure exerted by 6:2 FTS, favoring taxa with pollutant tolerance and degrading capabilities. Prior studies have demonstrated the degradative roles of *Gordonia*,²⁵ *Rhodococcus*,¹⁸ and *Dietzia*¹⁶ in 6:2 FTS biotransformation, as well as *Mycobacterium* in 6:2 FTOH degradation.³² *Brucella*, previously linked to polyethylene terephthalate degradation,⁷⁷ showed the highest abundance during 6:2 FTS biodegradation in this study, along with *Agrobacterium* and *Achromobacter*, which are known to degrade terbuthylazine⁷⁸ and polycyclic aromatic hydrocarbons,⁷⁹ respectively. Furthermore, *Rhodococcus* and *Achromobacter* have been observed in AFFF-impacted soil microcosms and are implicated being involved in the 6:2 FTS biotransformation.⁴⁷ In the current study, we hypothesize that *Brucella*, along with other dominant genera, including *Rhodococcus*, *Pseudoclavibacter*, *Agrobacterium*, and *Achromobacter*, collectively or individually play a role in the biotransformation of 6:2 FTS.

The Sankey diagram depicts the distribution of functional gene-coded enzymes across abundant bacterial species potentially involved in 6:2 FTS biodegradation (Fig. 4B, Table S12). We identified 19 suspected functional gene-coded enzymes, which are linked to key processes such as desulfonation, defluorination, and biotransformation. Genes associated with suspected desulfonation potential, including the *ssuEADCB* cluster, sulfite reductase (*cysI*, K00381), sulfate transport system binding protein (*sbp*, K23163), and cysteine desulfurase (*iscS*, K04487), were primarily linked to *Brucella*, *Achromobacter*, *Agrobacterium*, and *Rhodococcus* (Fig. 4B). These bacteria likely facilitate sulfate group assimilation under sulfur-limiting conditions. Similarly, defluorination-associated gene-coded enzymes, such as alkane monooxygenase (*ladA*, K20938), were predominantly linked to *Achromobacter*, *Rhodococcus*, and *Agrobacterium*. In contrast, *Brucella* was associated with haloacid dehalogenase

(K01560), haloacetate dehalogenase (*dehH*, K01561), and fluoride exporter (*crcB*, K06199), which may facilitate defluorination (Fig. 4B). Additional enzymes, such as aldehyde dehydrogenase (K00128), alcohol dehydrogenase (*adh*, K00001) and butyryl-CoA dehydrogenase (*bcd*, K00248) were shared among *Brucella*, *Achromobacter*, *Rhodococcus*, and *Agrobacterium*, and these enzymes likely play significant roles in aldehyde, alcohol and fatty acid metabolism during 6:2 FTS degradation biotransformation.

Additionally, the most upregulated DEGs on Day 7 (Fig. 3A) were further identified (Table S13), including genes such as *cfa* (K00574), *cspA* (K03704), and *rpoE* (K03088), among others. Their associated bacteria are visualized in the Sankey diagram (Fig. S11, Table S14). While the specific roles of these highly upregulated genes in 6:2 FTS biotransformation remain unclear, they are notably associated with *Brucella*, *Rhodococcus*, and *Achromobacter*. Further research is needed to elucidate their roles in 6:2 FTS biotransformation. The Sankey diagram suggests the functional diversity within the microbial community, illustrating how specific genera may specialize in distinct degradation pathways and desulfonation and defluorination functional genes. This interconnectedness suggests complementary roles among microbial taxa in breaking down complex pollutants, emphasizing the importance of microbial interactions in environmental bioremediation.

3.4. 6:2 FTS metabolism by pure bacterial isolates

Five single bacterial strains were isolated from the consortium SXC01 and could biodegrade 6:2 FTS in the current study. The metatranscriptomic analysis revealed that *Brucella*, *Rhodococcus*, and *Pseudoclavibacter* were dominant microbes during the biotransformation of 6:2 FTS (Fig. 4A), and the desulfonation and defluorination

functional genes were found to be abundant in *Brucella* (Fig. 4B). Therefore, a pure culture of *Brucella anthropi* SX009 was chosen to demonstrate the ability of 6:2 FTS defluorination by a single species. The colony morphology on LB agar is presented in Fig. 5A, and A phylogenetic tree depicting the sequence similarity of SX009 with closely related other species is shown in Fig. 5B, constructed using the neighbor-joining method. The tree clusters SX009 within the *Brucella anthropi* clade, indicating its close evolutionary relationship with other *Brucella* strains.

The degradation kinetics of 6:2 FTS (5 mg/L) by strain SX009 were assessed for over 28 days, as shown in Fig. 5C. In the sterile control, no significant change in 6:2 FTS concentration was observed throughout the incubation, indicating that the compound remains stable without bacterial activity. In contrast, strain SX009 demonstrated 65% degradation of 6:2 FTS by day 7, and near-complete degradation (99.5%) was achieved by day 14. The degradation of 6:2 FTS by single pure bacterial strains has also been observed in *D. aurantiaca* J3,¹⁶ *Gordonia* sp. NB4-1Y,¹⁷ *R. jostii* RHA1,¹⁸ and *Pseudomonas* sp. D2.²⁴ However, this is the first study to report members of the *Brucella* genus as a degrader of 6:2 FTS.

Fluoride ions were generated in the presence of bacterial strain SX009 under sulfur-limiting conditions, achieving 3% of defluorination ratio on day 7 and 6.5 % on day 28 (Fig. 5C). However, the ratio was lower than that observed for SXC01. We hypothesize that although *Brucella anthropi* SX009 can achieve near-complete biodegradation of 6:2 FTS, multiple bacterial strains within the consortium have participated in the defluorination of 6:2 FTS, contributing to its much higher defluorination ratio by the consortium. No fluoride generation was observed throughout the experiment in the control group. The identification of *Brucella* and its associated degradation and defluorination activities provides further evidence of the collaborative roles of different

strains in the SXC01 microbial communities in the biotransformation of 6:2 FTS. We believe these findings provide a foundation for future investigations into the enzymatic and genetic mechanisms underlying PFAS biotransformation by this strain.

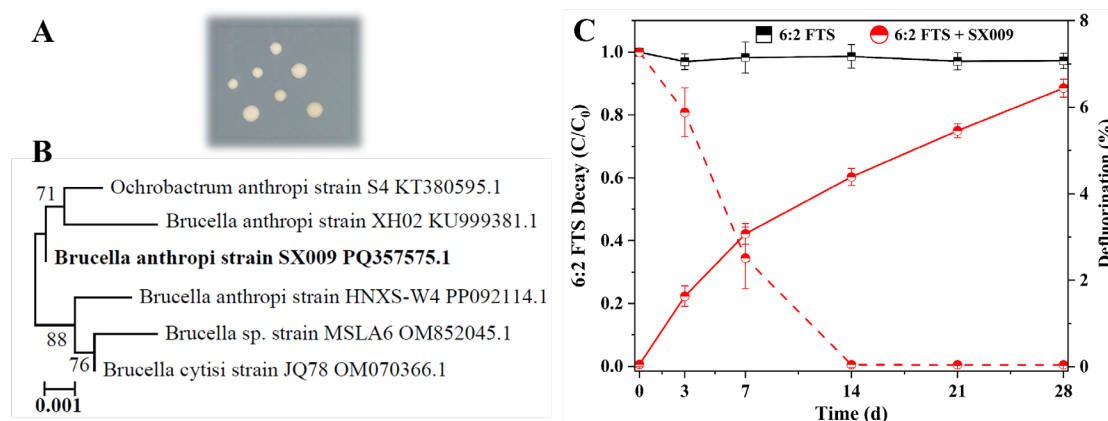


Fig. 5. Pure bacterial isolate and its role in 6:2 FTS degradation. Colony morphology of *Brucella anthropi* SX009 (A); phylogenetic relationship of SX009 based on 16S rRNA gene analysis (B); and degradation efficiency and defluorination ratio of 6:2 FTS over time (C). Degradation efficiency is represented in the dashed line, while the defluorination ratio is in the solid line. Error bars represent the standard error of three replicates.

4. Environmental implications

This study investigates the aerobic biotransformation of 6:2 FTS, an environmental contaminant, under sulfur-limiting conditions using an enrichment culture. The research achieved high degradation and defluorination efficiencies, uncovering extensive intermediate products formed during the process. The accumulation of 6:2 FTUCA implies that further 6:2 FTUCA transformation might be the rate-limiting step in the 6:2 FTS biodegradation pathway. Furthermore, the generation of end-product PFHxA is much more significant than that of PFBA, suggesting the alternative branching transformation via the 5:2 sFTOH pathway is a significant pathway for 6:2

FTUCA. Metatranscriptomic analysis revealed the upregulation of functional genes and microbial genera responsible for critical desulfonation and defluorination pathways, providing novel insights into the underlying mechanisms of PFAS biodegradation. Notably, the isolation of a *Brucella* species as a pure strain capable of degrading 6:2 FTS represents a valuable resource for further investigations into the specific functional genes involved in biodefluorination. This research advances our understanding of PFAS biodefluorination, and future studies should focus on the elucidation of the molecular mechanisms of key functional genes and enzymatic pathways involved in desulfonation and defluorination processes.

Supporting information

The Supporting Information is available free of charge at <https://pubs.acs.org/>

Details regarding medium composition, stability of the enrichment culture, metatranscriptomic analysis process, experimental conditions, consortium and single bacteria growth monitoring, targeted PFAS standards, instrument parameters, biotransformation product identification and quantifications, and metatranscriptomic analysis: KEGG pathway analysis of gene cluster, correlation of functional genes and abundance of microbes (docx).

Additional spreadsheets contain bacterial taxa information and upregulated genes and corresponding pathways identified by differentially expressed genes (DEGs) (XLSX).

Credit author contribution statement

Md. Golam Kabir: Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Visualization, Conceptualization.

Zhiwen Tang: Methodology, Visualization, Writing – review & editing. **Xiqian**

Zhang: Formal analysis, Investigation. **Frederic Coulon:** Writing – review & editing.

Andreas Tiehm: Writing – review & editing. **Xin Song:** Writing – review & editing, Methodology, Visualization, Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This research was funded by the National Natural Science Foundation of China (NSFC)-EU Environmental Biotechnology Joint Program (No. 32061133001), and the National Natural Science Foundation of China (No. 42277029). We acknowledge the cooperation between China and the EU through the EiCLaR project (European Union's Horizon 2020, No. 965945). We also acknowledge the help of Chen Hong from the Soil and Environment Analysis Center for quantitatively analyzing biotransformation product quantifications.

References

- (1) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; Voogt, P. De; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr. Environ. Assess. Manag.* **2011**, 7 (4), 513–541.
- (2) Sheng, N.; Zhou, X.; Zheng, F.; Pan, Y.; Guo, X.; Guo, Y.; Sun, Y.; Dai, J. Comparative hepatotoxicity of 6:2 fluorotelomer carboxylic acid and 6:2 fluorotelomer sulfonic acid, two fluorinated alternatives to long-chain perfluoroalkyl acids, on adult male Mice. *Arch. Toxicol.* **2017**, 91 (8), 2909–2919.
- (3) Backe, W. J.; Day, T. C.; Field, J. A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, 47 (10), 5226–5234.

