

**TWO-STEP TRANSFORMATION  
OF PER- AND POLYFLUOROALKYL  
SUBSTANCES (PFAS)**

by

Miranda Marini

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Microbiology

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## **ABSTRACT**

In this project, I evaluated a two-step approach to breakdown of long-chain PFAS chemicals into innocuous byproducts. Ideally, UV-activated nanoparticles will cleave long-chain PFAS chemicals into shorter chains, and microbes will remove the fluorines from the smaller compounds. PFAS causes a variety of health problems, and current methods of PFAS transformation have high energy requirements. In contrast, a combination of UV-activated nanoparticles and microbes could transform PFAS with lower energy requirements and would take advantage of infrastructure already in place at wastewater treatment plants. I found that perfluorooctanoic acid (PFOA) can be transformed by 100-nm nanoparticles in a basic pH solution. In addition, microbes from wastewater treatment plants defluorinated smaller PFAS chemicals like MFA. In conclusion, this work provides promising results that a two-step transformation process of long-chain PFAS chemicals could be feasible.

## Chapter 1

### INTRODUCTION

#### 1.1 Per-and Polyfluoroalkyl Substance Structure, Health Effects and Exposure

Per- and polyfluoroalkyl substances (PFAS) are a class of > 12,000

anthropogenic chemicals that have multiple carbon-fluorine bonds. PFAS chemicals are often amphipathic. The chemicals have a saturated hydrophobic tail with carbon-fluorine bonds and a polar head group. The carbon-fluorine bond is the strongest organic bond because fluorine, the most electronegative atom, attracts carbon's valence electrons more than any other element. As a result, the carbon-fluorine bond is extremely stable. PFAS chemicals were designed to be non-reactive (1). PFAS chemicals are called "forever chemicals" because of their ability to resist transformation (2). PFAS were initially produced because PFAS are highly stable and chemically inert, however those properties are also linked to human and environmental harm (1).

PFAS exposure is linked to testicular and kidney cancer, hormone disturbances, low antibody response to pathogens, decreased fertility, and asthma in humans (3). Furthermore, long-chain PFAS chemicals, like PFOA, are known to increase the risk of hypercholesterolemia, which is the accumulation of cholesterol in the bloodstream (4). In addition, PFAS chemicals can bioaccumulate in birds, fish, and marine mammals, including animals that live far away from point sources of PFAS (5). Despite all of the dangers associated with PFAS chemicals, the compounds are still used in cooking supplies, food packaging, firefighting foams, cosmetics,

waterproofing equipment including clothing, stain-resistant flooring, and many other consumer products (6). How did PFAS chemicals become so ubiquitous and what is the major source of PFAS entry into the environment?

## **1.2 Sources of PFAS into the Environment**

The major source of PFAS into the environment is directly from PFAS manufacturing plants that contaminate both the atmosphere and the water systems. Producing PFAS chemicals volatilizes both the final PFAS products and smaller PFAS intermediates produced during the production process (7). In fact, greater than 95% of PFAS chemicals volatilized from chemicals plants travel farther than 150 km (93.2 miles) according to modeling done by Community Multiscale Air Quality (CMAQ) model (7). The atmosphere is the main route of long-distance transport of PFAS chemicals, which causes the PFAS chemicals to be deposited far away from manufacturing sites (7). Even more concerning than PFAS in the atmosphere is PFAS in drinking water. The long-chain PFAS chemical, PFOA, is discharged into the environment at a rate of approximately 10-20 tons per year globally with 95% of the PFOA contributing to pollution in waterways (8). In addition, C4–C14 perfluoroalkyl carboxylic acids (PFCA), a group of PFAS chemicals, are discharged into water at a rate of 130 tons per year globally (8). PFAS exposure from atmospheric sources and water sources is currently unavoidable, however humans can remove PFAS from drinking water at water and wastewater treatment plants (WWTP).



Figure 1.1: PFAS exposure routes from manufacturers to the environment. Figure is based on image from PFAS Chemicals Overview | ASTDR (6).

### 1.3 Removal Methods and Disposal of PFAS Contaminated Waste

Removal of PFAS often occurs at WWTP or pump stations (9). Although there are many ways to remove PFAS from water, granulated activated carbon filters are by far the most common method (10). Activated carbon filters are the preferred method over other PFAS removal processes, like ion exchange resins and reverse osmosis, because of the lower cost and better PFAS removal at concentrations found in the environment i.e., ng/L range (11). Activated carbon filters capture PFAS and other organic pollutants via adsorption. The two methods of activated carbon disposal are in landfills or to reuse the activated carbon through regeneration (12). In fact, landfills account for up to 12% of the total PFAS emissions into the environment (8). In the landfill environment, activated carbon contaminated with PFAS is a risk for leaching and may contribute to the PFAS emissions from landfills. However, estimates of how

much PFAS released from landfills with municipal waste vs industrial waste with activated carbon filters are not publicly available. A better method of PFAS disposal involves recycling the activated carbon in a process called regeneration.

