JOURNAL OF
UNDERGRADUATE RESEARCH

PFAS in Wastewater Treatment Plants: Understanding Aggregation and Its Effect on Removal Processes

Sobanjo, Olutimilehin

College of Engineering, Honors Thesis

Dr. Katherine Deliz Quiñones, Department of Environmental Engineering Sciences

Abstract

Toxic per- and polyfluoroalkyl substances (PFAS) have emerged as a significant environmental concern across multiple Scientific, Technology, Engineering, and Mathematics (STEM) disciplines. Their widespread adoption in commercial and defense manufacturing stems from their exceptional properties including strong C-F bonds which confers high chemical stability making them resistant to heat, water, and friction. However, these same characteristics have led to unintended consequences, including environmental persistence, widespread contamination, and bioaccumulation in organisms. The challenge is particularly acute in wastewater treatment, where many facilities lack adequate treatment processes to effectively manage and reduce PFAS contamination. This research addresses this critical gap by investigating PFAS aggregation in water systems, with a specific focus on optimizing parameters that enhance aggregation capabilities. The study targets short-chained PFAS, which present unique challenges due to their lower aggregation propensity compared to longer-chained compounds and their resistance to current treatment technologies. The findings will contribute to our understanding of PFAS aggregation mechanisms and inform the development of more efficient separation technologies, particularly foam fractionation methods.

Keywords: PFAS Aggregation, WWTPs, Wastewater, Critical Micelle Concentration, Foam Fractionation, Short-chain PFAS, Micelles,

Introduction

Problem Statement

Per- and polyfluoroalkyl substances, better known as PFAS, represent a large family of synthetic chemicals that have evolved into a pressing global concern for public health and environmental protection. Originally developed in the 1930s as a replacement for carbon-hydrogen bond-based chemicals, PFAS quickly gained widespread industrial adoption, with their applications exponentially expanding over the past century. Today, PFAS serves as a fundamental component in both defense and commercial industries, appearing in diverse products such as fire-fighting foams, electronics, packaging materials, aerospace machinery, food processing equipment, and various other common consumer items. Their integration into different manufacturing processes is owed to their molecular structure, characterized by a carbon-fluorine (C-F) chain, which confers strong chemical stability, and provides unique properties that make PFAS compounds resistant to heat, water, oil, friction, and natural degradation (Brennan et al., 2021; Leung et al., 2023) . While these attributes initially made PFAS highly valuable for industrial applications and consumer products, their extraordinary chemical stability has revealed itself as a double-edged sword. The same properties that make PFAS beneficial for product performance now present environmental challenges due to their persistence in ecosystems and resistance to degradation.

PFAS enters wastewater systems from both industrial and domestic sources. Industrial facilities, namely manufacturing plants and chemical production sites, generate significant PFAS-laden wastewater through their operations. Additionally, consumer products containing PFAS contribute to contamination through normal usage and disposal. Once in wastewater systems, these compounds are extremely persistent and resist conventional wastewater treatment processes (Kurwadkar et al., 2022; Thompson et al., 2022; US EPA, 2020). As a result, treated effluent often carries residual PFAS concentrations into receiving waters, initiating their journey through environmental systems.

Despite variations in regional climate conditions, industrial development, and environmental policies, these contaminants have been identified in multiple environmental matrices including water, soil, sediments, and atmospheric samples across the globe, with detectable levels confirmed on all continents. Their persistence, mobility and bioaccumulation potential have facilitated their accumulation within living organisms. Their presence has been documented throughout entire food chains, from vegetation and wildlife to humans through means of consumption (Kurwadkar et al., 2022). This is imperative as current scientific evidence links PFAS exposure to several adverse health outcomes, including decreased fertility, elevated blood pressure, developmental delays during fetal development, immunotoxin effects, and potential carcinogenic properties. Although the negative ecological, environmental impacts of PFAS remain less understood due to complexities in assessing food chain dynamics and consumption patterns, there has been evidence of cell damage and structural wear to vegetation with PFAS bioaccumulation, primarily due to an excessive production of reactive oxygen species in response to PFAS contamination (Klingelhöfer et al., 2024; US EPA, 2020).

The complexity of PFAS remediation stems from the diverse nature of these compounds. Rather than representing a single chemical entity, PFAS encompasses thousands of distinct chemicals, each exhibiting distinct structures, unique chemistry and environmental behavior patterns that require specialized treatment approaches (Klingelhöfer et al., 2024). Understanding these compositional differences is crucial for developing effective remediation strategies tailored to specific PFAS groups. Advanced separation techniques such as sorption-based methodologies and fractionation offer scalable solutions for PFAS management. These approaches can effectively reduce environmental transport of PFAS compounds when implemented under optimized conditions. However, successful remediation requires careful consideration of how compounds with specific properties interact with different treatment mechanisms under changing environmental conditions. Moving forward, understanding these physicochemical relationships would be essential for designing effective PFAS remediation strategies.

PFAS Background

PFAS are identified as fully (per-) or partially fluorinated (Poly-) substances but, they must contain at least one fully fluorinated methyl (-CF3) or methylene (-CF2) carbon atom, meaning all hydrogen atoms in these groups are replaced with fluorine atoms. These compounds can incorporate either alkanediyl moiety and/or an aromatic ring within their structures. PFAS also exhibits a characteristic molecular architecture consisting of a perfluorinated alkyl chain (tail) and a terminal functional group (head), see Figure 1. The complete fluorination of the alkyl chain is manifested in the general chemical formula C_nF_{2n+1}—R., where n denotes the carbon chain length and R represents the functional group moiety (Buck et al., 2011). This structural configuration arises from the complete replacement of hydrogen atoms with fluorine atoms along the entire carbon chain, creating a fully fluorinated backbone that terminates in a specific functional group. The extent of fluorination, represented by the number of C-F bonds that the PFAS contains as well as its tail structure, affects each contaminant's physical and chemical characteristics (Barbosa et al., 2024; Leung et al., 2023).

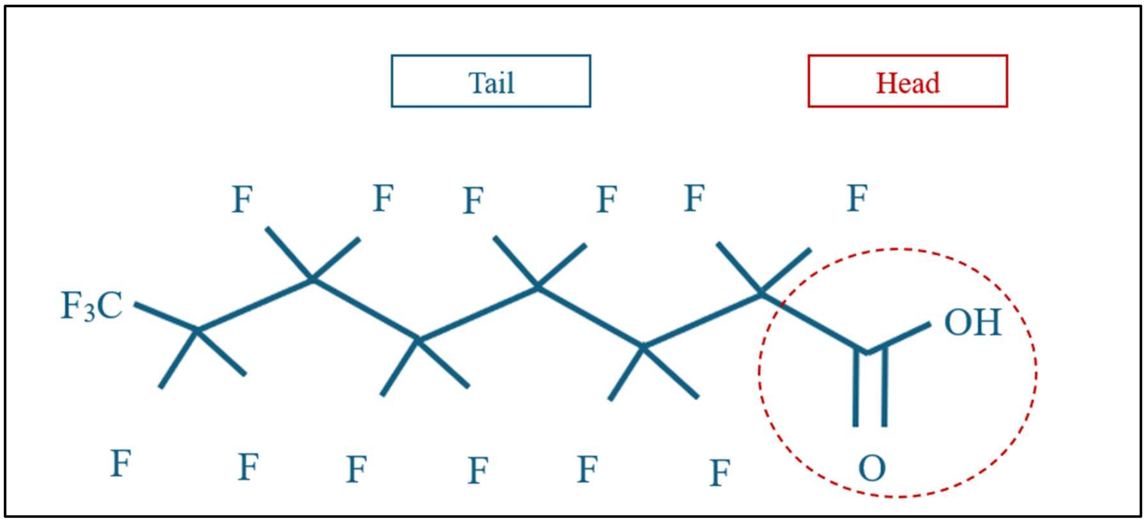


Figure 1. Structure diagram of the PFAS, PFOA, a commonly used and detected PFAS.

The PFAS family comprises thousands of structurally diverse organic compounds, encompassing multiple distinct subcategories that share common fluorinated characteristics (Diagram 1). These compounds are systematically organized into well-defined subgroups, polymers and non-polymers, each possessing unique structural features that influence their chemistry, environmental behavior, toxicity, and remediation approach (Hammel et al., 2022).