- (4) Moody, C. A.; Field, J. A. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ. Sci. Technol.* **2000**, *34* (18), 3864–3870.
- (5) Hamid, H.; Li, L. Y.; Grace, J. R. Formation of perfluorocarboxylic acids from 6:2 fluorotelomer sulfonate (6:2 FTS) in landfill leachate: role of microbial communities. *Environ. Pollut.* **2020**, *259*, 113835.
- (6) Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ. Sci. Technol.* **2013**, *47* (15), 8187–8195.
- (7) Kärrman, A.; Elgh-Dalgren, K.; Lafossas, C.; Møskeland, T. Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination. *Environ. Chem.* **2011**, *8* (4), 372.
- (8) Schultz, M. M.; Barofsky, D. F.; Field, J. A. Quantitative determination of fluorotelomer sulfonates in groundwater by LC-MS/MS. *Environ. Sci. Technol.* **2004**, *38* (6), 1828–1835.
- (9) Boiteux, V.; Dauchy, X.; Bach, C.; Colin, A.; Hemard, J.; Sagres, V.; Rosin, C.; Munoz, J. F. Concentrations and patterns of perfluoroalkyl and polyfluoroalkyl substances in a river and three drinking water treatment plants near and far from a major production source. *Sci. Total. Environ.* **2017**, *583*, 393–400.
- (10) Jin, L.; Jiang, C.; Zhang, P. Photochemical decomposition of 1H,1H,2H,2H-perfluorooctane sulfonate (6:2FTS) induced by ferric ions. *J. Environ. Sci.* **2017**, *51*, 120–127.
- (11) Zhuo, Q.; Li, X.; Yan, F.; Yang, B.; Deng, S.; Huang, J.; Yu, G. Electrochemical oxidation of 1H,1H,2H,2H-perfluorooctane sulfonic acid (6:2 FTS) on DSA electrode: operating parameters and mechanism. *J. Environ. Sci.* **2014**, *26* (8), 1733–1739.
- (12) Fath, A.; Sacher, F.; McCaskie, J. E. Electrochemical decomposition of fluorinated wetting agents in plating industry waste water. *Water Sci. Technol.* **2016**, *73* (7), 1659–1666.
- (13) Fernandez, N. A.; Rodriguez-Freire, L.; Keswani, M.; Sierra-Alvarez, R. Effect of chemical structure on the sonochemical degradation of perfluoroalkyl and polyfluoroalkyl substances (PFASs). *Environ. Sci.* **2016**, *2* (6), 975–983.
- (14) Yang, X.; Huang, J.; Zhang, K.; Yu, G.; Deng, S.; Wang, B. Stability of 6:2 fluorotelomer sulfonate in advanced oxidation processes: degradation kinetics and pathway. *Environ. Sci. Pollut. Res.* **2014**, *21* (6), 4634–4642.
- (15) Park, S.; Lee, L. S.; Medina, V. F.; Zull, A.; Waisner, S. Heat-activated persulfate oxidation of PFOA, 6:2 fluorotelomer sulfonate, and PFOS under conditions suitable for in-situ groundwater remediation. *Chemosphere* **2016**, *145*, 376–383.

- (16) Méndez, V.; Holland, S.; Bhardwaj, S.; McDonald, J.; Khan, S.; O'Carroll, D.; Pickford, R.; Richards, S.; O'Farrell, C.; Coleman, N.; Lee, M.; Manefield, M. J. Aerobic biotransformation of 6:2 fluorotelomer sulfonate by *Dietzia aurantiaca* J3 under sulfur-limiting conditions. *Sci. Total. Environ.* **2022**, 829, 154587.
- (17) Van Hamme, J. D.; Bottos, E. M.; Bilbey, N. J.; Brewer, S. E. Genomic and proteomic characterization of *Gordonia* Sp. NB4-1Y in relation to 6:2 fluorotelomer sulfonate biodegradation. *Microbiology* **2013**, 159 (8), 1618–1628.
- (18) Yang, S. H.; Shi, Y.; Strynar, M.; Chu, K. H. Desulfonation and defluorination of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) by *Rhodococcus jostii* RHA1: carbon and sulfur sources, enzymes, and pathways. *J. Hazard. Mater.* **2022**, 423, 127052.
- (19) Wang, N.; Liu, J.; Buck, R. C.; Korzeniowski, S. H.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M. 6:2 fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* **2011**, 82 (6), 853–858.
- (20) Zhang, S.; Lu, X.; Wang, N.; Buck, R. C. Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. *Chemosphere* **2016**, 154, 224–230.
- (21) Hamid, H.; Li, L. Y.; Grace, J. R. Aerobic biotransformation of fluorotelomer compounds in landfill leachate-sediment. *Sci. Total. Environ.* **2020**, 713, 136547.
- (22) Yan, P. F.; Dong, S.; Woodcock, M. J.; Manz, K. E.; Garza-Rubalcava, U.; Abriola, L. M.; Pennell, K. D.; Cápiro, N. L. Biotransformation of 6:2 fluorotelomer sulfonate and microbial community dynamics in water-saturated one-dimensional flow-through columns. *Water Res.* **2024**, 252, 121146.
- (23) Kertesz, M. A. Riding the sulfur cycle metabolism of sulfonates and sulfate esters in gram-negative bacteria. *FEMS Microbiol Rev.* **2000**, 24, 135–175.
- (24) Key Blake D; Howell Robert D; Criddle Craig S. Defluorination of organofluorine sulfur compounds by *Pseudomonas* Sp. Strain D2. *Environ. Sci. Technol.* **1998**, 32, 2283–2287.
- (25) Shaw, D. M. J.; Munoz, G.; Bottos, E. M.; Duy, S. V.; Sauvé, S.; Liu, J.; Van Hamme, J. D. Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by *Gordonia* Sp. Strain NB4-1Y under sulfur-limiting conditions. *Sci. Total. Environ.* **2019**, 647, 690–698.
- (26) Van Der Ploeg, J. R.; Eichhorn, E.; Leisinger, T. Sulfonate-sulfur metabolism and its regulation in *Escherichia coli*. *Arch. Microbiol.* **2001**, 176 (1–2), 1–8.

- (27) Chan, W. Y.; Wong, M.; Guthrie, J.; Savchenko, A. V.; Yakunin, A. F.; Pai, E. F.; Edwards, E. A. Sequence- and activity-based screening of microbial genomes for novel dehalogenases. *Microb. Biotechnol.* **2010**, 3 (1), 107–120.
- (28) Smith, J. M.; Harrison, K.; Colby, J. Purification and characterization of d-2-haloacid dehalogenase from *Pseudomonas putida* Strain AJ1/23. *J. Gen. Microbiol.* **1990**, 136 (5), 881–886.
- (29) Van Der Ploeg, J.; Van Hall, G.; Janssen, D. B. Characterization of the haloacid dehalogenase from *Xanthobacter autotrophicus* GJ10 and sequencing of the *DhlB* gene isolation of a dehalogenase-overproducing mutant. mutants of *X. autotrophicus* GJ10 resistant to monobromoacetate. *J. Bacteriol.* **1991**, 173, 7925–7933.
- (30) Kharasch, E. D.; Thummel, K. E. Identification of cytochrome p450 2e1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. *Anesthesiology* **1993**, 79 (4), 795–807.
- (31) Murphy, C. D. Microbial Degradation of Fluorinated Drugs: Biochemical pathways, impacts on the environment and potential applications. *Appl. Microbiol. Biotechnol.* **2016**, 100 (6), 2617–2627.
- (32) Kim, M. H.; Wang, N.; Chu, K. H. 6:2 Fluorotelomer alcohol (6:2 FTOH) biodegradation by multiple microbial species under different physiological conditions. *Appl. Microbiol. Biotechnol.* **2014**, 98 (4), 1831–1840.
- (33) Lewis, M.; Kim, M. H.; Liu, E. J.; Wang, N.; Chu, K. H. Biotransformation of 6:2 polyfluoroalkyl phosphates (6:2 PAPs): effects of degradative bacteria and co-substrates. *J. Hazard. Mater.* **2016**, 320, 479–486.
- (34) Huang, S.; Jaffé, P. R. Defluorination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by *Acidimicrobium* Sp. Strain A6. *Environ. Sci. Technol.* **2019**, 53, 11410–11419.
- (35) Harding-Marjanovic, K. C.; Houtz, E. F.; Yi, S.; Field, J. A.; Sedlak, D. L.; Alvarez-Cohen, L. Aerobic biotransformation of fluorotelomer thioether amido sulfonate (Lodyne) in AFFF-amended microcosms. *Environ. Sci. Technol.* **2015**, 49 (13), 7666–7674.
- (36) MacKay, G. M.; Zheng, L.; Van Den Broek, N. J. F.; Gottlieb, E. Analysis of cell metabolism using LC-MS and isotope tracers. *Methods. Enzymol.* **2015**, 561, 171–196.
- (37) Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, 34, i884–i890.
- (38) Kopylova, E.; Noé, L.; Touzet, H. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* **2012**, 28 (24), 3211–3217.

- (39) Haas, B. J.; Papanicolaou, A.; Yassour, M.; Grabherr, M.; Blood, P. D.; Bowden, J.; Couger, M. B.; Eccles, D.; Li, B.; Lieber, M.; Macmanes, M. D.; Ott, M.; Orvis, J.; Pochet, N.; Strozzi, F.; Weeks, N.; Westerman, R.; William, T.; Dewey, C. N.; Henschel, R.; Leduc, R. D.; Friedman, N.; Regev, A. De Novo Transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. *Nat. Protoc.* **2013**, *8* (8), 1494–1512.
- (40) Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **2012**, *28* (23), 3150–3152.
- (41) Fang, S.; Cao, W.; Wu, Q.; Cheng, S.; Yang, Y.; Liu, J.; Wu, Y.; Fang, F.; Feng, Q.; Cao, J.; Luo, J. Multifaceted roles of methylisothiazolinone intervention in sludge disintegration and acidogenic and methanogenic pathways for efficient carboxylate production during anaerobic fermentation. *Chem. Eng. J.* **2023**, *472*, 145022.
- (42) Love, M. I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15* (12), 550.
- (43) Buchfink, B.; Xie, C.; Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60.
- (44) Davis, S. N.; Klumker, S. M.; Mitchell, A. A.; Coppage, M. A.; Labonté, J. M.; Quigg, A. Life in the PFAS Lane: The impact of perfluoroalkyl substances on photosynthesis, cellular exudates, nutrient cycling, and composition of a marine microbial community. *Sci. Total Environ.* **2024**, *927*, 171977.
- (45) Stockbridge, R. B.; Wackett, L. P. The link between ancient microbial fluoride resistance mechanisms and bioengineering organofluorine degradation or synthesis. *Nat. Commun.* **2024**, *15* (1), 4593.
- (46) Dodge, A. G.; Thoma, C. J.; O'Connor, M. R.; Wackett, L. P. Recombinant *Pseudomonas* growing on non-natural fluorinated substrates shows stress but overall tolerance to cytoplasmically released fluoride anion. *mBio* **2024**, *15* (1).
- (47) Yan, P. F.; Dong, S.; Manz, K. E.; Woodcock, M. J.; Liu, C.; Mezzari, M. P.; Abriola, L. M.; Pennell, K. D.; Cápiro, N. L. Aerobic biotransformation of 6:2 fluorotelomer sulfonate in soils from two aqueous film-forming foam (AFFF)-impacted sites. *Water Res.* **2024**, *249*, 120941.
- (48) Van Hamme, J. D.; Fedorak, P. M.; Foght, J. M.; Gray, M. R.; Dettman, H. D. Use of a novel fluorinated organosulfur compound to isolate bacteria capable of carbon-sulfur bond cleavage. *Appl. Environ. Microbiol.* **2004**, *70* (3), 1487–1493.
- (49) Bottos, E. M.; AL-shabib, E. Y.; Shaw, D. M. J.; McAmmond, B. M.; Sharma, A.; Suchan, D. M.; Cameron, A. D. S.; Van Hamme, J. D. Transcriptomic response of *Gordonia* Sp. strain NB4-1Y when provided with 6:2 fluorotelomer sulfonamidoalkyl betaine or 6:2 fluorotelomer sulfonate as sole sulfur source. *Biodegradation* **2020**, *31* (4–6), 407–422.
- (50) Ren, Y.; Manefield, M. Evolution of pollutant biodegradation. *App. microb. biotechnol.* **2025**, *109* (1), 36.