Activated carbon is regenerated by heating the carbon and attached contaminants up to 1800°F. The heating process thermally desorbs PFAS from the activated carbon, which can then be reused (12). Surprisingly, the regeneration process can lead to long chain PFAS transformation. In fact, the regeneration process transformed 99.999% of the hardest to degrade PFAS chemicals like PFOA, perfluorosulfonic acid (PFOS), hexafluoropropylene oxide dimer acid (GenX), and perfluorobutane sulfonate (PFBS) chemicals (12).

#### **1.4 Why Use Nanoparticles and Microbes to Transform PFAS Over the Regeneration of Activated Carbon?**

Although the regeneration process transforms PFAS, the regeneration process also requires high energy expenditure to overcome the extremely strong bond between carbon and fluorine atoms in long chain PFAS molecules. To regenerate activated carbon filters, the filter material must be removed and transported to the regeneration facility, regenerated, then moved back to the wastewater treatment facility. A lower energy method of PFAS destruction is needed. Ultraviolet (UV)-activated nanoparticles (NP) could potentially decrease energy costs while utilizing pre-existing infrastructure, UV light treatment, in WWTP. In addition, microbial cultures will take advantage of energy sources already within the WWTP, like dissolved organic carbon, to transform shorter-chain PFAS chemicals. Combining UV-activated NP and microorganisms can potentially result in complete PFAS remediation.

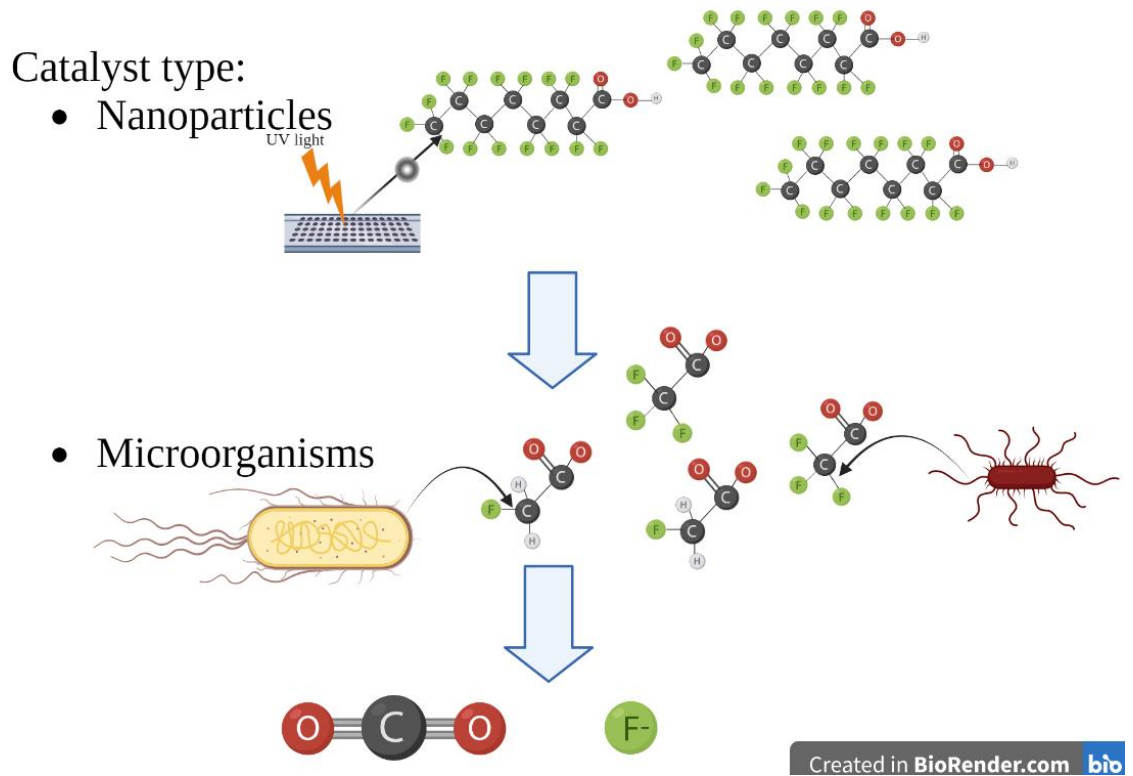


Figure 1.2: Graphical summary of project. UV-activated NPs will break down long-chain PFAS chemicals, i.e., PFOA. Microorganisms will break down shorter-chain PFAS chemicals, e.g., monofluoroacetate and trifluoroacetate. Figure made using BioRender.com (13).