Polymer PFAS consists of extremely long molecular chains (can have chains that reach thousands of carbons), typically comprised of smaller monomers connected in a repeating pattern and are considered relatively stable in the environment. On the other hand, non-polymer PFAS, such as PFOA and PFOS, have shorter chains (typically between 4-12, but can be shorter or longer as well), and are considered more mobile in the environment. Both polymer and non-polymer PFAS can be created from the degradation products of other PFAS polymers. Polymer

PFAS subclasses include Fluoropolymer, Polymetric Perfluoropolyether (PFPE), and sidechain fluorinated polymers. Fluoropolymer PFAS have been designated as "polymers of low concern," leading researchers to approach their assessment differently regarding environmental impacts, regulatory frameworks, and risk analysis. This classification has significantly influenced research priorities, resulting in limited scientific investigation of polymer PFAS in environmental contexts. Consequently, the majority of academic research has focused on non-polymer PFAS variants, creating an imbalance in our understanding of these complex polymer contaminants and their environmental implications, particularly through their breakdown and contribution to non-polymer PFAS loading in the environment(Buck et al., 2021; Henry et al., 2018; Wang et al., 2015).

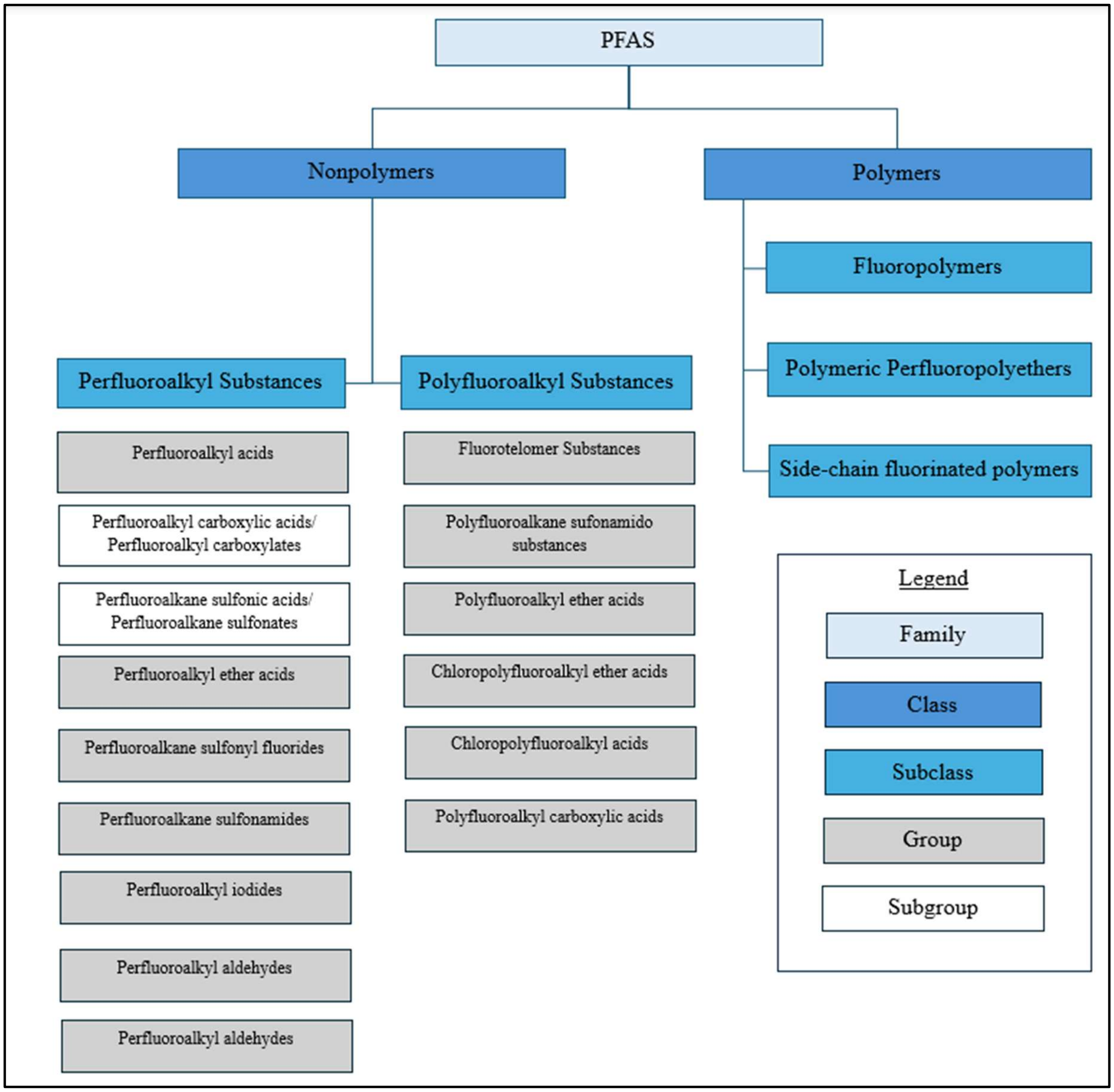


Diagram 1. Hierographic Showcasing the Categorization of PFAS

To our knowledge, the non-polymer class is comparatively bigger than its polymer counterpart. The class also contains well-known PFAS that are commonly used in consumer products. The two subclasses of non-polymers are categorized as Perfluoroalkyl and Polyfluoroalkyl substances. Perfluoroalkyl substances are fully fluorinated compounds that follow the architecture described in the formula C_nF_{2n+1}—R. A common example of a perfluoroalkyl substance is PFOA (Figure 1).

In contrast, polyfluoroalkyl substances are not fully fluorinated. This nonfluorinated bond between the carbon and hydrogen allows for polyfluoroalkyl molecules to be more prone to chemical transformations. This characteristic of the substance can lead to the transformation of polyfluoroalkyls into perfluoroalkyl acids under the right conditions.

Within the Perfluoroalkyl subclass, the PFAS is divided further into groups and subgroups. These group distinctions are dependent on the type of molecular arrangement the PFAS has and features the contaminants display. Perfluoroalkyl acids (PFAAs) are the most studied PFAS group and contain the majority of traceable PFAS. This is due to the non-degradability of these contaminants in the environment. When analyzed, PFAAs are less complex compared to other PFAS. The PFAA group can be divided into Perfluoroalkyl carboxylic acids (PFCAs) and Perfluoroalkyl sulfonic acid (PFSAs). Since both PFCAs and PFSAs were created for commercial use, and resist natural environmental degradation, these compounds have remained the primary focus for scientific studies and regulatory actions, even after their ban in the USA (Buck et al., 2011; Ng & Hungerbühler, 2014).

The lengths of PFAAs' chain are another way the group is classified. This supplementally identification serves as a means of additional distinction between the PFCAs and PFSAs subgroups. To be considered long chain PFAS, PFCAs must have at least eight or more carbons with seven or more carbons being perfluorinated. Meanwhile, PFSAs must have six or more carbons, with six or more being perfluorinated. Anything less than those described carbon amounts are classified as short chain or ultra short chain (Brendel et al., 2018; Ng & Hungerbühler, 2014).

While an extensive number of studies have reported PFAS distribution within the various environmental mediums, including air, water, sediment, soils, and biological, water emerges as a primary route for their transport. Currently, many water treatment facilities lack standardized procedures for managing PFAS, prompting significant investment in developing effective water treatment technologies (Barbosa et al., 2024).

PFAS Remediation Technologies in Water Treatment Plants

As research on PFAS has grown, two main approaches have emerged for treating contaminated water: technologies that focus on PFAS removal and technologies that focus on PFAS degradation. Despite having access to both methods, water treatment facilities often prioritize PFAS removal as opposed to degradation due to factors such as cost, supported research, and wide-scale applicability. Because of this, the PFAS removal technology used in these treatment plants principally falls under the category of sorption removal (Cai et al., 2022).

Sorption represents a water treatment methodology wherein PFAS contaminants are sequestered through binding interactions with externally introduced matrices or substrates. The effectiveness of this process is influenced by multiple parameters, including pH levels, ionic strength, temperature, and competitive interactions with co-present contaminants. This multi-factorial nature necessitates diverse sorption configurations to accommodate varying PFAS contamination scenarios (Cai et al., 2022). Depending on the specific type of sorption being used, this component can vary in size, material, and state of matter.