- (51) Yu, Y.; Che, S.; Ren, C.; Jin, B.; Tian, Z.; Liu, J.; Men, Y. Microbial defluorination of unsaturated per- and polyfluorinated carboxylic acids under anaerobic and aerobic conditions: a structure specificity study. *Environ. Sci. Technol.* **2022**, *56* (8), 4894–4904.
- (52) Nabb, D. L.; Szostek, B.; Himmelstein, M. W.; Mawn, M. P.; Gargas, M. I.; Sweeney, L. M.; Stadler, J. C.; Buck, R. C.; Fasano, W. J. In vitro metabolism of 8-2 fluorotelomer alcohol: interspecies comparisons and metabolic pathway refinement. *Toxicol. Sci.* **2007**, *100* (2), 333–344.
- (53) Fang, B.; Zhang, Y.; Chen, H.; Qiao, B.; Yu, H.; Zhao, M.; Gao, M.; Li, X.; Yao, Y.; Zhu, L.; Sun, H. Stability and biotransformation of 6:2 fluorotelomer sulfonic acid, sulfonamide amine oxide, and sulfonamide alkylbetaine in aerobic sludge. *Environ. Sci. Technol.* **2024**, *58* (5), 2446–2457.
- (54) Liu, J.; Wang, N.; Buck, R. C.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M.; Bellin, C. A. Aerobic biodegradation of [14C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system. *Chemosphere* **2010**, *80* (7), 716–723.
- (55) Yu, Y.; Zhang, K.; Li, Z.; Ren, C.; Chen, J.; Lin, Y. H.; Liu, J.; Men, Y. Microbial cleavage of c-f bonds in two c6per- and polyfluorinated compounds via reductive defluorination. *Environ. Sci. Technol.* **2020**, *54* (22), 14393–14402.
- (56) Wang, N.; Buck, R. C.; Szostek, B.; Sulecki, L. M.; Wolstenholme, B. W. 5:3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel “one-carbon removal pathways.” *Chemosphere* **2012**, *87* (5), 527–534.
- (57) Yin, T.; Tran, N. H.; Huiting, C.; He, Y.; Gin, K. Y. H. Biotransformation of polyfluoroalkyl substances by microbial consortia from constructed wetlands under aerobic and anoxic conditions. *Chemosphere* **2019**, *233*, 101–109.
- (58) Tang, Z.; Vogel, T. M.; Wang, Q.; Wei, C.; Ali, M.; Song, X. Microbial defluorination of TFA, PFOA, and HFPO-DA by a native microbial consortium under anoxic conditions. *J. Hazard. Mater.* **2024**, *465*, 133217.
- (59) Jiao, J.; Wang, S.; Tian, H.; Xu, X.; Zhang, Y.; Liu, B.; Zhang, W. Decreased expression of *LRA4*, a key gene involved in rhamnose metabolism, caused up-regulated expression of the genes in this pathway and autophagy in *Pichia pastoris*. *AMB Express* **2020**, *10* (1), 37.
- (60) An, X.; Li, N.; Zhang, L.; Xu, Z.; Zhang, S.; Zhang, Q. New insights into the typical nitrogen-containing heterocyclic compound-quinoline degradation and detoxification by microbial consortium: integrated pathways, meta-transcriptomic analysis and toxicological evaluation. *J. Hazard. Mater.* **2024**, *465*, 133158.
- (61) Wang, H.; Kim, H.; Ki, J. S. Transcriptome survey, molecular identification, and expression analysis of stress-responsive genes in the toxic dinoflagellate *Alexandrium pacificum* under algicidal agents and metal stresses. *J. Appl. Phycol.* **2021**, *33* (5), 3139–3151.

- (62) Dong, S.; Yan, P. F.; Mezzari, M. P.; Abriola, L. M.; Pennell, K. D.; Cápiro, N. L. Using network analysis and predictive functional analysis to explore the fluorotelomer biotransformation potential of soil microbial communities. *Environ. Sci. Technol.* **2024**, *58* (17), 7480–7492.
- (63) Van Der Ploeg, J. R.; Iwanicka-Nowicka, R.; Bykowski, T.; Hryniewicz, M. M.; Leisinger, T. The *Escherichia coli* *SsuEADCB* gene cluster is required for the utilization of sulfur from aliphatic sulfonates and is regulated by the transcriptional activator Cbl*. *J. Biol. Chem.* **1999**, *274* (41), 29358–29365.
- (64) Eichhorn, E.; Leisinger, T. *Escherichia coli* utilizes methanesulfonate and l-cysteate as sole sulfur sources for growth. *FEMS Microbiol. Lett.* **2001**, *205* (2), 271–275.
- (65) Koch, D. J.; Rückert, C.; Key, D. A.; Mix, A.; Pühler, A.; Kalinowski, J. Role of the *ssu* and *seu* genes of *Corynebacterium glutamicum* ATCC 13032 in utilization of sulfonates and sulfonate esters as sulfur sources. *Appl. Environ. Microbiol.* **2005**, *71* (10), 6104–6114.
- (66) Thakur, A.; Somai, S.; Yue, K.; Ippolito, N.; Pagan, D.; Xiong, J.; Ellis, H. R.; Acevedo, O. Substrate-dependent mobile loop conformational changes in alkanesulfonate monooxygenase from accelerated molecular dynamics. *Biochemistry* **2020**, *59* (38), 3582–3593.
- (67) Kabil, O.; Banerjee, R. Characterization of patient mutations in human persulfide dioxygenase (ETHE1) involved in H₂S catabolism. *J. Biol. Chem.* **2012**, *287* (53), 44561–44667.
- (68) Zeghouf, M.; Fontecave, M.; Coves, J. A. Simplified functional version of the *Escherichia coli* sulfite reductase. *J. Biol. Chem.* **2000**, *275* (48), 37651–37656.
- (69) Jacobson, B. L.; He, J. J.; Vermersch, P. S.; Lemon, D. D.; Quioco, F. A. Engineered interdomain disulfide in the periplasmic receptor for sulfate transport reduces flexibility: site-directed mutagenesis and ligand-binding studies. *J. Biol. Chem.* **1991**, *266* (8), 5220–5225.
- (70) Marelja, Z.; Stöcklein, W.; Nimtz, M.; Leimkühler, S. A. Novel role for human *nfs1* in the cytoplasm: *nfs1* acts as a sulfur donor for *mocs3*, a protein involved in molybdenum cofactor biosynthesis. *J. Biol. Chem.* **2008**, *283* (37), 25178–25185.
- (71) Schmitz, J.; Wuebbens, M. M.; Rajagopalan, K. V.; Leimkühler, S. Role of the C-terminal Gly-Gly motif of *Escherichia coli* MoaD, a molybdenum cofactor biosynthesis protein with a ubiquitin fold. *Biochemistry* **2007**, *46* (3), 909–916.
- (72) Marek, J.; Vevodova, J.; Smatanova, I. K.; Nagata, Y.; Svensson, L. A.; Newman, J.; Takagi, M.; Damborsky, J. Crystal structure of the haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26. *Biochemistry* **2000**, *39* (46), 14082–14086.

- (73) Kim, M. H.; Wang, N.; McDonald, T.; Chu, K.-H. Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane-degrading *Pseudomonas* strains. *Biotechnol. Bioeng.* **2012**, *109* (12), 3041–3048.
- (74) Wackett, L. P. Why is the biodegradation of polyfluorinated compounds so rare? *mSphere* **2021**, *6* (5).
- (75) Skibbe, D. S.; Liu, F.; Wen, T.-J.; Yandea, M. D.; Cui, X.; Cao, J.; Simmons, C. R.; Schnable, P. S. Characterization of the aldehyde dehydrogenase gene families of *Zea mays* and *Arabidopsis*. *Plant Mol. Biol.* **2002**, *48*, 751–764.
- (76) Matsubara, Y. O.; Indo, Y.; Naito, E.; Ozasaj, H.; Glassberg, R.; Vockley, J.; Ikedall, Y.; Kraus, J.; Tanakall, K. Molecular cloning and nucleotide sequence of cDNAs encoding the precursors of rat long-chain acyl-coenzyme A short-chain acyl-coenzyme A, and isovaleryl-coenzyme A dehydrogenase. *J. Biol. Chem.* **1989**, *264* (21), 16321–16331.
- (77) Srivastava, P.; Saji, J.; Manickam, N. Biodegradation of polyethylene terephthalate (PET) by *Brucella intermedia* IITR130 and its proposed metabolic pathway. *Biodegradation.* **2024**, *35* (5), 671–685.
- (78) Liu, Y.; Liu, W.; Li, M.; Liu, S.; Peng, D.; Zhao, F.; Wu, X.; Tan, H. Biodegradation characteristics and mechanism of terbutylazine by the newly isolated *Agrobacterium rhizogenes* strain AT13. *J. Hazard. Mater.* **2023**, *456*, 131664.
- (79) Nzila, A.; Ramirez, C. O.; Musa, M. M.; Sankara, S.; Basheer, C.; Li, Q. X. Pyrene biodegradation and proteomic analysis in *Achromobacter xylosoxidans*, PY4 strain. *Int. Biodeterior. Biodeg.* **2018**, *130*, 40–47.

Supporting Information

Metatranscriptomic Insights into Aerobic Biotransformation of 6:2 Fluorotelomer Sulfonate by an Enrichment Culture under Sulfur-Limiting Conditions

Md G. Kabir,^{a,b} Zhiwen Tang,^a Xiqian Zhang,^a Frederic Coulon,^c Andreas Tiehm,^d Xin Song^{a,b,*}

^aState Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 211135, China.

^bUniversity of Chinese Academy of Sciences, Beijing 100049, China

^cSchool of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, U.K.

^dDepartment of Water Microbiology, TZW: DVGW-Technologiezentrum Wasser, 76139 Karlsruhe, Germany

*Corresponding author: Xin Song, Email: xsong@issas.ac.cn, Phone: +86-25-86881543, Fax: +86-25-86881126, 298 Chuangyou Road, Nanjing 211135, China

Summary of Supporting Information Contents:

Number of pages 29, Texts S1-S4, Tables S1-S10, Figures S1-S11

Text of Contents

Text S1: Sulfur-free acetate (SFA) medium.....	S4
Text S2: Stability of the enrichment culture.....	S4
Text S3: Metatranscriptomic analysis.....	S4
Text S4: Quality Evaluation of RNA-Sequenced Data.....	S6

Table of Contents

Table S1. Biotransformation experimental conditions of 6:2 FTS.....	S7
Table S2. Chemical names, acronyms, molecular structure, CAS no, and suppliers of target poly- and perfluoroalkyl substances (PFAS) used in this study for LC-MS/MS quantitative analysis.....	S8
Table S3. Instrument conditions and LC gradient used for the detection and quantification of PFASs by UPLC-MS/MS.....	S9
Table S4. Multiple reaction monitoring (MRM) parameters for the detection and quantification of PFASs by UPLC-MS/MS.....	S10
Table S5. Limits of detection (LODs) and limits of quantification (LOQs) of target PFAS.....	S11
Table S6. Comparison of 6:2 FTS aerobic biotransformation efficiency across enrichment cultures and environmental matrices.....	S12
Table S7. List of targeted biotransformation products identified by LC-QTOF-MS.....	S13
Table S8. List of non-targeted biotransformation products identified by Q-TOF.....	S14
Table S9. Changes in concentration (μM) of 6:2 FTS and its biotransformation products over time.....	S15
Table S10. The quality evaluation of sequenced sample data.....	S16
Tables S11-S14.....	Separate Excel file

Figure of contents

Fig. S1 Evaluation of the consortium SXC01 stability was conducted across successive enrichments with 6:2 FTS.....	S17
Fig. S2 Evaluation of the consortium (SXC01) for its ability to utilize 6:2 FTS as a carbon source.....	S18
Fig. S3 Growth curves of SXC01 consortium with 6:2 FTS at different concentrations for the evaluation of its toxicity, and 6:2 FTS as the sole sulfur source.....	S19
Fig. S4 Growth of SXC01 consortium on different concentrations of 6:2 FTS.....	S20
Fig. S5 Growth curve of <i>Brucella anthropi</i> SX009	S21
Fig. S6 Degradation efficiency of 6:2 FTS at different concentrations in microcosm by the consortium at different time intervals.....	S22
Fig. S7 Identification of biotransformation products of 6:2 FTS.....	S23
Fig. S8 Detection of the extracted-ion chromatogram (XIC) of the parent compound and biotransformed products of 6:2 FTS in the control and biodegradation group at 5 mg/L....	S24
Fig. S9 Putative Identification of target biotransformation products using LC-Q-TOF-MS.....	S25
Fig. S10 KEGG pathway analysis of <i>ssuEADCB</i> gene cluster.....	S26
Fig. S11 Sankey diagram represents the 6:2 FTS degradation functional top-upregulated DEG genes in different bacteria of SXC01.....	S27
Reference.....	S28

Text of Contents

Text S1: Sulfur-Free Acetate (SFA) Medium

The sulfur-free acetate (SFA) medium^{1,2} with the following composition per liter: 0.4 g potassium dihydrogen phosphate (KH_2PO_4), 1.6 g dipotassium phosphate (K_2HPO_4), 1.55 g ammonium chloride (NH_4Cl), 5 g Sodium acetate (NaCH_3COO), 0.165 g magnesium dichloride (MgCl_2), 0.09 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$).