## Chapter 2

### METHOD TO TRANSFORM LONG-CHAIN PFAS CHEMICALS VIA UV ACTIVATED NPs

#### 2.1 Introduction

##### 2.1.1 Long-Chain PFAS Chemicals

Long chain PFAS chemicals are typically defined as perfluorinated chemicals that have a chain of >7 carbon bonds fully saturated with fluorines (14). Long chain PFAS molecules are more stable, recalcitrant, and tend to bioaccumulate more than shorter PFAS chemicals (14). In addition, research beginning in the 1950s and continuing today on long chain PFAS chemicals, like PFOA and PFOS, suggests that the perfluorinated chemicals pose risks to human and environmental health (14).

##### 2.1.2 Evolving NP Technology

Although NP technology is not new, many applications for NP technology have only recently been realized. Key to these applications is that NPs can be energy conduits in the form of electricity. The NP's property of carrying electric charge is valuable. This project will use UV light to excite the NP electrons to initiate long-chain PFAS transformation.

Aluminum was chosen to be the NP material for a few reasons. First, aluminum is a metal that has 3 valence electrons (15). Aluminum is most stable when the outer electron shell is full, so aluminum tends to donate electrons during chemical reactions (15). When the UV light excites the aluminum NP's electrons, a plasmon is produced. The plasmon releases an electric field which we hypothesize can break the bonds in PFOA or PFOS (Figure 2.1) (16). In addition, aluminum is inexpensive, so

scaling up experimentation is more cost-effective than using other NP materials like silver or platinum.

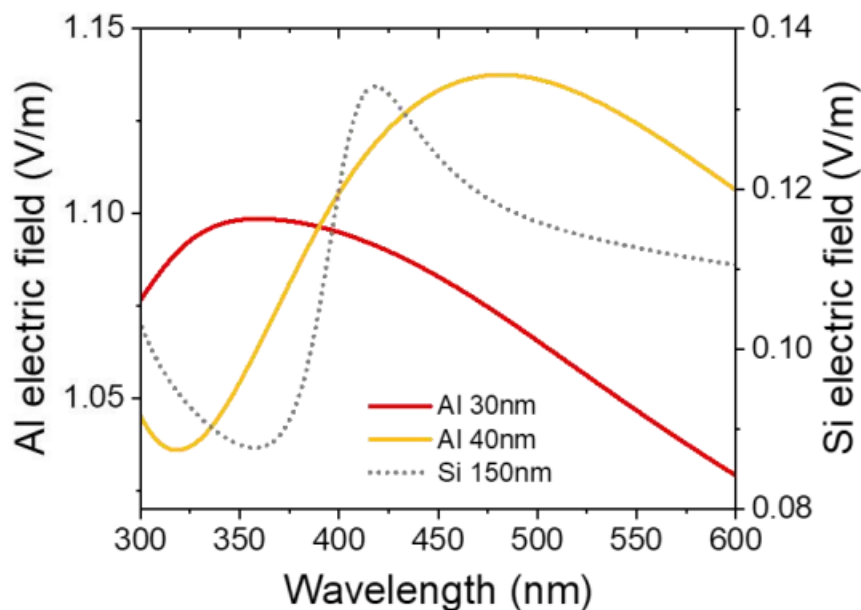


Figure 2.1: Predicted electric field strength of silicon and aluminum NPs in response to light of different wavelengths. NP diameter is indicated in the figure legend. Courtesy of Dongxia Wei and Stephanie Law, Department of Materials Science and Engineering.

### 2.1.3 Building on Previous Research

Experiments from a former master's student, Emma Smith, provided promising results on the potential of degrading PFOA using UV light and NPs. Over the course of 8 hour experiments, 50 mg/L of PFOA was partially transformed in the presence of both UV light alone and UV light plus 100 nm NP (Figure 2.2) (16). The 0.5 mg/L PFOA solution exposed to 8 hours of UV light had 13% PFOA transformation (Figure 2.2) (16). PFOA solution exposed to 75 nm NP for 8 hours transformed PFOA by 16% and 0.5 mg/L PFOA solution exposed to 100 nm NP for 8 hours transformed 0.5 mg/L PFOA by 24% (Figure 2.2) (16). Interestingly, increasing the time of UV light and NP

exposure did not increase PFOA transformation (Figure 2.2) (16). Although the results are encouraging, Emma provided suggestions for improvement: more replicates, decreasing the initial volume of the PFAS solution to maximize PFAS contact with the NPs, and testing the effects of pH and decreased UV exposure time (Figure 2.2) (16). Here, my goals were to confirm the previous results and identify parameters that improved PFOA transformation by UV-activated NPs.