Adsorption (surface binding) and ion exchange (selective replacement of ions of similar charge between two phases) are popular sorption methods used in water treatments. Using force-based mass transfer, adsorption uses weak ionic forces to bind PFAS to curated adsorptive media like granular activated carbon (GAC) (Appleman et al., 2013). Ion exchange focuses on the alteration of ionic relationships within the solutions. The process involves the precise exchange of charged functional groups, where the PFAS tail ions are selectively replaced by similarly charged counter-ions from the resin phase, maintaining the solution's overall electrochemical balance (Cai et al., 2022). Comparably, outside of ion transfer focused applications, pressure-based technologies are being implemented for PFAS treatment. High-pressure membrane technologies, specifically reverse osmosis (RO) and nanofiltration (NF), demonstrate exceptional efficiency in PFAS removal. While RO achieves nearly complete removal with rejection rates exceeding 99%, NF systems maintain consistently high performance with removal efficiencies ranging from 90% to 99%. However, the majority of existing studies focus on a limited selection of well-known long-chain PFAS [perfluoroalkyl carboxylic acids (PFCA) with ≥ 7 carbons and perfluoroalkyl sulfonic acids (PFSA) containing ≥ 6 carbons], particularly PFOS and PFOA (Chen et al., 2020; Hang et al., 2015; Steinle-Darling & Reinhard, 2008; Tang et al., 2007); while the removal efficacy of NF/RO on other PFAS species remains unclear (Liu et al., 2022). Membrane fouling stands as the primary limitation for NF and RO systems, though proper pretreatment and membrane modifications can help mitigate this challenge (Liu et al., 2022). However, these technologies also face several interconnected constraints: they require substantial infrastructure investment for pretreatment units, materials, energy, and chemicals, which significantly elevates capital costs and operational expenses. Additionally, both processes generate highly concentrated retentate streams that demand specialized handling and treatment solutions for PFAS and other retained contaminants. While established methods remain in use, innovative technologies continue to emerge as valuable additions to PFAS treatment protocols (Appleman et al., 2013).

Foam fractionation is a relatively new approach for PFAS removal, with earliest publications within the last 6 years. Previously used for the separation of other substances within water (We et al., 2024), favorable results have been found when the technology has been used to treat PFAS (Robey et al., 2020). Foam fractionation captures PFAS compounds through a multi-step bubble interaction process that leverages surface tension properties and chemical affinities. The process begins with bubble formation at the bottom of a column, where air is introduced into the contaminated water. When the bubbles rise through the liquid phase, they create a dynamic interface between air and water due to PFAS amphiphilic nature - containing both hydrophobic (water-repelling) and hydrophilic (water-attracting) regions. This dual characteristic allows PFAS to position themselves at the air-water interface of rising bubbles, where they become stabilized through surface tension and concentration effects, and bubble-PFAS interactions. The bubbles facilitate separation of PFAS through bubbles that can reach the surface, resulting in a PFAS concentrated foam that can be easily harvested. Just like previous sorption techniques, foam fractionation can be applied to large volumes of water and is cost effective. In addition, foam fractionation shows promise as experimental studies have proven that the treatment procedure has high PFAS removal efficiency, with the procedure removing 90% of long chain PFAS (Buckley et al., 2022; Garg et al., 2021; Robey et al., 2020).

Despite the progress made in PFAS removal technologies, significant challenges remain to be addressed. Recent research has revealed significant variations in PFAS removal efficiency across different treatment approaches and compound types. While sorption-based treatments have demonstrated substantial effectiveness for certain PFAS compounds, emerging evidence suggests that short-chain PFAS and lesser-studied compounds outside the PFAAs group exhibit notably lower removal rates (Tow et al., 2021). This disparity in treatment effectiveness is particularly evident in foam fractionation, where removal efficiency varies considerably based on the specific PFAS compound being targeted (We et al., 2024). The type of water treatment plant is another factor that can play an adverse critical role in the effectiveness of PFAS removal. Different treatment plants handle distinct concentrations and diversity of PFAS and contain varying levels of particulate matter and co-contaminants that can significantly affect PFAS behavior and treatment efficiency of contaminated water. Wastewater treatment plants (WWTPs) face particularly challenging conditions due to their complex influent composition, making them more susceptible to PFAS removal issues compared to drinking water and other water treatment plants (Barbosa et al., 2024; Kurwadkar et al., 2022).

The removal of short-chain PFAS compounds continues to present significant challenges in water treatment technologies, particularly in foam fractionation processes. Despite substantial research and resources investments allocated to improve the efficiency of short-chain PFAS removal in PFAS technologies like foam fractionation, removal efficiencies remain inconsistent across different treatment scenarios. Recent studies suggest that PFAS aggregation behavior in water bodies may offer a promising avenue for improvement, particularly when integrated with existing foam fractionation technologies. This emerging area of research focuses on understanding and manipulating the parameters that influence PFAS aggregation patterns, with potential applications for enhancing removal rates in wastewater treatment plants (WWTPs).

This literature review seeks to improve our understanding of theoretical parameters that improve PFAS aggregation in water, which can be validated through a proposed experimental design. This knowledge can then be applied to develop more effective removal methods that integrate controlled aggregation processes with foam fractionation technology, potentially addressing the current limitations in short-chain PFAS treatment.

PFAS Aggregation

Hydrocarbon chained surfactants like PFAS tend to aggregate when certain environmental conditions are met (Hu et al., 2024). The aggregation aims to protect the PFAS from environmental degradation. It also helps with the PFAS’ stability and movement through the environment, giving the contaminant the ability to travel longer distances compared to its nonaggregate form (Hu et al., 2024; Krafft & Riess, 2015). When examining the reasons why PFAS and other surfactants behave like this, research heavily shows that this is due to their amphiphilic structures that allow them to surround fluid-fluid interfaces. There are many fluid-fluid interfaces where PFAS self assembles at the boundary such as octanol-water, and water-lipids. Nevertheless, PFAS accumulates the highest at air-water interfaces, with PFAS such as PFOA, PFOS, and other PFAAs showing high concentrations greater than two times the number of other interfaces. Another probable reason PFAS may accumulate near air-water interfaces is because of the contaminants' potential movement from the atmosphere into the water (Costanza et al., 2019).

Regardless of how the contaminant gets to the interface, once they reach the surface, the PFAS tails point towards the air due to its hydrophobic properties. Meanwhile, the hydrophilic head remains in the water, as displayed in Figure 2. While all surfactants display this behavior, what differentiates PFAS is that their carbon-fluorine bond allows for them to have a stronger affinity for these interfaces (Costanza et al., 2019). This hydrophobic and hydrophilic nature of PFAS structures correlates directly to the contaminants' ability to concentrate at the air-water interfaces. PFAS groups whose tails are comparatively more hydrophobic and have lower solvation energies such as PFSAs have a higher tendency to concentrate and accumulate at the surface. Without external interruption, the PFAS will continue to accumulate around the surface until eventual aggregation (Hu et al., 2024).

In terms of PFAS aggregation, PFAS can either aggregate through means of micellization or through supramolecular aggregation. Micelles are the traditionally assumed shape that PFAS form when they aggregate (Figure 3). These types of aggregates form once the amount of PFAS reaches a certain concentration within the fluid-fluid interface. As this desired concentration is met, the hydrophobic tails of PFAS attach to each other to form a sphere around the border of the interface (Figure 3). The specific concentration where this occurs is called Critical Micelle Concentration (CMC) (Hu et al., 2024). PFAS micelle spheres tend to be smaller than other surfactants' micelles due to the contaminant being a fluorinate surfactant. Fluorinated surfactants also tend to have lower CMCs, signifying that they form micelles at lower concentrations (Bhhatarai & Gramatica, 2011). Fluorinated surfactants have also been recorded to occasionally deviate from usual micellar behaviors under certain environmental conditions (Bhhatarai & Gramatica, 2011). These external conditions can be mimicked through experimentation to change the CMC and other characteristics of the micelles and how they form.