Sulfur-free trace elements solution² (1 mL) was added, which contained the following composition per liter, acidified with 10 mL of 25% HCl: 1.5 g ferrous chloride tetrahydrate, 0.19 g cobalt chloride hexahydrate, 0.1 g manganese chloride tetrahydrate, 0.07 g zinc chloride, 0.006 g boric acid, 0.036 g sodium molybdate dehydrate, 0.024 g nickel chloride hexahydrate, 0.002 g copper chloride dihydrate.

Wolin vitamin solution² (1 mL) was added, which contained per liter (pH 7): 0.02 g biotin, 0.02 g folic acid, 0.1 g pyridoxine hydrochloride, 0.05 g riboflavin, 0.05 g thiamine, 0.05 g nicotinic acid, 0.05 g pantothenic acid, 0.001 g vitamin B12, 0.05 g p-aminobenzoic acid and 0.05 g thiotic acid.

Text S2: Stability of the Enrichment Culture

The stability of the enrichment culture was evaluated by monitoring 6:2 FTS degradation and fluoride generation over five successive enrichment cycles, as shown in Fig. S1. The enrichment was transferred once at each concentration before increasing to the next level. Fig. S1 A illustrates the decline in 6:2 FTS concentration over time in the presence of the enriched consortium, whereas the control showed no degradation. A consistent decrease in 6:2 FTS concentration across successive enrichments indicates the sustained metabolic activity of the consortium. Fluoride ion generation is a key indicator of defluorination. Fig. S1 B depicts a progressive increase in fluoride concentration over successive enrichments, suggesting that the consortium maintained its ability to degrade 6:2 FTS and catalyze defluorination over time. These results confirm the stability and enrichment of a microbial community capable of 6:2 FTS biotransformation.

Text S3: Metatranscriptomic Analysis

Metatranscriptomic sequencing analysis was employed to investigate the active microbial community and functional gene expression profiles during 6:2 FTS biotransformation.

Microbial samples were collected from a duplicate biodegradation experiment using 5 mg/L of 6:2 FTS, where 6:2 FTS biodegradation and fluoride release closely mirrored those observed in the main study. 10 mL of samples were collected in triplicate on Days 0, 7, and 14. Samples from Days 7 and 14 represented the active transformation phase of 6:2 FTS, while Day 0 was used as the control. However, the Day 14 sample did not pass the RNA quality control and was therefore excluded from sequencing.

Collected samples were filtered through 0.22 μm sterilized membrane filters and immediately transported to MajorBio (Shanghai, China) for total RNA extraction, rRNA removal, and library preparation. RNA was extracted and purified using a Soil RNA Extraction Kit (Majorbio, Shanghai, China), following the manufacturer's protocol. The integrity of the extracted RNA was assessed using an Agilent 5300 Bioanalyzer (Agilent Technologies, USA), while the quality was measured using a Qubit 2.0 Fluorometer (Thermo Scientific, Palo Alto, CA, USA). The cDNA libraries were prepared using the Illumina Stranded mRNA Prep, Ligation (Illumina, San Diego, CA, USA), while rRNA was depleted using a RiboCop rRNA Depletion Kit (Lexogen, USA) prior to sequencing. PCR amplification was performed on a T100 Thermal Cycler with the following thermocycling conditions: initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 10 min. Sequencing was performed on an Illumina Novaseq Xplus sequencer (Illumina, San Diego, CA, USA) using paired-end sequencing with a read length of 400 bp at Majorbio (Shanghai, China).

The fastp³ tool (version 0.19.6) (<https://github.com/OpenGene/fastp>) was used for quality trimming of adapter sequences at the 3' and 5' ends of the reads. Reads shorter than 50bp, with a quality value lower than 20, or containing N bases after trimming were removed, ensuring the retention of high-quality reads. The reads were eliminated by aligning them to the SILVA SSU (16S/18S) and SILVA LSU (23S/28S) database using the SortMeRNA⁴ (version 2.1b) (<http://bioinfo.lifl.fr/RNA/sortmerna/>) and optimized de novo sequences were assembled using Trinity⁵ (version 2.2.0) (<http://trinityrnaseq.github.io/>). The CD-HIT⁶ (<http://www.bioinformatics.org/cd-hit/>) was used to cluster and remove redundancy in transcriptomes of length ≥ 300 bp assembled sequence with 95 % identity and 90% coverage. The longest sequence in each cluster was selected as the representative sequence to construct a Unigene set for subsequent species and functional annotation. Clean reads were aligned to the Unigene set, and gene expression levels (RPKM values) were calculated by RSEM (<http://deweylab.biostat.wisc.edu/rsem/>) using transcripts per million (TPM) based on the

number of aligned reads. The assembled sequences of upregulated differentially expressed genes (DEGs) were annotated by comparison to the NCBI Non-redundant Protein (NR) database using the BLASTP algorithm with an e-value threshold of $<1e^{-5}$ to predicted gene descriptions.⁷

For taxonomic annotation, the best-hit taxonomy of unigenes was determined by aligning them against the NCBI NR (non-redundant) database using DIAMOND⁸ (version 0.8.35; <http://www.diamondsearch.org/index.php>) with the same e-value $<1e^{-5}$ cutoff. Species composition and relative abundance of microbes were determined by comparing unigenes with taxonomic information. In contrast, relative abundance was expressed as a percentage of the total annotated transcript reads within a sample. Similar transcript-based methods for taxonomic profiling have been applied in previous studies.^{9,10} Open reading frames (ORFs) were predicted using TransGeneScan¹¹ (<http://sourceforge.net/projects/transgenescan/>). Functional annotation of unigenes was conducted using the KEGG (Kyoto Encyclopedia of Genes and Genomes, <http://www.genome.jp/kegg/>) database. Differentially expressed genes (DEGs) between samples were identified based on unigene expression levels using DESeq2,¹² with statistical significance determined at P-adjust < 0.05 and ≥ 2 log2-fold change (FC) threshold.

Text S4: Quality Evaluation of RNA-Sequenced Data

The quality of RNA-sequenced data was assessed through multiple parameters to ensure the reliability of downstream analyses (Table S10). Raw reads represent the total number of sequencing reads obtained directly from the sequencing platform before preprocessing. Clean reads were generated after implementing quality control measures, including adapter trimming, removal of low-quality reads, and elimination of sequences containing ambiguous bases (N), utilizing the fastp tool. Non-rRNA reads were obtained by filtering out ribosomal RNA sequences using the SILVA SSU (16S/18S) and SILVA LSU (23S/28S) databases to retain only mRNA sequences for transcriptomic analysis. The GC content (%) was calculated as the proportion of guanine (G) and cytosine (C) bases relative to the total bases in the clean reads, using the fastq tool. Additionally, sequencing accuracy was evaluated through $>Q20$ (%) and $>Q30$ (%) scores, which indicate the proportion of bases with quality scores exceeding 20 and 30, respectively, calculated using the fastq tool.

Supporting Tables

Table S1: Biotransformation Experimental Conditions of 6:2 FTS

	Condition name	Spiking	Consortium	Spiking concentration (mg/L)
Blank	SXC01		SXC01	
Control	6:2 FTS	6:2 FTS		0.5, 2.5, 5.0, 25.0
Biodegradation	SXC01 + 6:2 FTS	6:2 FTS	SXC01	0.5, 2.5, 5.0, 25.0

Table S2. Chemical Names, Acronyms, Molecular Structure, CAS No., and Suppliers of Target Poly- and Perfluoroalkyl Substances (PFAS) Used in This Study For LC-MS/MS Quantitative Analysis

Chemical name	Acronym	Molecular structure	Molecular weight (g/mol)	CAS No.	Supplier
6:2 fluorotelomer sulfonate	6:2 FTS	$F(CF_2)_6CH_2CH_2SO_3$	428.16	27619-97-2	Sigma-aldrich (St. Louis, USA)
6:2 fluorotelomer sulfonate	6:2 FTS	$F(CF_2)_6CH_2CH_2SO_3$	428.16	27619-97-2	PFAC-HIF-ES stock, Wellington Laboratories
Perfluorobutanoic acid	PFBA	$F(CF_2)_3COOH$	214.039	375-22-4	
Perfluoropentanoic acid	PFPeA	$F(CF_2)_4COOH$	264.05	2706-90-3	
Perfluorohexanoic acid	PFHxA	$F(CF_2)_5COOH$	314.054	307-24-4	
Perfluoroheptanoic acid	PFHpA	$F(CF_2)_6COOH$	364.06	375-85-9	
6:2 Fluorotelomer Alcohol	6:2 FTOH	$F(CF_2)_6CH_2CH_2OH$	364.11	647-42-7	Weng Jiang Reagent
6:2 fluorotelomer saturated carboxylic acid	6:2 FTCA	$F(CF_2)_6CH_2COOH$	378.09	53826-12-3	Wellington Laboratories
6:2 fluorotelomer unsaturated carboxylic acid	6:2 FTUCA	$F(CF_2)_5CF=CHCOOH$	358.08	70887-88-6	Weng Jiang Reagent
5:3 fluorotelomer carboxylic acid	5:3 FTCA	$F(CF_2)_5CH_2CH_2COOH$	342.11	914637-49-3	Weng Jiang Reagent
5:3 Fluorotelomer unsaturated carboxylic acid	5:3 FTUCA	$F(CF_2)_5CH=CHCOOH$	340.09	875878-70-9	-
6:2 fluorotelomer aldehyde	6:2 FTAL	$F(CF_2)_6CHCHOH$	362.09	56734-81-7	-
4:2 Fluorotelomer carboxylic acid	4:2 FTCA	$F(CF_2)_4CH_2COOH$	278.07	-	-

Table S3. Instrument Conditions and LC Gradient Were Used For The Detection and Quantification of PFASs By UPLC-MS/MS