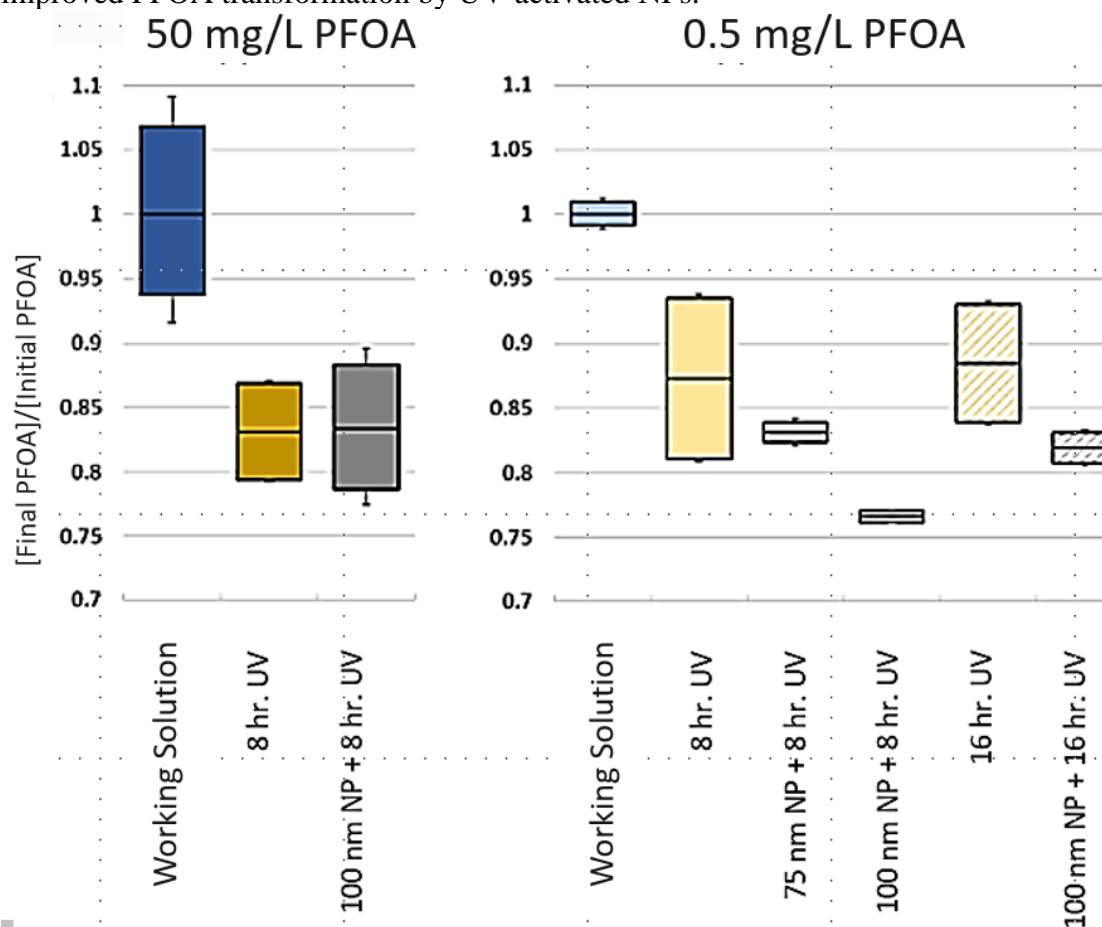


Figure 2.2: Transformation of 50 mg/L and 0.5 mg/L PFOA when exposed to UV light, 75 nm NP, and 100 nm NP. The ratio of final PFOA concentration to initial PFOA concentration is plotted. Figure from Emma Smith's thesis (16)

Table 2.1: Comparing previous experiments to experiments described here.

