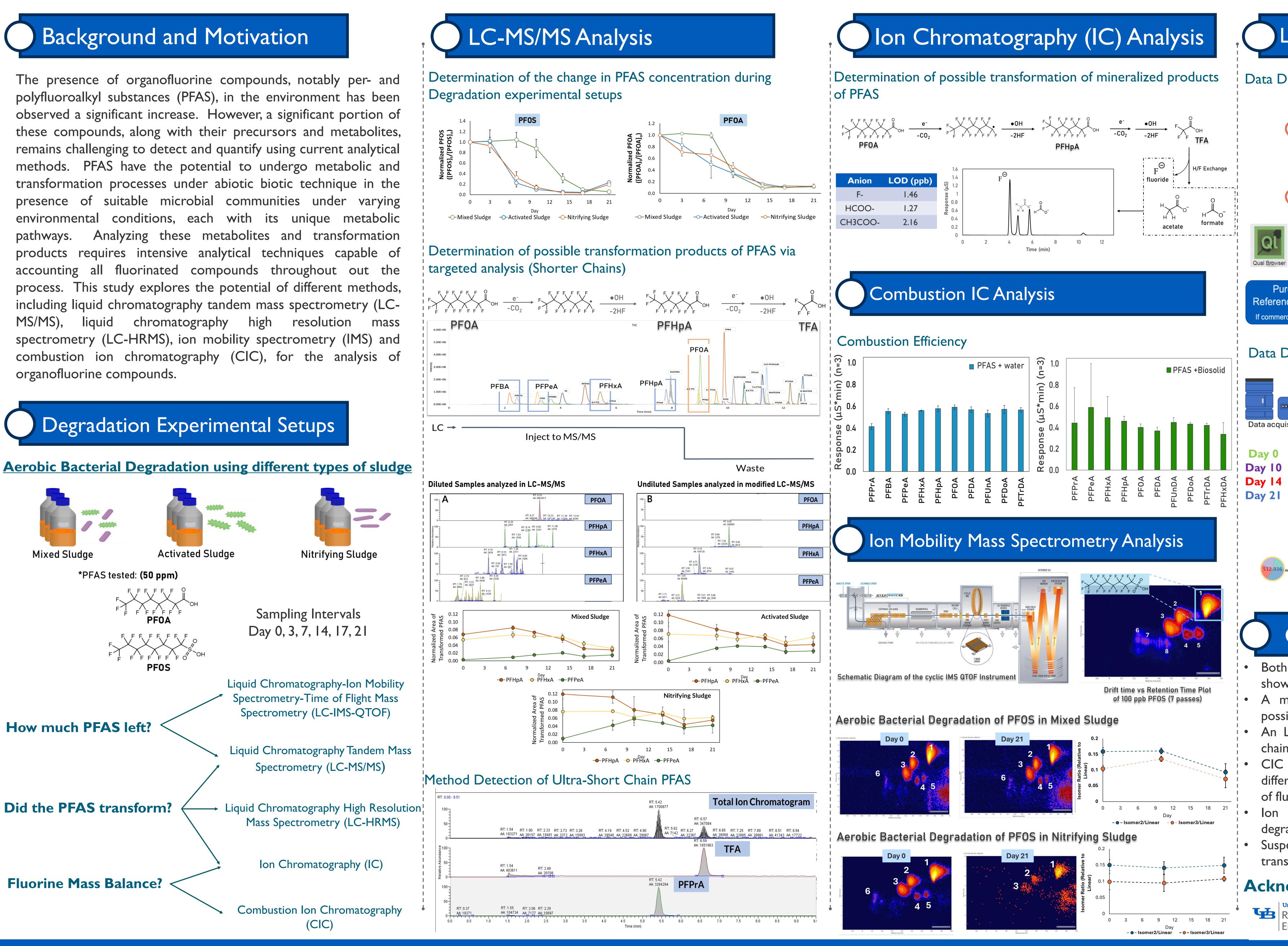# A Comprehensive Analytical Approach and Potential Solutions in Understanding Microbial Transformation of Legacy PFAS in Wastewater

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Background and Motivation

The presence of organofluorine compounds, notably per- and polyfluoroalkyl substances (PFAS), in the environment has been observed a significant increase. However, a significant portion of these compounds, along with their precursors and metabolites, remains challenging to detect and quantify using current analytical methods. PFAS have the potential to undergo metabolic and transformation processes under abiotic biotic technique in the presence of suitable microbial communities under varying environmental conditions, each with its unique metabolic pathways. Analyzing these metabolites and transformation products requires intensive analytical techniques capable of accounting all fluorinated compounds throughout out the process. This study explores the potential of different methods, including liquid chromatography tandem mass spectrometry (LC-MS/MS), liquid chromatography high resolution mass spectrometry (LC-HRMS), ion mobility spectrometry (IMS) and combustion ion chromatography (CIC), for the analysis of organofluorine compounds.

Degradation Experimental Setups

Aerobic Bacterial Degradation using different types of sludge

Mixed Sludge   Activated Sludge   Nitrifying Sludge

*PFAS tested: (50 ppm)

PFOA

PFOS

Sampling Intervals
Day 0, 3, 7, 14, 17, 21

How much PFAS left?
— Liquid Chromatography-Ion Mobility Spectrometry-Time of Flight Mass Spectrometry (LC-IMS-QTOF)
— Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

Did the PFAS transform?
— Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS)
— Ion Chromatography (IC)

Fluorine Mass Balance?
— Combustion Ion Chromatography (CIC)

LC-MS/MS Analysis

Determination of the change in PFAS concentration during Degradation experimental setups

Determination of possible transformation products of PFAS via targeted analysis (Shorter Chains)

Method Detection of Ultra-Short Chain PFAS

Ion Chromatography (IC) Analysis

Determination of possible transformation of mineralized products of PFAS

Anion	LOD (ppb)
F-	1.46
HCOO-	1.27
CH3COO-	2.16

Combustion IC Analysis

Combustion Efficiency

Ion Mobility Mass Spectrometry Analysis

Schematic Diagram of the cyclic IMS QTOF Instrument

Drift time vs Retention Time Plot of 100 ppb PFOS (7 passes)

Aerobic Bacterial Degradation of PFOS in Mixed Sludge

Aerobic Bacterial Degradation of PFOS in Nitrifying Sludge

LC-HRMS Analysis (Suspect Screening)

Data Dependent Acquisition – Fluoromatch Workflow

6846 candidates

Data Dependent Acquisition – GNPS Workflow

Conclusions

- Both abiotic and biotic degradation/removal experimental setups showed efficient removal of PFAS.
- A modified targeted LC-MS/MS analysis was done to determine possible transformed products of shorter chain homologues.
- An LC-MS/MS method was developed to determine the ultra-short chain PFAS (TFA and PFPrA) as possible degradation product.
- CIC analysis showed no variability in the combustion efficiencies of different PFAS homologues and can be used to quantify different forms of fluorine that can be used for mass balance approach.
- Ion Mobility separation exhibited the regioselectivity of PFOS degradation
- Suspect screening using different workflows showed possible transformed products

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