Lc system	LC-MS AB 5500 (Sciex, USA)																																				
Column	Water BEH C-18 analytical column (100 mm × 3.0 mm, 1.7 μm dia)																																				
Column temp.	40 ⁰ C																																				
Sample temp.	15 ⁰ C																																				
Injection volume	3 μL																																				
Mobile phase A	2 mM Ammonium acetate																																				
Mobile phase B	Acetonitrile																																				
LC gradient conditions																																					
	<table><tr><th>Time (Min)</th><th>Flow rate (mL/min)</th><th>A %</th><th>B %</th></tr><tr><td>0.00</td><td>0.300</td><td>78</td><td>22</td></tr><tr><td>1.00</td><td>0.300</td><td>78</td><td>22</td></tr><tr><td>12.00</td><td>0.300</td><td>10</td><td>90</td></tr><tr><td>14.00</td><td>0.300</td><td>10</td><td>90</td></tr><tr><td>14.50</td><td>0.300</td><td>0</td><td>100</td></tr><tr><td>15.50</td><td>0.300</td><td>0</td><td>100</td></tr><tr><td>16.00</td><td>0.300</td><td>78</td><td>22</td></tr><tr><td>20.00</td><td>0.300</td><td>78</td><td>22</td></tr></table>	Time (Min)	Flow rate (mL/min)	A %	B %	0.00	0.300	78	22	1.00	0.300	78	22	12.00	0.300	10	90	14.00	0.300	10	90	14.50	0.300	0	100	15.50	0.300	0	100	16.00	0.300	78	22	20.00	0.300	78	22
Time (Min)	Flow rate (mL/min)	A %	B %																																		
0.00	0.300	78	22																																		
1.00	0.300	78	22																																		
12.00	0.300	10	90																																		
14.00	0.300	10	90																																		
14.50	0.300	0	100																																		
15.50	0.300	0	100																																		
16.00	0.300	78	22																																		
20.00	0.300	78	22																																		
MS conditions																																					
MS system	AB TripleQuad																																				
Ionization mode	ESI-																																				
Capacitv voltage	0.5 kV																																				
Desolvation temp	350 ⁰ C																																				
Desolvation gas flow	650 L/hr																																				
Source temp	150 ⁰ C																																				
Cone gas flow	20 L/hr																																				

Table S4. Multiple Reaction Monitoring (MRM) Parameters For The Detection and Quantification of PFASs by UPLC-MS/MS AB 5500 (Sciex, USA)

Analytes	Molecular	Cone	Collision	Quant.	Cone	Collision
	Ion (m/z)	Voltage (V)	Energy (V)	Ion (m/z)	Voltage (V)	Energy (V)
6:2 FTS	427	-20	-34	407	-20	-52
5:3 FTCA	341	-40	-15	237	-40	-35
5:3 FTUCA	339	-20	-25	269	-20	-25
6:2 FTCA	377	-40	-15	293	-40	-35
6:2 FTUCA	357	-20	-25	293	-20	-25
6:2 FTOH	423	-70	-27	236.9	-70	-27
4:2 FTCA	291	-30	-30	187	-30	-30
6:2 FTAL	361	-40	-30	141	-40	-30
PFBA	213	-30	-11	168.9	-30	-13
PFPeA	263	-40	-11	218.9	-40	-11
PFHxA	312.9	-35	-11	268.9	-35	-11
PFHpA	362.9	-30	-13	318.9	-30	-13

Table S5. Limits of Detection (LODs) and Limits of Quantification (LOQs) of Target PFAS

Analytes	LOD (µg/L)	LOQ (µg/L)
6:2FTS	0.002	0.005
5:3 FTCA	0.024	0.081
5:3 FTUCA	0.059	0.195
6:2 FTCA	0.043	0.143
6:2 FTUCA	0.020	0.068
6:2 FTOH	0.34	6.01
PFBA	0.004	0.013
PFPeA	0.003	0.011
PFHxA	0.004	0.013
PFHpA	0.003	0.010

Table S6. Comparison of 6:2 FTS Aerobic Biotransformation Efficiency Across Enrichment Cultures and Environmental Matrices

Source of Microbial consortium	Culture condition	Initial spiking (mg/L)	Biotransformation Efficiency	Duration	Biotransformation products	Reference
AFFF Soil (Robins)	-	1.7 mg/L	36.30%	224 d	6:2 FTUA, 6:2 FTCA, 5:2 sFTOH, 5:3 acid, PFBA, PFPeA, PFHxA	13
AFFFSoil (Loring)	-	1.7 mg/L	99.80%	224 d	6:2 FTUA, 5:2 sFTOH, 5:3 acid, PFBA, PFPeA, PFHxA, PFHpA	13
Aerobic sludge	-	1.2 mg/L	≥ 99%	60 d	6:2 FTUSA, 4:3 FTCA, 5:3 FTCA, PFBA, PFPeA, PFHxA, PFHpA	14
Landfill leachate	-	0.65 mg/L	21%	60 d	PFBA, PFPeA, PFHxA, PFHpA	15
Wetland slurry	-	8 mg/L	8.90%	142 d	5:3 acid, PFPeA, PFHxA	16
River sediment	-	1.3 mg/L	98.10%	90 d	6:2 FTOH, 6:2 FTCA, 5:2 sFTOH, 5:2 ketone, 5:3 acid, PFPeA, PFHxA, PFHpA	17
Activated sludge	-	1.8-2.6 mg/L	36.30%	90 d	5:2 ketone, 5:2 sFTOH, 5:3 acid, PFBA, PFPeA, PFHxA	18
Acclimatized enrichment consortium	S-free conditions	0.5 - 5 mg/L	≥ 99%	14 d	6:2 FTOH, 5:3 FTCA, 5:3 FTUCA, 6:2 FTCA, 6:2 FTUCA, 6:2 FTAL, 4:2 FTCA, PFBA, PFPeA, PFHxA, PFHpA	This study

Table S7. List of Targeted Biotransformation Products Identified by LC-QTOF-MS

Sl. No.	Acronym	Formula	m/z		Mass Error (ppm)	RT (min)	Peak area
Parent compound							
1	6:2 FTS	C ₈ H ₅ F ₁₃ O ₃ S	426.96790	426.96796	+0.12	12.01	1.06E+05
Biotransformation products							
1	PFBA	C ₄ HF ₇ O ₂	212.97920	212.97926	+0.27	3.58	6.85E+03
2	PFPeA	C ₅ HF ₉ O ₂	262.97601	262.97599	-0.06	6.57	1.98E+04
3	PFHxA	C ₆ HF ₁₁ O ₂	312.97281	312.97292	+0.35	8.98	1.18E+05
4	PFHpA	C ₇ HF ₁₃ O ₂	362.96962	362.96967	+0.15	10.93	7.16E+04
5	4:2 FTCA	C ₆ H ₃ F ₉ O ₂	276.99166	276.98860	-1.90	0.87	6.25E+05
6	5:3 FTCA	C ₈ H ₅ F ₁₁ O ₂	341.00411	341.00400	-0.33	10.29	2.17E+05
7	5:3 FTUCA	C ₈ H ₃ F ₁₁ O ₂	338.98846	338.98860	+0.4	10.37	1.02E+06
8	6:2 FTCA	C ₈ H ₃ F ₁₃ O ₂	376.98527	376.98498	-0.76	10.46	2.21E+04
9	6:2 FTUCA	C ₈ H ₂ F ₁₂ O ₂	356.97904	356.97908	+0.11	10.36	5.59E+05
10	6:2 FTOH	C ₈ H ₅ F ₁₃ O	363.00600	363.00571	-1.27	0.88	3.73E+04
11	6:2 FTAL	C ₈ H ₃ F ₁₃ O	360.99035	360.98956	-2.20	0.96	5.63E+05

Table S8. List of Non-targeted Biotransformation Products Identified by Q-TOF

Sl. No.	Peak Area	Retention Time	Precursor Mass	Formula Finder Results	Formula Finder Score
1	3.07E+06	10.36	292.983	C ₇ HF ₁₁	95.776
2	6.01E+05	0.99	246.982	C ₅ HF ₉ O	85.154
3	3.45E+05	8.98	268.984	C ₅ HF ₁₁	93.273
4	2.17E+05	10.29	341.004	C ₈ H ₅ F ₁₁ O ₂	96.672
5	1.87E+05	10.29	236.996	C ₇ H ₂ F ₈	97.519
6	1.19E+05	8.98	312.974	C ₆ HF ₁₁ O ₂	94.005
7	6.23E+04	6.57	218.987	C ₄ HF ₉	82.581
8	5.45E+04	8.98	246.981	C ₅ HF ₉ O	91.255
9	1.75E+04	1.49	147.021	C ₇ H ₄ N ₂ O ₂	85.442
10	1.47E+04	4.76	316.987	C ₈ H ₃ F ₉ O ₃	92.649

Table S9. Changes in Concentration (µm) of 6:2 FTS and its Biotransformation Products Over Time

Day	6:2 FTS	6:2 FTOH	5:3 FTCA	5:3 FTUCA	6:2 FTCA	6:2 FTUCA	6:2 FTAL	4:2 FTCA	PFBA	PFPeA	PFHxA	PFHpA
Control group												
0	12.799 ± 0.420	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.001	0.010 ± 0.002	0.010 ± 0.003
3	12.519 ± 0.317	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.001	0.010 ± 0.001	0.009 ± 0.001
7	12.892 ± 0.306	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.001	0.011 ± 0.002	0.009 ± 0.000
14	13.064 ± 0.221	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.001	0.012 ± 0.000	0.009 ± 0.002
21	12.799 ± 0.227	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.001	0.012 ± 0.002	0.009 ± 0.001
28	12.869 ± 1.087	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.002 ± 0.001	0.012 ± 0.001	0.008 ± 0.002
Biodegradation group												
0	12.301 ± 0.054	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.001 ± 0.003	0.012 ± 0.003	0.011 ± 0.004
3	10.417 ± 0.679	0.027 ± 0.013	0.006 ± 0.003	0.208 ± 0.025	0.059 ± 0.030	0.030 ± 0.002	N.D.	N.D.	N.D.	0.003 ± 0.002	0.010 ± 0.002	0.009 ± 0.005
7	3.153 ± 0.210	0.164 ± 0.062	0.207 ± 0.064	0.475 ± 0.062	0.540 ± 0.041	1.170 ± 0.410	N.D.	N.D.	0.001 ± 0.001	0.004 ± 0.001	0.027 ± 0.018	0.015 ± 0.005
14	0.056 ± 0.005	0.326 ± 0.124	0.414 ± 0.127	0.944 ± 0.124	1.080 ± 0.082	2.340 ± 0.821	N.D.	N.D.	0.002 ± 0.002	0.008 ± 0.004	0.054 ± 0.035	0.030 ± 0.014
21	0.033 ± 0.000	0.449 ± 0.113	0.526 ± 0.148	1.187 ± 0.152	1.116 ± 0.143	3.048 ± 0.925	N.D.	N.D.	0.005 ± 0.002	0.015 ± 0.004	0.106 ± 0.044	0.043 ± 0.019

28	0.033 ± 0.000	0.459 ± 0.138	0.511 ± 0.054	1.164 ± 0.055	1.091 ± 0.059	3.680 ± 0.358	N.D.	N.D.	0.005 ± 0.001	$0.017 \pm$	$0.144 \pm$	$0.064 \pm$
										0.005	0.063	0.009

Table S10. The Quality Evaluation of Sequenced Sample Data

Samples	Raw reads	Clean reads	Non- rRNA reads	GC content (%)	> Q20 (%)	> Q30 (%)
Day 0 R1	70,753,964	81,660,126	47,466,488	45.54	98.93	96.48
Day 0 R2	82,657,976	68,300,908	39,861,914	46.58	98.88	96.33
Day 0 R3	73,060,758	72,247,888	41,383,556	45.42	98.94	96.51
Day 7 R1	97,199,860	95,413,618	63,216,536	54.77	98.74	95.98
Day 7 R2	94,136,948	92,196,454	55,169,958	53.62	98.74	96.05
Day 7 R3	79,213,562	77,931,612	48,344,344	53.77	98.76	96.04

#R = Replicates

Tables S11-S14: Excel file

Table S11. KEGG-Based functional annotation of Differentially Expressed Genes (DEGs) and their associated metabolic pathways

Table S12. Differentially expressed transcripts with predicted functional genes and their taxonomic assignment

Table S13. KEGG-Based functional annotation of the most upregulated DEGs and their associated metabolic pathways on Day 7 of 6:2 FTS biotransformation

Table S14. Bacterial taxa associated with the most upregulated differentially expressed transcripts on Day 7 of 6:2 FTS biotransformation