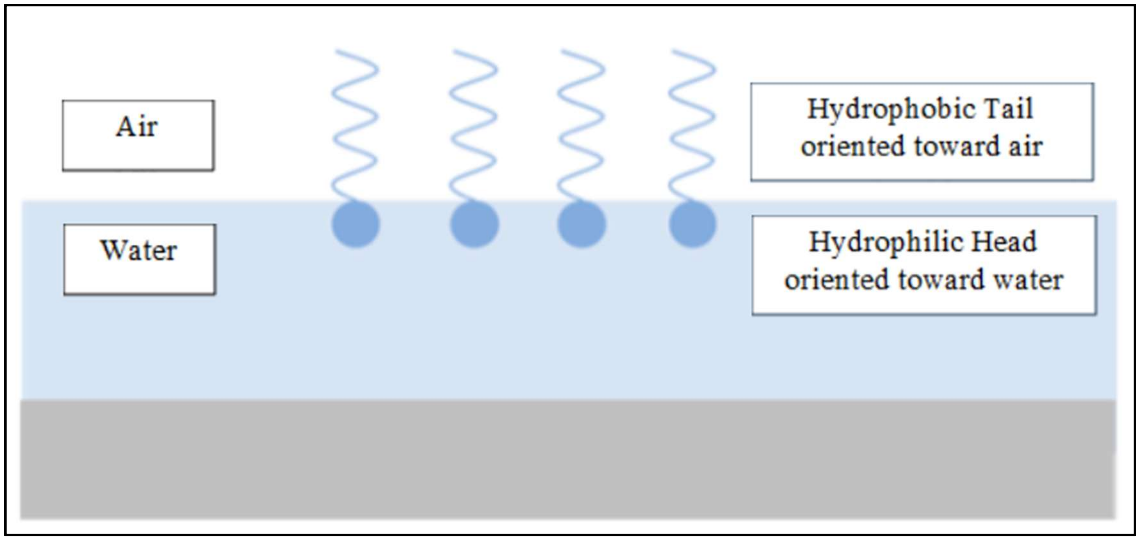


Figure 2. Example of expected orientation and accumulation of PFAS at air-water interface (Source: D. Adamson, GSI).

While traditional spherical micelles have dominated our understanding of PFAS aggregation, researchers have recently described another important form of aggregation - supramolecular aggregates. Unlike well-characterized micelles, these structures remain poorly understood and have only begun to emerge in recent experimental studies examining PFAS behavior. Rather than forming perfect spheres, supramolecular aggregates can manifest as diverse structures, and include hemi-micelles, submicelles, and other unusually shaped aggregates that deviate from the spherical micelle shape, shown in Figure 3 (Krafft & Riess, 2015). These aggregates look more clustered and textured, forming much more visually complex structures compared to micelles. Supramolecular aggregates also differ in size from micelles as the supramolecular aggregates are larger in size. This increased size affects the aggregates movement and lowers its mobility as a consequence of its increased radius and viscosity. When the air-water interface contains increased levels of positively charged adsorbents, aggregate structures begin to form. Notably, preliminary research suggests that these supramolecular aggregates can develop at concentrations even lower than the CMC typically associated with their parent PFAS compounds. However, further investigation is needed to fully understand these phenomena and their broader implications for PFAS behavior (Johnson et al., 2021).

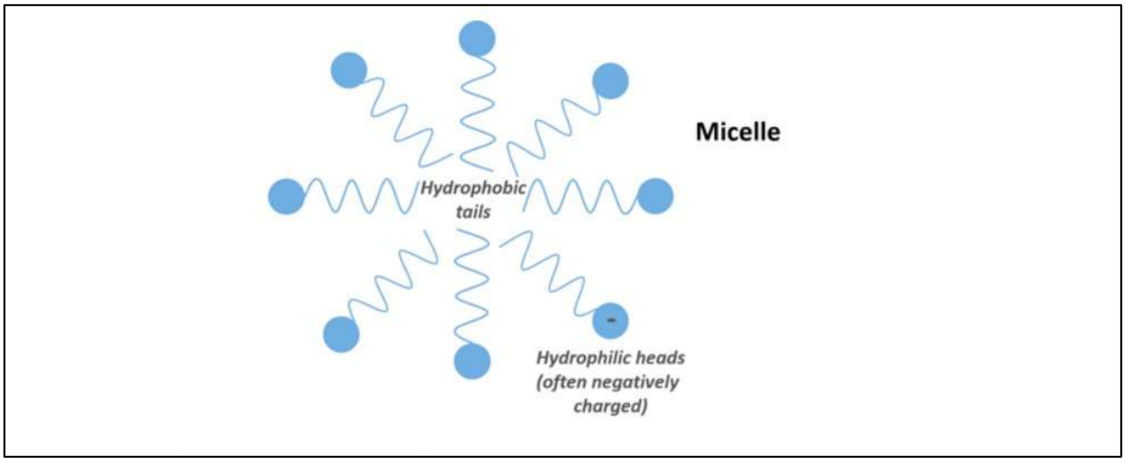


Figure 3. Formation of a micelle (Source: D. Adamson, GSI).

Chain length is another crucial factor influencing PFAS aggregation behavior. A direct relationship exists between chain length and accumulation potential at fluid-fluid interfaces, with longer chains showing progressively greater accumulation capacity. This enhanced accumulation stems from strengthened Van der Waals forces between PFAS molecules, which overcome the electrostatic repulsion that normally keeps them separated. The extended tail length of long-chain PFAS compounds enhances their hydrophobic character, thereby increasing their likelihood of aggregation. In contrast, short-chain PFAS exhibit low accumulation potential due to its shorter tail length and high diffusion coefficient (Brendel et al., 2018; Hu et al., 2024). These aspects of short-chain PFAS promote molecular exchange which keeps the PFAS from aggregating. However, recent research reveals interesting exceptions to this pattern. Under specific conditions, such as in lipid-water interfaces or environments with elevated salt concentrations, short-chain PFAS have displayed accumulative and aggregative properties. Furthermore, these conditions can lead to the formation of distinctly ellipsoidal shaped micelles (Leung et al., 2023).

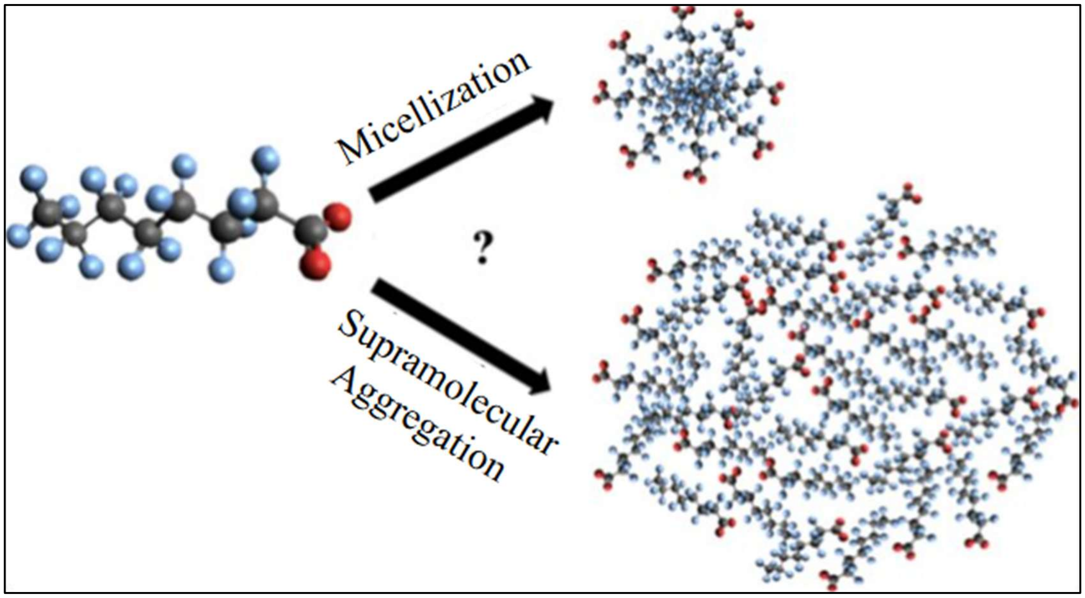


Figure 4. Difference in visuals between micellization and supramolecular aggregation (Sobolewski et al., 2024).

The ability of short-chain PFAS to form aggregates, under specific conditions that can be controlled (Table 1), presents a promising solution for removing these contaminants from water. Both long-chain and short-chain PFAS can be induced to form aggregates, which serves as a crucial preprocessing step that enhances subsequent treatment effectiveness. This approach holds promise for techniques such as foam fractionation, offering improved efficiency in PFAS removal applications.

Table 1. Theoretical and experimentally tested conditions that improve PFAS aggregation.

Parameters	Relation to Improving Aggregation
Salinity	Increase • High Salt Concentrations aggregate short-chain PFAS • Neutralizes the double negative electric properties of PFAS • Reference: Leungetal20
PH	Decrease • As pH decreases, PFAS binding capabilities increase • Reference: Pereiraetal2023
Fluid-Fluid Interface	Air-Water • Accumulation of PFAS in air-water interface were 2 to 16 times greater than at the NAPL-water interface, and up to 8 times greater than just water concentrations • Reference: KrafftetRiess2015
Alcohols	Medium-length Chain • Decreases head group repulsion • Solubilization • Can have adverse effects • Reference: Gargetal2021

Objectives and Methods

Objectives

- Collect and characterize wastewater treatment effluent to assess potential to induce PFAS aggregation by means of reducing the CMC.
- Propose an experimental method that implements surface tension and conductivity measurements as a proxy for CMC of a PFAS mixture.