Supporting figures

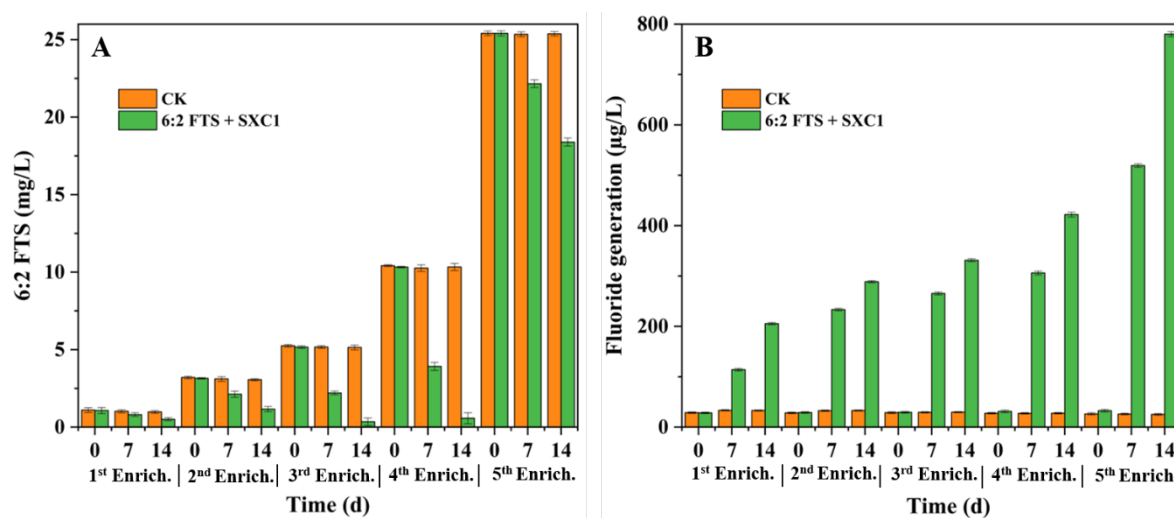


Fig. S1 Evaluation of the consortium SXC01 stability was conducted across successive enrichments with 6:2 FTS. (A) 6:2 FTS degradation across different enrichment stages. (B) Fluoride generation corresponds to different enrichment stages. Error bars represent the standard error of three replications.

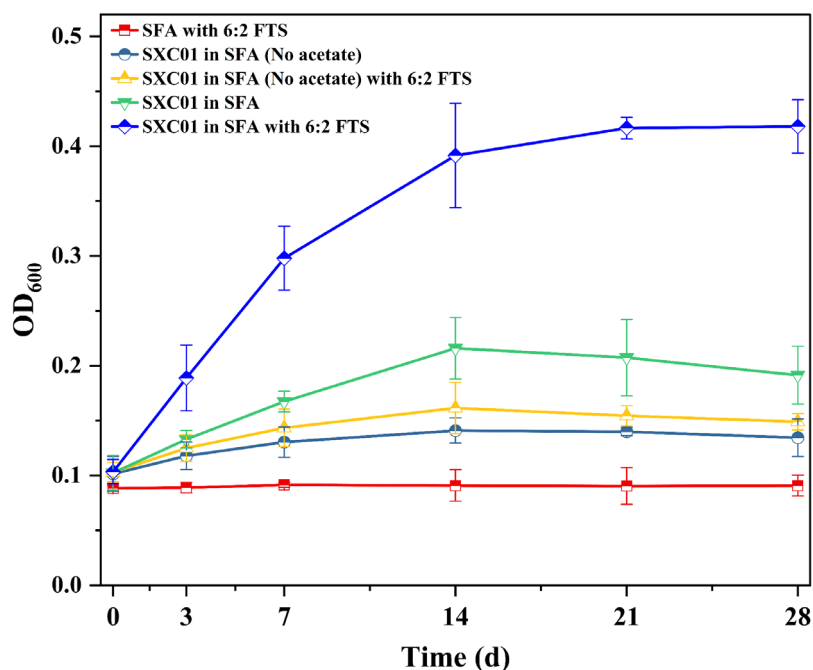


Fig. S2 Evaluation of the consortium (SXC01) for its ability to utilize 6:2 FTS as a carbon source. The consortia did not exhibit significant growth in the culture media without acetate (SFA (No acetate) with or without 6:2FTS, yellow and dark green lines), while significant growth was observed in the presence of acetate (SXC01 in SFA, bright green line), suggesting that acetate serves as the primary carbon source. The growth was further significantly increased with the presence of 6:2 FTS in the SFA media (SXC01 in SFA with 6:2FTS, blue line), suggesting 6:2FTS is the primary sulfur source, a key cofactor for the consortium growth.

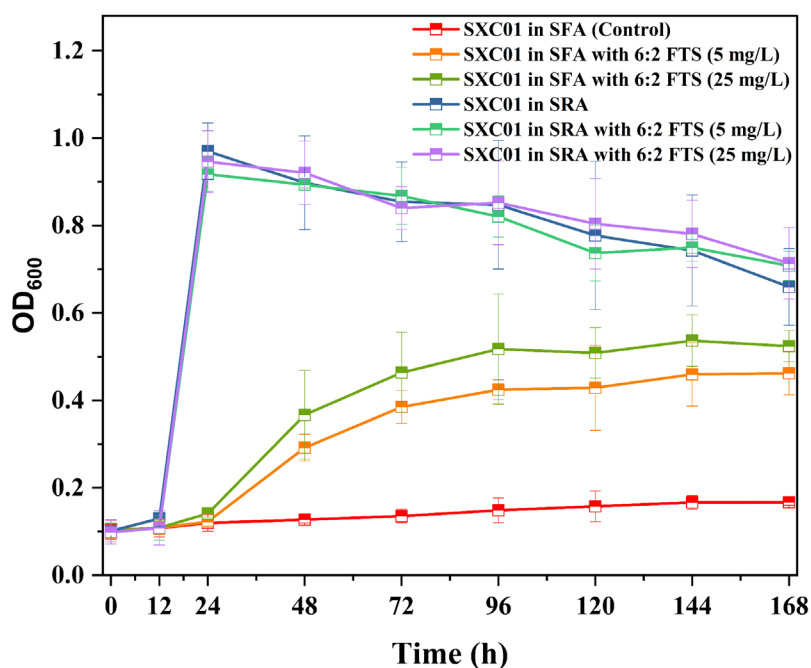


Fig. S3 Growth curves of SXC01 consortium with 6:2 FTS at different concentrations for the evaluation of its toxicity, and 6:2 FTS as the sole sulfur source. **Note:** SRA refers to the media in which MgCl_2 in the original SFA was replaced by MgSO_4 (0.165 g/L) with the equivalent of Mg concentration in MgCl_2 .

No significant growth was observed in sulfur-free medium without 6:2 FTS or MgSO_4 (red line), while robust growth was observed in the presence of MgSO_4 , with or without 6:2 FTS (blue, green, and purple lines), confirming that MgSO_4 serves as a readily assimilable sulfur source. Moderate growth was observed when 6:2 FTS was the sole sulfur source, with higher biomass at 25 mg/L (olive green line) than at 5 mg/L (orange line), indicating that 6:2 FTS supports growth in a concentration-dependent manner. Notably, co-supplementation with MgSO_4 further enhanced growth at both concentrations, suggesting that the cultures remained sulfur-limited and were not adversely affected by 6:2 FTS or its transformation products.

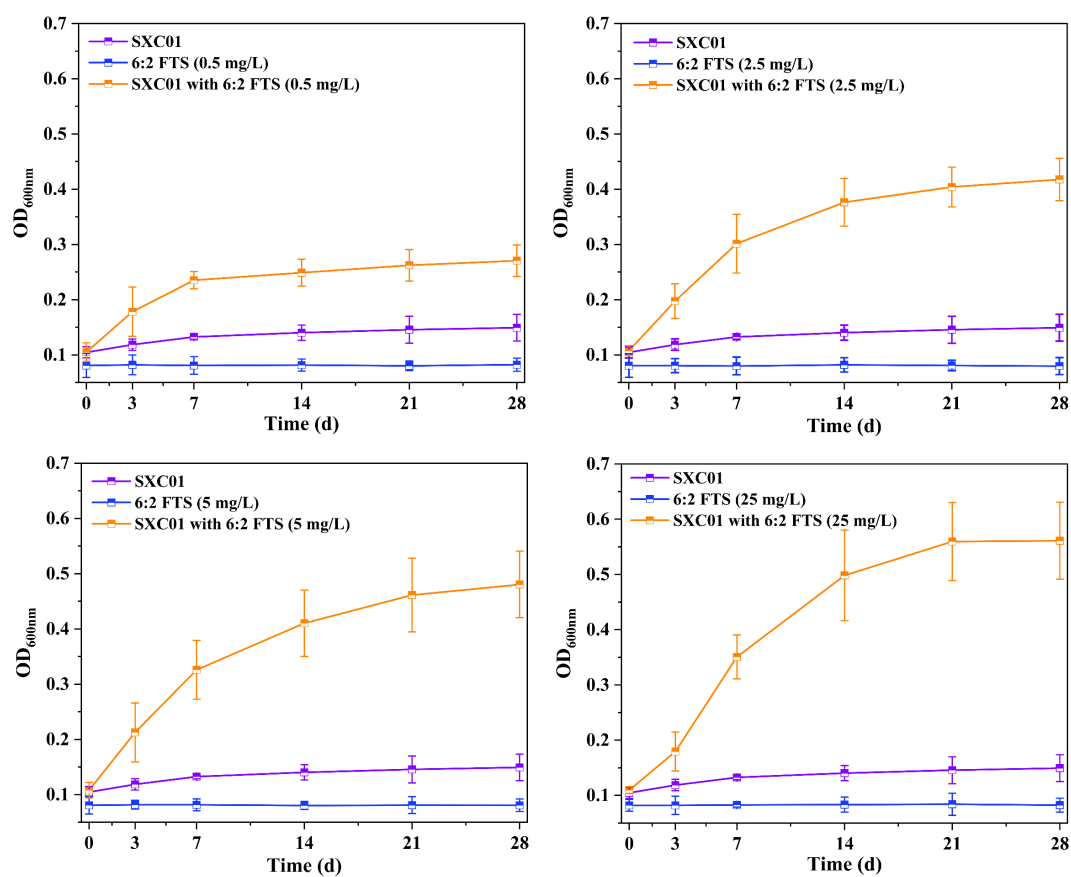


Fig. S4 Growth of SXC01 consortium on different concentrations of 6:2 FTS. A. 0.5 mg/L, B. 2.5 mg/L, C. 5 mg/L, D. 25 mg/L. Error bars represent the standard error of three replications.

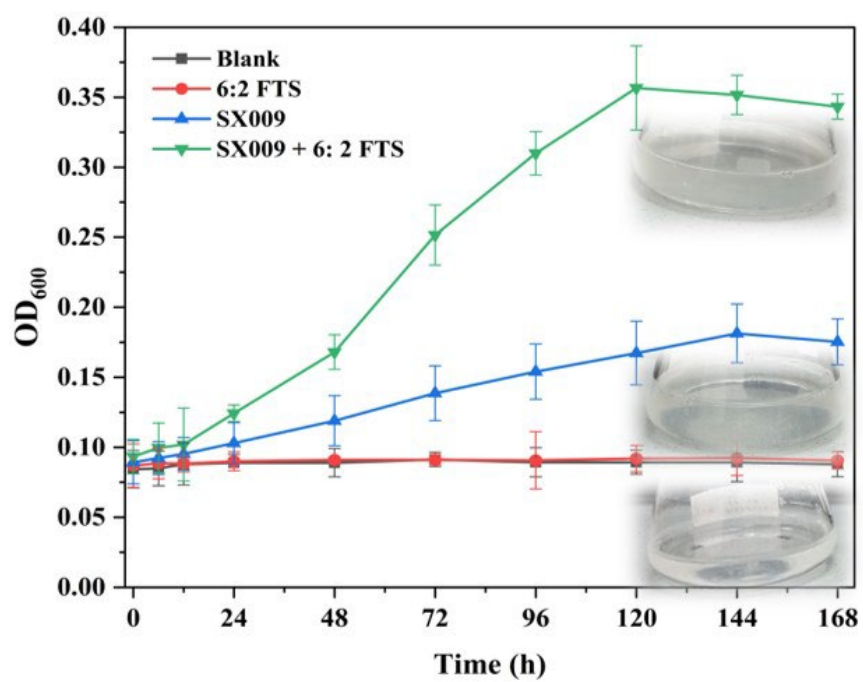


Fig S5. Growth curve of *Brucella anthropi* SX009

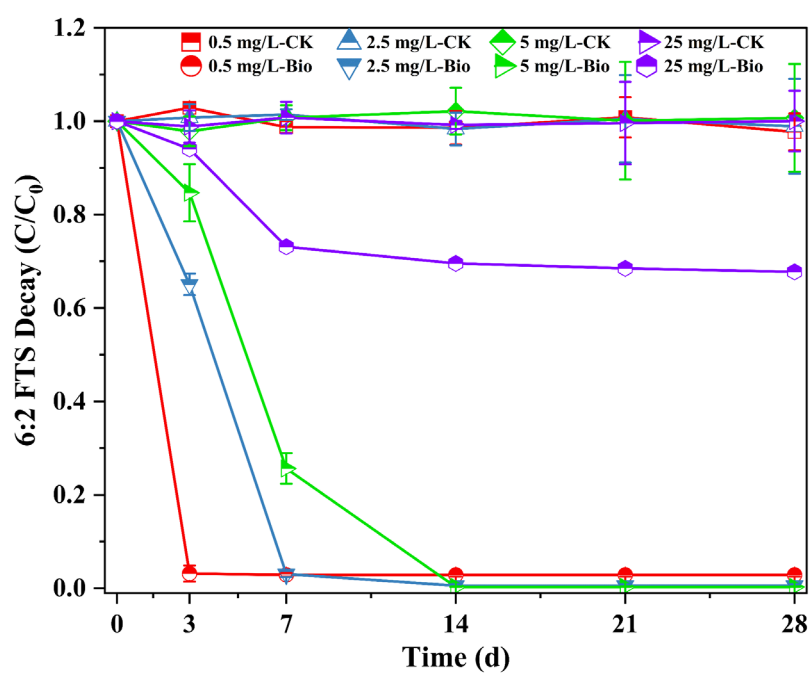
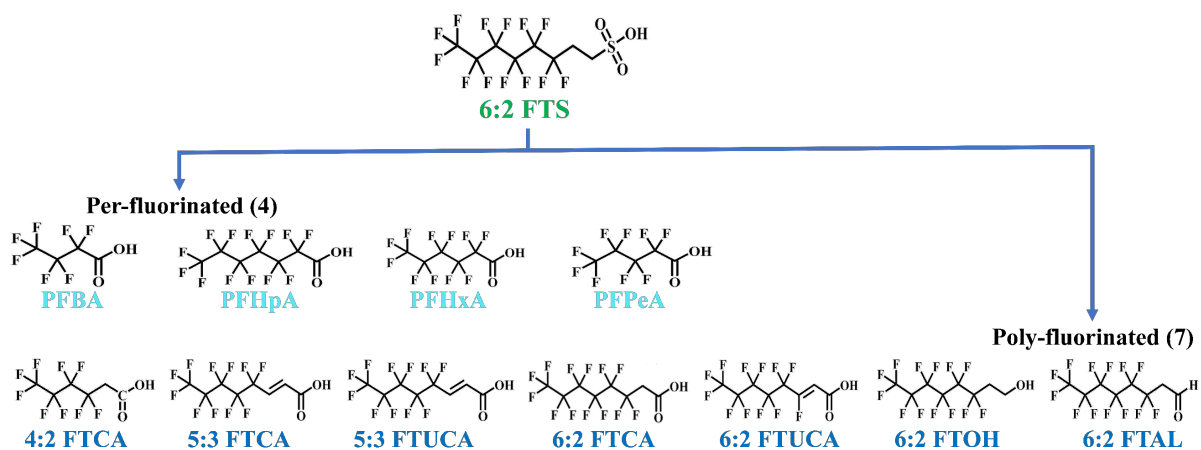


Fig. S6 Degradation efficiency of 6:2 FTS at different concentrations in microcosm by the consortium at different time intervals. Error bars represent the standard error of three replications.



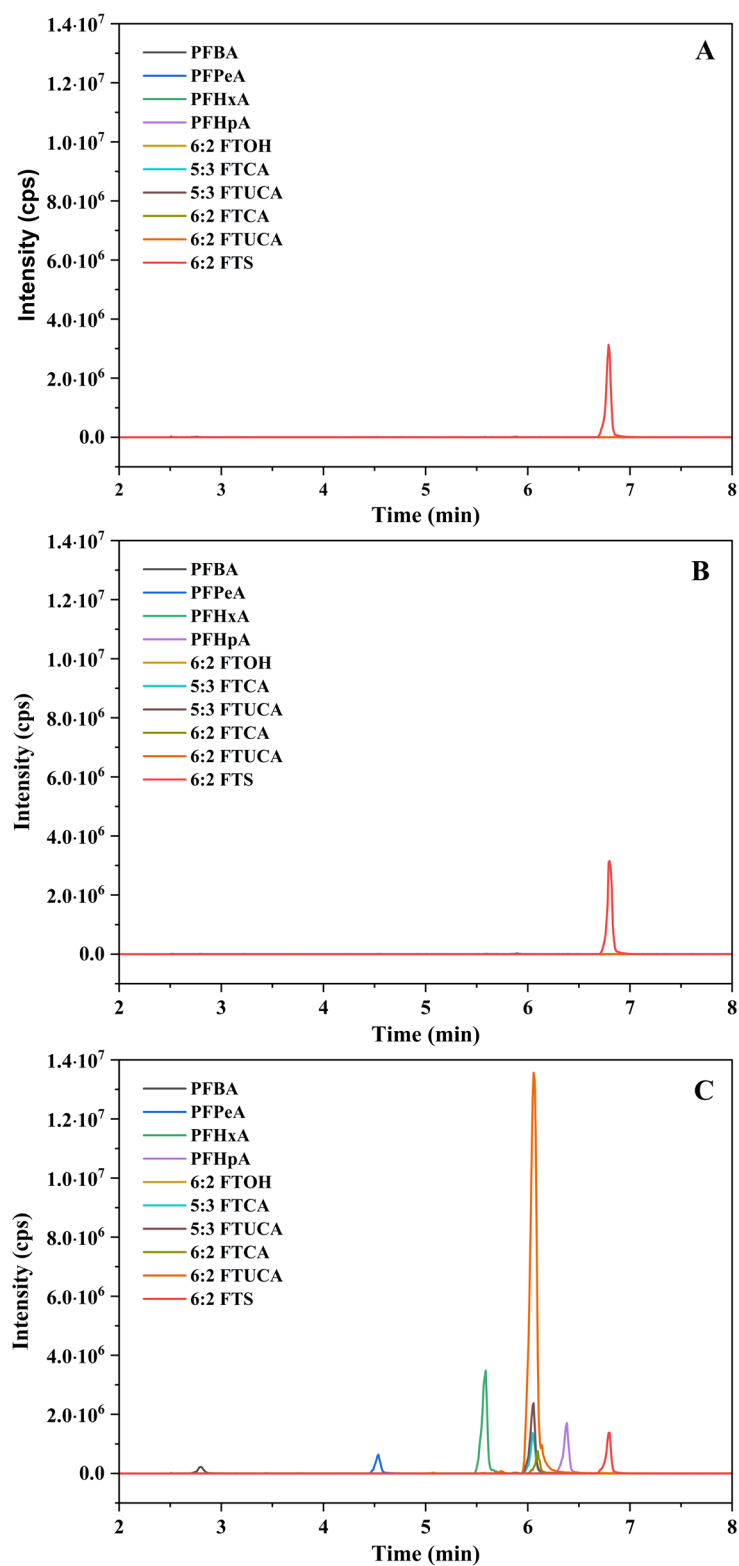


Fig. S8 Detection of the extracted-ion chromatogram (XIC) of the parent compound and biotransformation products of 6:2 FTS in the control and biodegradation group at 5 mg/L. A. Control (14 days); B. Biodegradation (0 day); C. Biodegradation (14 days)

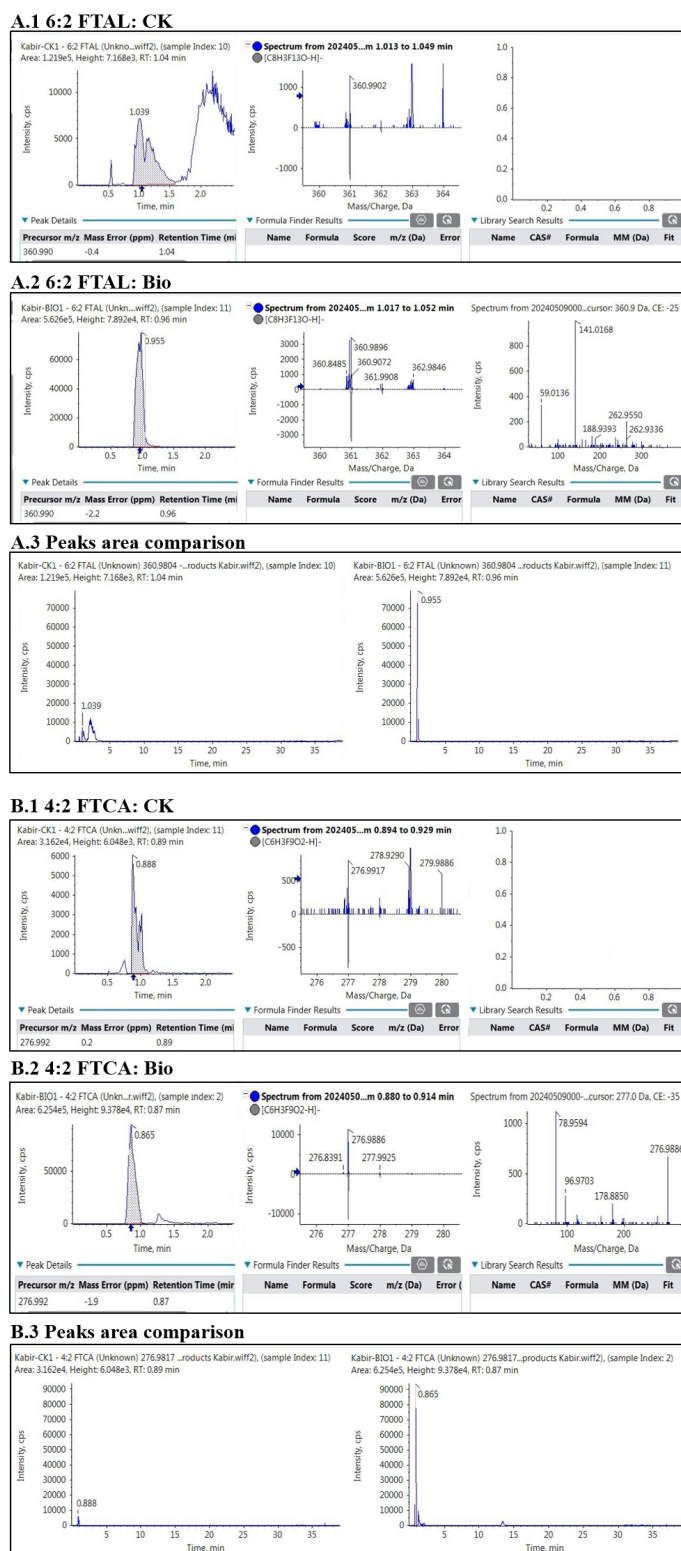


Fig. S9 Putative identification of target biotransformation products using LC-QTOF-MS. A.1-A.3: 6:2 FTAL, B.1-B.3: 4:2 FTCA. A1 and B1: Control and A2 and B2: biodegradation group, while A3 and B3 show peaks in area comparison between the control and biodegradation groups.