Methodology

Sample Collection and characterization – One-nine-liter sample *of pre-chlorinated wastewater* effluent was obtained from UF's Water Reclamation Facility on 02/18/25. Sample was taken to the lab and characterized for pH and salinity using a Seventh Excellence Multimeter (Figure 5*).* Sample was placed in the freezer until PFAS analysis.

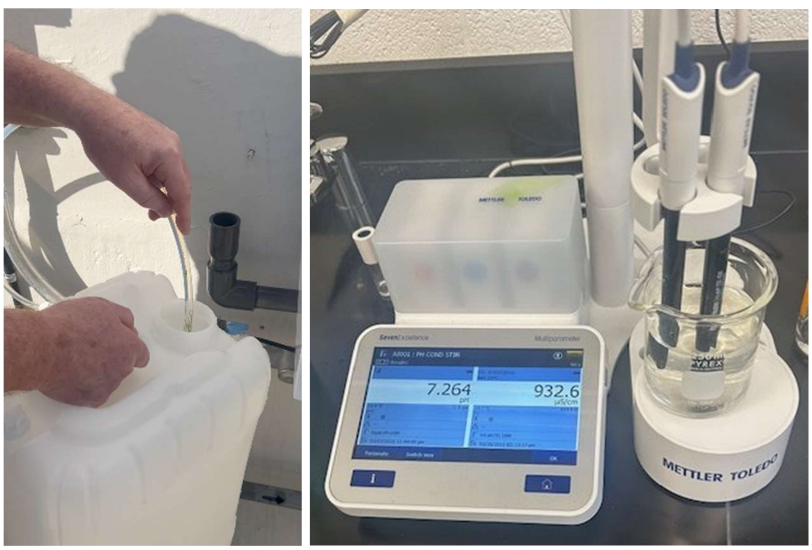


Figure 5. Picture of sample collection and analytical equipment used to analyze for pH and Salinity.

PFAS *Extraction through Solid Phase Extraction –*wastewater sample, field blank, and quality control samples were thawed and weighed gravimetrically. These samples were then spiked with 25 µL of an isotopically mass-labeled internal standard mixture and the pH was adjusted to 3 using glacial acetic acid. The extraction was conducted as described by (Santiago et al., 2024) via solid-phase extraction using Strata-XL-AW 100 um Polymeric Weak Anion 500 mg/6mL cartridges. The cartridges were conditioned by soaking them for two minutes in 4 mL of 0.3% ammonium hydroxide methanolic solution which was then passed through the cartridges by gravity flow. Next, 3 mL of methanol followed by 4 mL of acetic acid buffer were applied in the same manner.

Capillary tubing was attached to each of the cartridges and water samples were passed through the cartridges using a vacuum pump. After extraction, the cartridges were washed with 4 mL of acetic acid buffer and dried for 5 minutes under full vacuum. For PFAS elusion, 2 mL of methanol was added, followed by two additions of 3 mL of 0.3% ammonium hydroxide methanolic solution. The cartridges were soaked for 2 minutes in each solvent and then allowed to drain by gravity into 15 mL Falcon tubes. The resulting sample extracts were then evaporated down to 1 mL using ultra-high purity nitrogen gas and stored in -20 °C until further analysis. This method is described in Figure 6.

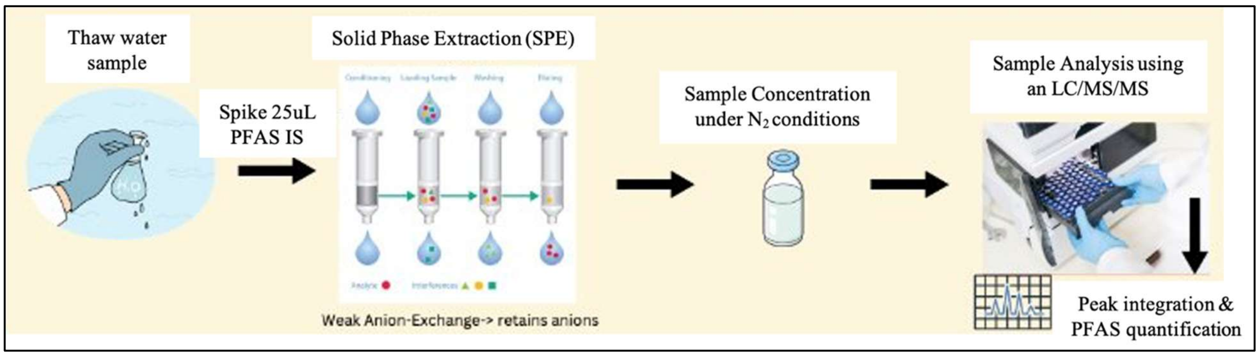


Figure 6. Protocol for PFAS extraction from water.

Method for Sample Cleaning and Analysis at UF Analytical Toxicology Core Laboratory (ATCL) – Extracted samples at 1ml volume were spiked with 10ul of after extraction internal standard mix (Wellington Laboratory; MPFAC-HIF-IS). After mixing by vortex, samples were filtered with Whatman GD/X 0.2um nylon, 13mm, syringe filters (6870-1302) to remove particulates observed in samples. An aliquot of 100ul filtered extract was transferred to a polypropylene autosampler vials and stored at 4C until analysis. Samples were analyzed by ultra-high-performance liquid chromatography-tandem mass spectrometry using a Shimadzu Nexera X2 UHPLC (Kyoto, Japan) and a triple quadrupole linear ion trap (QTRAP 6500, AB SCIEX, Redwood Shores, CA). The column used was Poroshell 120 EC-C18 2.1x100mm, 2.7um, with a delay column, Poroshell 120 EC-C18 2.1x50mm, 4um (Agilent Technologies). The column oven is 40C. The injection volume was 2uL. Mobile phases used were Optima, LCMS grade water (A) and Methanol (B), both containing 2mM ammonium acetate (Fisher Scientific). The flow rate is 0.3ml/min starting with 95%A and 5%B. Standard concentrations used were 0.05, 0.1, 0.5, 1,5,10, 50,100,200 ng/ml and contained 5ul each of extraction internal standard (Wellington laboratory; MPFAC-HIF-ES) and after extraction internal standard. ABSciex data processing software was used to quantify the acquired data (MultiQuant™ v.3.0.1).

Based on the review of various literature pertaining to PFAS aggregation, a methodology has been proposed. By increasing salinity and lowering pH of the tested wastewater, both short-chain PFAS and long-chain PFAS will aggregate more at a lower concentration than the PFAS' CMC. These parameters were selected because of the neutralizing effects of salts and pH on the electric double-repulsion between PFAS by the introduction of ions into the water system (Leung et al., 2023).

The minute size of PFAS leads to restrictions in traditional ways of measurement. Therefore, PFAS aggregation will be accounted for through the evaluation of its CMC. CMC, reciprocally, will be measured by surface tension and conductivity based on the correlation between PFAS accumulation, lowering of fluid surface tension, and increasing fluid conductivity (Hu et al., 2024). By graphing different PFAS concentration amounts and their respective surface tension and conductivity measurements, the CMC can be determined by sudden break points in the slope of the curated graphs, as shown in Graph 1 (KRÜSS Scientific, n.d.).

Results and Discussion

Results obtained from pH, salinity and PFAS analysis are summarized below and in Table 2. Overall, salinity (7.3 ppt) and pH (1 ppt) measurements fall within the typical range for other municipal wastewater treatment facilities in the USA, 0.5 – 3 ppt and 6.5 – 8.5 respectively. From the 35 PFAS analyzed 10 PFAS were detected in the wastewater effluent sample (Table 2). Of these, two were short chain, three had sulfonic functional groups and seven had carboxylic functional groups. Since there are no standards in place for PFAS levels in wastewater effluent, when compared to more stringent minimum contaminant levels (MCL) established by the Environmental Protection Agency (EPA) for drinking water, PFOA and the mixture containing PFHxS, PFNA, and PFBS exceed the established Hazard Index (Eq.1).

$$\text{Hazard Index (1 unitless)} = \left(\frac{[\text{HFPO} - \text{DA}_{\text{ppt}}]}{[10\ \text{ppt}]}\right) + \left(\frac{[\text{PFBS}_{\text{ppt}}]}{[2000\ \text{ppt}]}\right) + \left(\frac{[\text{PFNA}_{\text{ppt}}]}{[10\ \text{ppt}]}\right) + \left(\frac{[\text{PFHxS}_{\text{ppt}}]}{[10\ \text{ppt}]}\right) \tag{1}$$

Table 2. Concentration of PFAS (ppt) in wastewater effluent, and established EPA MCL for some of the Selected PFAS in drinking water.