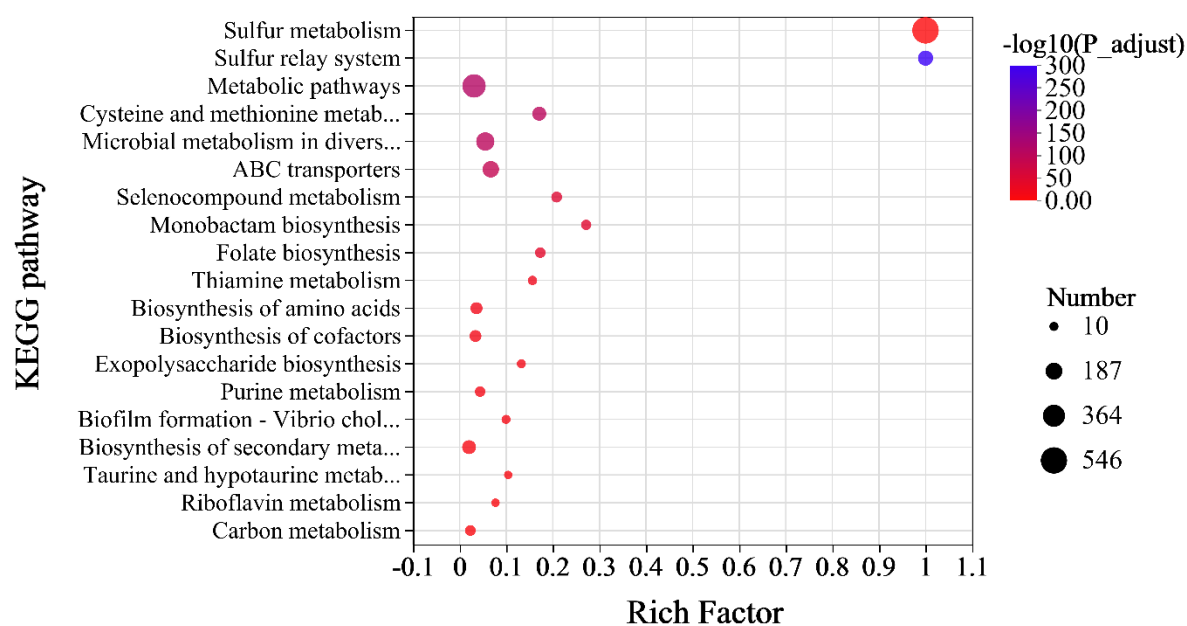


Fig. S10 KEGG pathway analysis of *ssuEADCB* gene cluster. Functional enrichment analysis using the KEGG pathway of the *ssu* gene cluster and the hypergeometric distribution algorithm to obtain the functions of significantly enriched genes in the gene set.

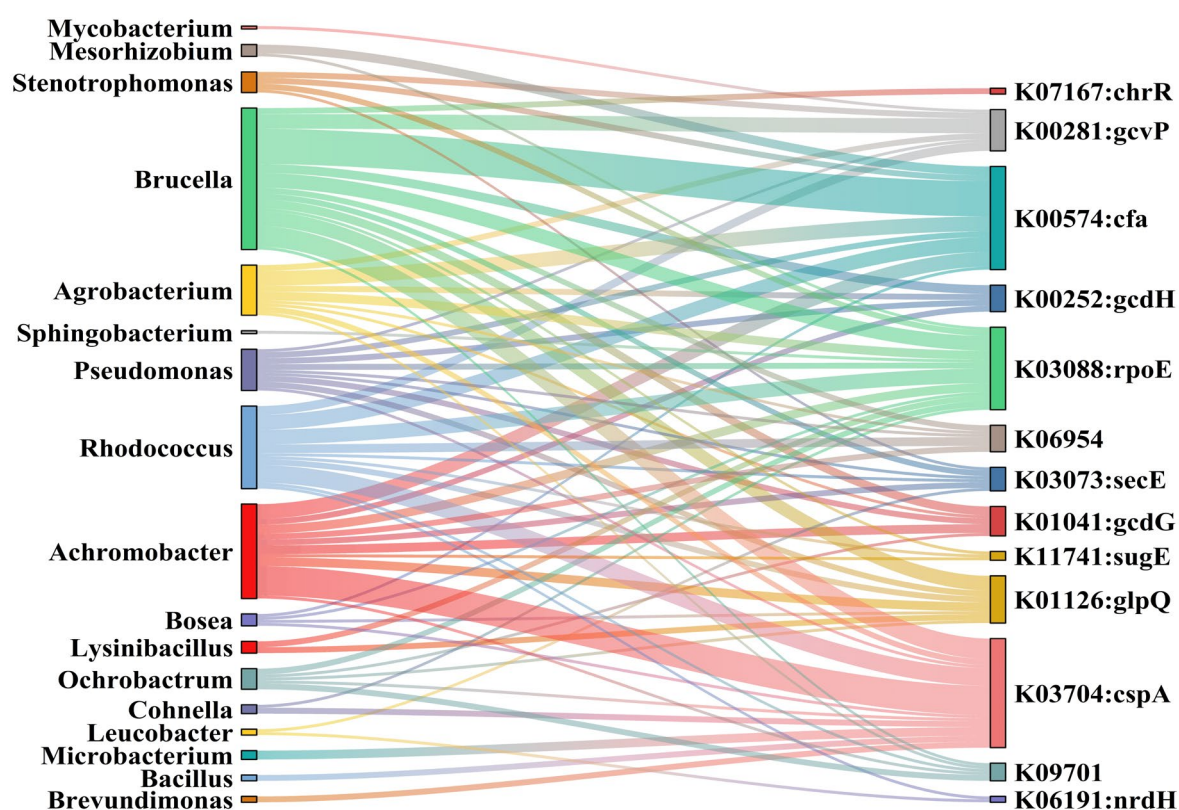


Fig. S11 Sankey diagram represents the 6:2 FTS degradation functional top-upregulated DEG genes in different bacteria of SXC01. Different colors represent different species, and the column length represents the proportion of the species.

Reference

- (1) Van Hamme, J. D.; Bottos, E. M.; Bilbey, N. J.; Brewer, S. E. Genomic and proteomic characterization of *Gordonia* Sp. NB4-1Y in relation to 6:2 fluorotelomer sulfonate biodegradation. *Microbiology* **2013**, *159* (8), 1618–1628.
- (2) Méndez, V.; Holland, S.; Bhardwaj, S.; McDonald, J.; Khan, S.; O’Carroll, D.; Pickford, R.; Richards, S.; O’Farrell, C.; Coleman, N.; Lee, M.; Manefield, M. J. Aerobic biotransformation of 6:2 fluorotelomer sulfonate by *Dietzia aurantiaca* J3 under sulfur-limiting conditions. *Sci. Total Environ.* **2022**, *829*, 154587.
- (3) Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An Ultra-Fast All-in-one FASTQ preprocessor. In *Bioinformatics*; Oxford University Press, **2018**, *34*, i884–i890.
- (4) Kopylova, E.; Noé, L.; Touzet, H. SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* **2012**, *28* (24), 3211–3217.
- (5) Haas, B. J.; Papanicolaou, A.; Yassour, M.; Grabherr, M.; Blood, P. D.; Bowden, J.; Couger, M. B.; Eccles, D.; Li, B.; Lieber, M.; Macmanes, M. D.; Ott, M.; Orvis, J.; Pochet, N.; Strozzi, F.; Weeks, N.; Westerman, R.; William, T.; Dewey, C. N.; Henschel, R.; Leduc, R. D.; Friedman, N.; Regev, A. De Novo transcript sequence reconstruction from RNA-Seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **2013**, *8* (8), 1494–1512.
- (6) Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **2012**, *28* (23), 3150–3152.
- (7) Conesa, A.; Götz, S.; García-Gómez, J. M.; Terol, J.; Talón, M.; Robles, M. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **2005**, *21* (18), 3674–3676.
- (8) Buchfink, B.; Xie, C.; Huson, D. H. Fast and Sensitive protein alignment using DIAMOND. *Nat. Methods.*, **2014**, *12*, 59–60.
- (9) Han, Y.; Zhang, M.; Chen, X.; Zhai, W.; Tan, E.; Tang, K. Transcriptomic evidences for microbial carbon and nitrogen cycles in the deoxygenated seawaters of Bohai Sea. *Environ. Int.* **2022**, *158*, 106889.
- (10) Mamtimin, T.; Han, H.; Khan, A.; Feng, P.; Zhang, Q.; Ma, X.; Fang, Y.; Liu, P.; Kulshrestha, S.; Shigaki, T.; Li, X. Gut microbiome of Mealworms (*Tenebrio Molitor*

- larvae) show similar responses to polystyrene and corn straw diets. *Microbiome* **2023**, *11* (1), 98.
- (11) Fang, S.; Cao, W.; Wu, Q.; Cheng, S.; Yang, Y.; Liu, J.; Wu, Y.; Fang, F.; Feng, Q.; Cao, J.; Luo, J. Multifaceted roles of methylisothiazolinone intervention in sludge disintegration and acidogenic and methanogenic pathways for efficient carboxylate production during anaerobic fermentation. *Chem. Eng. J.* **2023**, *472*, 145022.
 - (12) Love, M. I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15* (12), 550.
 - (13) Yan, P. F.; Dong, S.; Manz, K. E.; Woodcock, M. J.; Liu, C.; Mezzari, M. P.; Abriola, L. M.; Pennell, K. D.; Cápiro, N. L. Aerobic biotransformation of 6:2 fluorotelomer sulfonate in soils from two aqueous film-forming foam (AFFF)-impacted sites. *Water Res.* **2024**, *249*, 120941.
 - (14) Fang, B.; Zhang, Y.; Chen, H.; Qiao, B.; Yu, H.; Zhao, M.; Gao, M.; Li, X.; Yao, Y.; Zhu, L.; Sun, H. Stability and biotransformation of 6:2 fluorotelomer sulfonic acid, sulfonamide amine oxide, and sulfonamide alkylbetaine in aerobic sludge. *Environ. Sci. Technol.* **2024**, *58* (5), 2446–2457.
 - (15) Hamid, H.; Li, L. Y.; Grace, J. R. Aerobic biotransformation of fluorotelomer compounds in landfill leachate-sediment. *Sci. Total Environ.* **2020**, *713*, 136547.
 - (16) Yin, T.; Tran, N. H.; Huiting, C.; He, Y.; Gin, K. Y. H. Biotransformation of polyfluoroalkyl substances by microbial consortia from constructed wetlands under aerobic and anoxic conditions. *Chemosphere* **2019**, *233*, 101–109.
 - (17) Zhang, S.; Lu, X.; Wang, N.; Buck, R. C. Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. *Chemosphere* **2016**, *154*, 224–230.
 - (18) Wang, N.; Liu, J.; Buck, R. C.; Korzeniowski, S. H.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* **2011**, *82* (6), 853–858.



DSPACE

<https://dspace.org/>

Metatranscriptomic insights into aerobic biotransformation of 6:2 fluorotelomer sulfonate by an enrichment culture under sulfur-limiting conditions

Kabir, Md G.; Tang, Zhiwen; Zhang, Xiqian; Coulon, Frederic; Tiehm, Andreas ...

2025-09

American Chemical Society (ACS)

<https://doi.org/10.1021/acs.est.5c05531>

Kabir MG, Tang Z, Zhang X, et al., (2025) Metatranscriptomic insights into aerobic biotransformation of 6:2 fluorotelomer sulfonate by an enrichment culture under sulfur-limiting conditions. Environmental Science & Technology, Volume 59, Issue 35, September 2025, pp. 18722-18734