PFAS Compound	Average Concentration	Standard Deviation	Drinking Water MCL (EPA)
PFBS	10.053	0.919	Haz. Index
PFHxSΣ2	0.403	0.079	Haz. Index
PFBA	156.947	113.695	
PFHxA	19.008	4.378	
PFHpA	1.456	0.069	
PFOA	4.658	0.129	4.0
PFOS	3.430	2.969	4.0
PFNA	1.415	0.095	Haz. Index
PFDA	1.052	0.177	
PFUnDA	0.473	0.025	
Total PFAS	**200.104**		
	Hazard Index	**Measurement**	**MCL**
		0.187	1.0

Results show that current wastewater treatment methods at the UF Water Reclamation Facility fail to adequately remove PFAS compounds from effluent streams. This presents a critical concern, as the treated water is reused for campus irrigation purposes. Conditioning of treated wastewater could be implemented to achieve optimized aggregation processes that can enhance PFAS removal through foam fractionation. The proposed conditioning approach focuses on modifying treated wastewater characteristics to enhance PFAS aggregation, creating optimal conditions for subsequent foam fractionation. This method builds upon established principles of PFAS behavior at air-water interfaces, where these compounds naturally concentrate and form distinct layers. When properly conditioned, the wastewater can produce more stable foam structures that facilitate efficient PFAS removal, potentially achieving higher removal efficiencies than current treatment methods. Alternatively, foam fractionation could be introduced earlier in the treatment process when wastewater conditions align with theoretical specifications outlined in Table 1. Both approaches represent significant improvements over current methods, offering practical solutions for enhancing PFAS removal from wastewater.

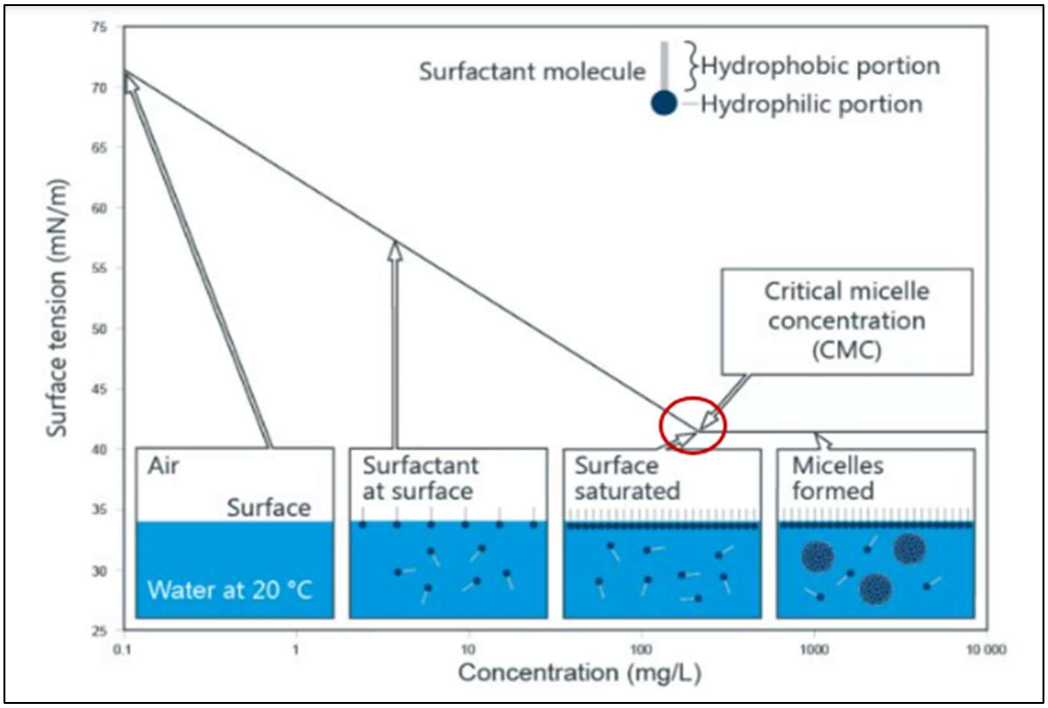
Proposed Experimental Design Implementing Surface Tension and Conductivity Measurements as a Proxy for PFAS CMC

The following experimental protocol is proposed to examine how environmental parameters influence the aggregation behavior of both short-chain and long-chain PFAS in wastewater treatment contexts. Based on established relationships between solution/water chemistry and PFAS behavior (described in Table 1), we focus on two primary environmental parameters: pH and salinity, which significantly impact PFAS aggregation and surface activity. PFAS CMC behavior will be characterized through surface tension and conductivity values.

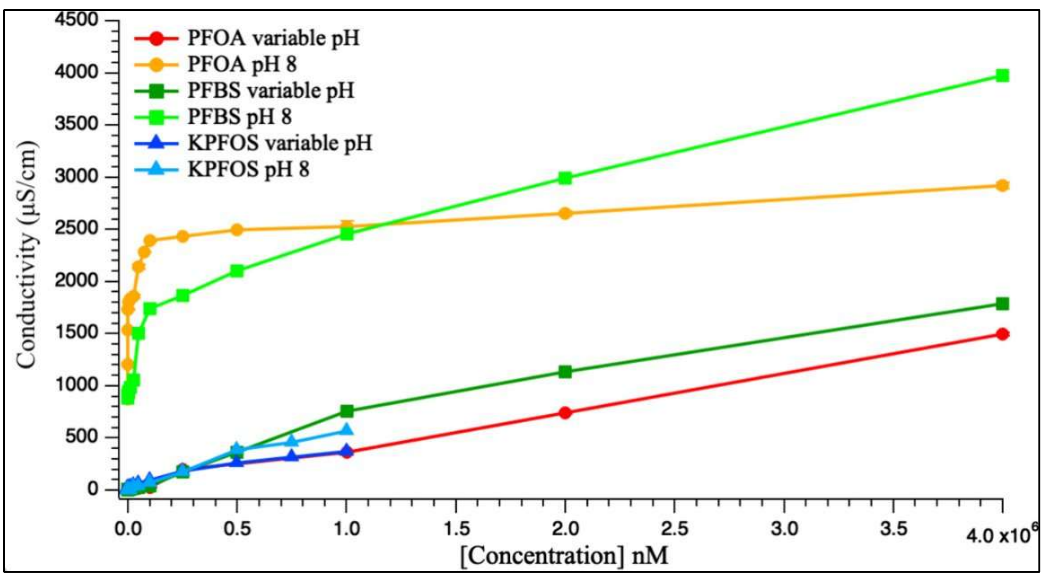
Salinity and pH modifications in wastewater effluent samples –the experimental design consists of systematic variations in both pH and salinity levels. For pH manipulation, four distinct levels (pH 3, 4, 5, and 6) will be achieved through controlled additions of sulfuric acid. Each pH-adjusted sample will undergo thorough mixing for at least one minute prior to measurement. Three replicates will be made for each pH sample, and the surface tension and conductivity will be measured at multiple time intervals 2hrs, 4hrs, 8hrs, and 24hr) to capture temporal effects on PFAS behavior.

A parallel experimentation following the same measurement intervals and replicates is proposed to evaluate the impacts of salinity on PFAS aggregation. Through the addition of Calcium Chloride, salinities of 1.5 ppt, 2 ppt, 2.5 ppt, and 3ppt will be established and the sample mixed for a minimum of 1 minute before being measured. An additional iteration, also following the same measurement intervals and replicates, is proposed to evaluate potential synergistic effects of the most extreme pH and salinity conditions evaluated on PFAS aggregation, using primarily surface tension as the proxy for aggregation.

Salinity and pH modifications in wastewater effluent samples with different PFAS concentrations – modifications of the PFAS concentration in the wastewater sample, will be implemented (e.g. spiking or diluting the sample) accordingly to adjust PFAS concentrations of selected PFAS towards their respective CMC values. The same experimental approach implemented for involving pH and salinity alteration will be implemented for 3 total spikes/dilutions for a 90 total sample. These steps will allow us to determine if the CMC for selected PFAS within a mixture can be assessed using surface tension and conductivity as proxies. Surface tension and conductivity measurements are plotted against PFAS concentration for each parameter combination. The CMC is identified at the breakpoint where the slope of these relationships' changes, providing quantitative insight into PFAS aggregation behavior under various environmental conditions. (Graph 1 and Graph 2).



Graph 1. Concentration of PFAS with Respect to Surface Tension and CMC Reading (Sobolewski et al., 2024).



Graph 2. Concentration of PFAS with Respect to Conductivity and CMC Reading (Sobolewski et al., 2024).

Conclusion

Current wastewater treatment methods demonstrate significant shortcomings in removing PFAS compounds from effluent streams, highlighting a critical need for more effective treatment solutions. To address this deficiency, foam fractionation emerges as a particularly promising approach that combines cost-effectiveness with scalability, making it an attractive addition to existing wastewater treatment trains. This method leverages the natural tendency of PFAS compounds to aggregate at air-water interfaces creating concentrated foam layers that can be efficiently removed from the treatment stream. Furthermore, foam fractionation can be optimized, through careful control of operating conditions that enhance PFAS aggregation, allowing operators to achieve higher PFAS removal efficiency, especially for short chain PFAS.

While foam fractionation concentrates PFAS compounds into more manageable (smaller volume) streams, it doesn't destroy these persistent pollutants – it merely transfers them from one phase to another. This raises concerns about proper handling and disposal of the concentrated foam waste, as well as potential environmental impacts if not managed correctly. Furthermore, the process generates a secondary waste stream requiring specialized storage and disposal protocols, adding operational complexity and environmental liability to treatment facilities. Implementation challenges extend beyond technical considerations. The lack of standardized measurement protocols and limited research data on PFAS aggregation behavior creates uncertainty in scaling up operations and evaluating effectiveness across different wastewater compositions. Accordingly, standardized measurement protocols represent a crucial first step for advancing our understanding and control of PFAS aggregation's far-reaching implications. As these protocols gain widespread acceptance, they will catalyze a surge in aggregation research, enabling scientists to study PFAS behavior across diverse environmental scenarios. This expanding knowledge base will foster crucial dialogue about two critical areas: the potential of PFAS aggregates to enhance remediation efforts, and their specific impacts on both organism health and ecosystem integrity. As research continues to uncover the unique properties and behaviors of aggregated PFAS compounds, regulatory frameworks can evolve to provide stronger protection for both human health and environmental systems. This systematic approach to understanding and managing PFAS aggregation will ultimately enable more effective prevention strategies and safer environmental management practices across various sectors, from wastewater treatment to ecosystem protection.

The proposed experimental design implementing surface tension and conductivity measurements as a proxy for PFAS aggregation as determined by their CMC, could serve as an important step towards improving our understanding of PFAS aggregation behavior and its potential integration into optimized foam fractionation methods for PFAS removal.

From this experiment, more knowledge can be uncovered pertaining to PFAS aggregation and the parameters that affect it. Additional experiments can also be conducted testing distinct aspects of the parameters: the type of salt, type of acid, and PFAS subgroup. Other factors can be experimented with as well: temperature/season, influent wastewater, alcohols, etc. Besides analyzing CMC, other aspects of aggregation can be studied, such as the aggregate shapes. Using technology like Cyro-EM imaging, enlarged detail visuals of PFAS aggregates can be created, and the aggregate structure themselves can be studied (Sobolewski et al., 2024).

In terms of application of PFAS aggregation properties, understanding optimal conditions for reducing surface tension and CMC play a crucial role in enhancing PFAS remediation and separation technologies such as foam fractionation. When accumulating and aggregating at air-water interfaces, the PFAS' tail angle is pushed away. This behavior increases the stability of foam when foam fractionation is applied to the water system (Yuan et al., 2023). This aspect accompanied by evidential aggregation of short-chain PFAS allows for the proposition of aggregation treatment before foam fractionation to aid in the foaming of short-chain PFAS and improved short-chain PFAS removal efficiency. Another technology that can take advantage of PFAS foam stability in the correct environment is called ozofractionation. This treatment method exploits the surfactant properties as well as micelle formation to encourage the foaming capabilities of PFAS. Incorporating aggregation treatment as a predecessor treatment as well will help improve efficiency of this treatment's PFAS removal (Garg et al., 2021).

Currently there is limited information and resources regarding PFAS aggregation. When searching for quantitative PFAS aggregation data, only a few environmental databases provided this information publicly; one of the companies with the most information was the Interstate Technology and Regulatory Council (ITRC). Within the ITRC database, the only data that can be found regarding PFAS aggregation was the CMC for a limited amount of PFAS within its PFAS

Technical and Regulatory Guidance Document (*PFAS — Per- and Polyfluoroalkyl Substances*, n.d.). Out of the thousands of PFAS, there were only about 50 PFAS' recorded CMCs. Moreover, when experimentation was done pertaining to certain PFAS, the CMC values that were derived were consistently different values than what was on record. Besides the CMC amount, there was no data about the type of aggregation, aggregate shape, dimension, and other crucial aspects to PFAS self-assembly. Another issue pertaining to the official CMC amounts was the fact that these values were not obtained through experimentation. Instead, they were calculated theoretically using Quantitative structure–activity relationship (QSAR) modeling.

Because of the lack of records and knowledge regarding PFAS aggregation, there are no regulations or legislation pertaining to the prevention of the natural formation of PFAS aggregation in the environment from the EPA. This needs to be addressed because PFAS aggregates are more concentrated than PFAS itself and could potentially have unique negative health effects on humans and the environment. Through the conduction of more experiments, a database of CMC values for desired PFAS can be collected and can help with the creation of cohesive and consistent measurements for PFAS CMCs. From there environmental agencies and databases can produce an official way to measure PFAS CMCs. Once the official methodology comes out, PFAS aggregation experiments will expand in numbers, with the overall knowledge about the subject matter growing. Additionally, discussions can start about how PFAS aggregates can affect organism health and appropriate legislation, and regulation can be put in place.

Acknowledgements

I would like to thank the University of Florida's Water Reclamation Facility for providing access and support collecting samples, and Malena Gonzalez Fernandez and Sanneri Santiago Borres for their support performing PFAS extraction from wastewater samples and calculating PFAS concentrations. Additional thanks to UF University Scholars Program for providing a stipend and to Dr. Katherine Deliz for funding this study.

References

Appleman, T. D., Dickenson, E. R. V., Bellona, C., & Higgins, C. P. (2013). Nanofiltration and granular activated carbon treatment of perfluoroalkyl acids. *Journal of Hazardous Materials*, *260*, 740–746. https://doi.org/10.1016/j.jhazmat.2013.06.033

Barbosa, G. D., Tavares, F. W., & Striolo, A. (2024). Molecular Interactions of Perfluorinated and Branched Fluorine-Free Surfactants at Interfaces: Insights from a New Reliable Force Field. *Journal of Chemical Theory and Computation*, *20*(16), 7300–7314. https://doi.org/10.1021/acs.jctc.4c00459

Bhhatarai, B., & Gramatica, P. (2011). Prediction of Aqueous Solubility, Vapor Pressure and Critical Micelle Concentration for Aquatic Partitioning of Perfluorinated Chemicals. *Environmental Science & Technology*, *45*(19), 8120–8128. https://doi.org/10.1021/es101181g

Brendel, S., Fetter, É., Staude, C., Vierke, L., & Biegel-Engler, A. (2018). Short-chain perfluoroalkyl acids: Environmental concerns and a regulatory strategy under REACH. *Environmental Sciences Europe*, *30*(1), 9. https://doi.org/10.1186/s12302-018-0134-4

Brennan, N. M., Evans, A. T., Fritz, M. K., Peak, S. A., & Holst, H. E. von. (2021). Trends in the Regulation of Per- and Polyfluoroalkyl Substances (PFAS): A Scoping Review. *International Journal of Environmental Research and Public Health*, *18*(20), 10900. https://doi.org/10.3390/ijerph182010900

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*, *7*(4), 513–541. https://doi.org/10.1002/ieam.258

Buck, R. C., Korzeniowski, S. H., Laganis, E., & Adamsky, F. (2021). Identification and classification of commercially relevant per- and poly-fluoroalkyl substances (PFAS). *Integrated Environmental Assessment and Management*, *17*(5), 1045–1055. https://doi.org/10.1002/ieam.4450

Buckley, T., Xu ,Xiaoyong, Rudolph ,Victor, Firouzi ,Mahshid, & and Shukla, P. (2022). Review of foam fractionation as a water treatment technology. *Separation Science and Technology*, *57*(6), 929–958. https://doi.org/10.1080/01496395.2021.1946698

Cai, W., Navarro, D. A., Du, J., Ying, G., Yang, B., McLaughlin, M. J., & Kookana, R. S. (2022). Increasing ionic strength and valency of cations enhance sorption through hydrophobic interactions of PFAS with soil surfaces. *Science of The Total Environment*, *817*, 152975. https://doi.org/10.1016/j.scitotenv.2022.152975

Chen, X., Vanangamudi, A., Wang, J., Jegatheesan, J., Mishra, V., Sharma, R., Gray, S. R., Kujawa, J., Kujawski, W., Wicaksana, F., & Dumée, L. F. (2020). Direct contact membrane distillation for effective concentration of perfluoroalkyl substances – Impact of surface fouling and material stability. Water Research, 182, 116010. https://doi.org/10.1016/j.watres.2020.116010

Costanza, J., Arshadi, M., Abriola, L. M., & Pennell, K. D. (2019). Accumulation of PFOA and PFOS at the Air–Water Interface. *Environmental Science & Technology Letters*, *6*(8), 487–491. https://doi.org/10.1021/acs.estlett.9b00355

Garg, S., Wang, J., Kumar, P., Mishra, V., Arafat, H., Sharma, R. S., & Dumée, L. F. (2021). Remediation of water from per-/poly-fluoroalkyl substances (PFAS) – Challenges and perspectives. *Journal of Environmental Chemical Engineering*, *9*(4), 105784. https://doi.org/10.1016/j.jece.2021.105784

Hammel, E., Webster, T. F., Gurney, R., & Heiger-Bernays, W. (2022). Implications of PFAS definitions using fluorinated pharmaceuticals. *iScience*, *25*(4). https://doi.org/10.1016/j.isci.2022.104020

Hang, X., Chen, X., Luo, J., Cao, W., & Wan, Y. (2015). Removal and recovery of perfluorooctanoate from wastewater by nanofiltration. Separation and Purification Technology, 145, 120–129. https://doi.org/10.1016/j.seppur.2015.03.013

Henry, B. J., Carlin, J. P., Hammerschmidt, J. A., Buck, R. C., Buxton, L. W., Fiedler, H., Seed, J., & Hernandez, O. (2018). A critical review of the application of polymer of low concern and regulatory criteria to fluoropolymers. *Integrated Environmental Assessment and Management*, *14*(3), 316–334. https://doi.org/10.1002/ieam.4035

Hu et al., et al. (2024). (PDF) Bioaccumulation Mechanisms of Perfluoroalkyl Substances (PFASs) in Aquatic Environments: Theoretical and Experimental Insights. *ResearchGate*. https://doi.org/10.1016/j.jhazmat.2024.136283

Johnson, M. S., Buck, R. C., Cousins, I. T., Weis, C. P., & Fenton, S. E. (2021). Estimating Environmental Hazard and Risks from Exposure to Per- and Polyfluoroalkyl Substances (PFASs): Outcome of a SETAC Focused Topic Meeting. *Environmental Toxicology and Chemistry*, *40*(3), 543–549. https://doi.org/10.1002/etc.4784

Klingelhöfer, D., Braun, M., Groneberg, D. A., & Brüggmann, D. (2024). The "forever" per- and polyfluoroalkyl substances (PFAS): A critical accounting of global research on a major threat under changing regulations. *Chemosphere*, *354*, 141694. https://doi.org/10.1016/j.chemosphere.2024.141694

Krafft, M. P., & Riess, J. G. (2015). Selected physicochemical aspects of poly- and perfluoroalkylated substances relevant to performance, environment and sustainability—Part one. *Chemosphere*, *129*, 4–19. https://doi.org/10.1016/j.chemosphere.2014.08.039

KRÜSS Scientific. (n.d.). *Critical micelle concentration (CMC) and surfactant concentration*. KRÜSS Scientific. Retrieved April 2, 2025, from https://www.kruss-scientific.com/en-US/know-how/glossary/critical-micelle-concentration-cmc-and-surfactant-concentration

Kurwadkar, S., Dane, J., Kanel, S. R., Nadagouda, M. N., Cawdrey, R. W., Ambade, B., Struckhoff, G. C., & Wilkin, R. (2022). *Per*- and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. *Science of The Total Environment*, *809*, 151003. https://doi.org/10.1016/j.scitotenv.2021.151003

Leung, S. C. E., Wanninayake, D., Chen, D., Nguyen, N.-T., & Li, Q. (2023). Physicochemical properties and interactions of perfluoroalkyl substances (PFAS)—Challenges and opportunities in sensing and remediation. *Science of The Total Environment*, *905*, 166764. https://doi.org/10.1016/j.scitotenv.2023.166764

Liu, C., Zhao, X., Faria, A. F., Deliz Quiñones, K. Y., Zhang, C., He, Q., Ma, J., Shen, Y., & Zhi, Y. (2022). Evaluating the efficiency of nanofiltration and reverse osmosis membrane processes for the removal of per- and polyfluoroalkyl substances from water: A critical review. *Separation and Purification Technology*, *302*, 122161. https://doi.org/10.1016/j.seppur.2022.122161

Ng, C. A., & Hungerbühler, K. (2014). Bioaccumulation of Perfluorinated Alkyl Acids: Observations and Models. *Environmental Science & Technology*, *48*(9), 4637–4648. https://doi.org/10.1021/es404008g

PFAS — Per- and Polyfluoroalkyl Substances. (n.d.). Retrieved April 2, 2025, from https://pfas-1.itrcweb.org/

Robey, N. M., da Silva, B. F., Annable, M. D., Townsend, T. G., & Bowden, J. A. (2020). Concentrating Per- and Polyfluoroalkyl Substances (PFAS) in Municipal Solid Waste Landfill Leachate Using Foam Separation. Environmental Science & Technology, 54(19), 12550–12559. https://doi.org/10.1021/acs.est.0c01266

Sobolewski, T. N., Findlay, J. L., Hemphill, J. E., & Walker, R. A. (2024). Aggregation, Not Micellization: Perfluorooctanoic Acid, Perfluorobutanesulfonic Acid, and Potassium Perfluorooctanesulfonate Behavior in Aqueous Solution. *Langmuir*, *40*(47), 24820–24831. https://doi.org/10.1021/acs.langmuir.4c02566

Steinle-Darling, E., & Reinhard, M. (2008). Nanofiltration for Trace Organic Contaminant Removal: Structure, Solution, and Membrane Fouling Effects on the Rejection of Perfluorochemicals. Environmental Science & Technology, 42(14), 5292–5297. https://doi.org/10.1021/es703207s

Tang, C. Y., Fu, Q. S., Criddle, C. S., & Leckie, J. O. (2007). Effect of Flux (Transmembrane Pressure) and Membrane Properties on Fouling and Rejection of Reverse Osmosis and Nanofiltration Membranes Treating Perfluorooctane Sulfonate Containing Wastewater. Environmental Science & Technology, 41(6), 2008–2014. https://doi.org/10.1021/es062052f

Thompson, K. A., Mortazavian, S., Gonzalez, D. J., Bott, C., Hooper, J., Schaefer, C. E., & Dickenson, E. R. V. (2022). Poly- and Perfluoroalkyl Substances in Municipal Wastewater Treatment Plants in the United States: Seasonal Patterns and Meta-Analysis of Long-Term Trends and Average Concentrations. *ACS ES&T Water*, *2*(5), 690–700. https://doi.org/10.1021/acsestwater.1c00377

US EPA, O. (2020, December 15). *Interim Guidance on the Destruction and Disposal of PFAS and Materials Containing PFAS* [Other Policies and Guidance]. https://www.epa.gov/pfas/interim-guidance-destruction-and-disposal-pfas-and-materials-containing-pfas

Wang, Z., Xie, Z., Mi, W., Möller, A., Wolschke, H., & Ebinghaus, R. (2015). Neutral Poly/Per-Fluoroalkyl Substances in Air from the Atlantic to the Southern Ocean and in Antarctic Snow. *Environmental Science & Technology*, *49*(13), 7770–7775. https://doi.org/10.1021/acs.est.5b00920

We, A. C. E., Zamyadi, A., Stickland, A. D., Clarke, B. O., & Freguia, S. (2024). A review of foam fractionation for the removal of per- and polyfluoroalkyl substances (PFAS) from aqueous matrices. *Journal of Hazardous Materials*, *465*, 133182. https://doi.org/10.1016/j.jhazmat.2023.133182

Yuan, S., Wang, X., Jiang, Z., Zhang, H., & Yuan, S. (2023). Contribution of air-water interface in removing PFAS from drinking water: Adsorption, stability, interaction and machine learning studies. *Water Research*, *236*, 119947. https://doi.org/10.1016/j.watres.2023.119947