	PFAS Solution Volume (mL)	Experimental Time (hrs.)	pH	Number of Replicates	Buffered Solution	Stir Bar Material	Quartz Attachment
Previous Experiments	50	8	7	2	No	Teflon coated	Clear nail polish
		16					
This Study	2	0.25	7	4	Yes (inorganic buffers)	Stainless steel	N/A
		1					
		2					
		4					
		0.25	10				
		1					
		2					
		4					

## 2.2 Materials and Methods

### 2.2.1 PFAS Solutions

Prior to experimentation, the glassware was cleaned by swirling 100% methanol in bottles to make sure that the methanol was able to touch every part of the bottle, pouring out the methanol, and allowing the rest of the methanol to evaporate in the fume hood. The PFAS solutions were made in the lab with the PFOA or PFOS solutions being measured out on a mass balance for PFOA or with a P20 micropipette for the PFOS solution.

The experimental conditions tested in the NP experiments varied by PFAS substrate, concentration, pH, type of solvent, and UV exposure time. Transformation of both PFOA and PFOS was tested. Variations in PFAS transformation were measured in solutions at pH = 7.00 and pH = 10.00, buffered with 0.1 mM phosphate buffer or 0.1 mM carbonate buffer, respectively. Milli-Q water was used to dilute the samples. In the November 2022 experiments, three ratios of dimethyl sulfoxide (DMSO): water were tested, 8:1, 1:1, 1:8 v/v. Low temperature mineralization of PFCAs in a DMSO: water solution of 8:1 (v/v) was possible (17), so the November

experiments tested if DMSO and water could facilitate PFAS transformation in the presence of NPs. Concentrated “natural” water came from reverse osmosis permeation from contaminated groundwater and had ~0.14 µg/L PFOA and ~ 1.8 µg/L PFOS.

Table 2.1. Experimental Parameters.

<b>Experiment Date</b>	July, 2021	January, 2022	July, 2022	November, 2022
<b>Chemical</b>	PFOA	PFOS	PFOA	PFOA
<b>Concentration</b>	50 mg/L	100 µg/L	20 µg/L	12 µg/L
<b>Solvent</b>	Water	Water	Water	DMSO & Water
<b>Water Type</b>	Deionized	Deionized	Deionized	Deionized & Natural
<b>pH variation</b>	7 & 10	7 & 10	7 & 10	10
<b>Sample Order</b>	Non-random	Non-random	Randomized	Randomized
<b>Experiment time (hrs.)</b>	0, 0.25, 1	0, 0.25, 1	0,2,4	0,2
<b>Replicate Number</b>	4	4	4	1
<b>NP Size</b>	Control, 75 and 100 nm NP	Control, 75 and 100 nm NP	Control, 75 and 100 nm NP	Control, and 100 nm NP
<b>Experimental Design</b>	Manual	Manual	Design of Experiments	Design of Experiments
<b>UV Exposure</b>	Only samples exposed to UV	Only samples exposed to UV	Only samples exposed to UV	Samples with and without UV exposure

### 2.2.2 Experimental Variables and Design

To design experiments that could isolate the effect of each variable, the July 2022 and November 2022 experimental plans were made using JMP’s Design of Experiments software (18). The experimental parameters tested in the experiments were variations in NP size, pH, UV exposure, and time. A quartz slide without NPs

(control) was only exposed to UV light. The NP size used in the experiments were 75 and 100 nm NPs in diameter. The NP were secured on a 1 cm<sup>2</sup> quartz slide. Each quartz slide either had all 75 nm NP or 100 nm NP. The effect of pH on PFAS transformation was tested at pH = 7 and pH = 10. For assessing the effect of UV exposure, samples were put under UV light or put in a dark cabinet for the experimental duration. The length of experimental time varied from 0 to 4 hours.

### **2.2.3 Experimental Setup**

First, a string level was placed between the UV lamp and the mirror to make sure that the mirror was level with the UV lamp. A square water filter filled with Milli-Q water was placed about 1 inch in front of the UV lamp. The water filter was used to filter out longer wavelengths of light that could cause heat and therefore increased evaporation. After passing through the water filter, the UV light was reflected onto the NPs by a UV-enhanced aluminum coated mirror from Thor Labs. The UV lamp was turned on and the dial was set to 40 Amps.

The materials in direct contact with the PFAS solution were a quartz slide with or without NPs on the surface, a stainless-steel stir bar, silicone tweezers, and a glass 20 mL beaker. The quartz slide, the beaker, and the stir bar were washed with 100% methanol before and after each replicate. After cleaning the experimental supplies, 2 mL of PFAS solution was added to the beaker. The PFAS solution was stirred at 300 rpm with the stainless-steel stir bar throughout the experiment. At the end of the experiment, the PFAS solution, the beaker, the stir bar, and quartz slide with or without NPs were removed from the UV light source.

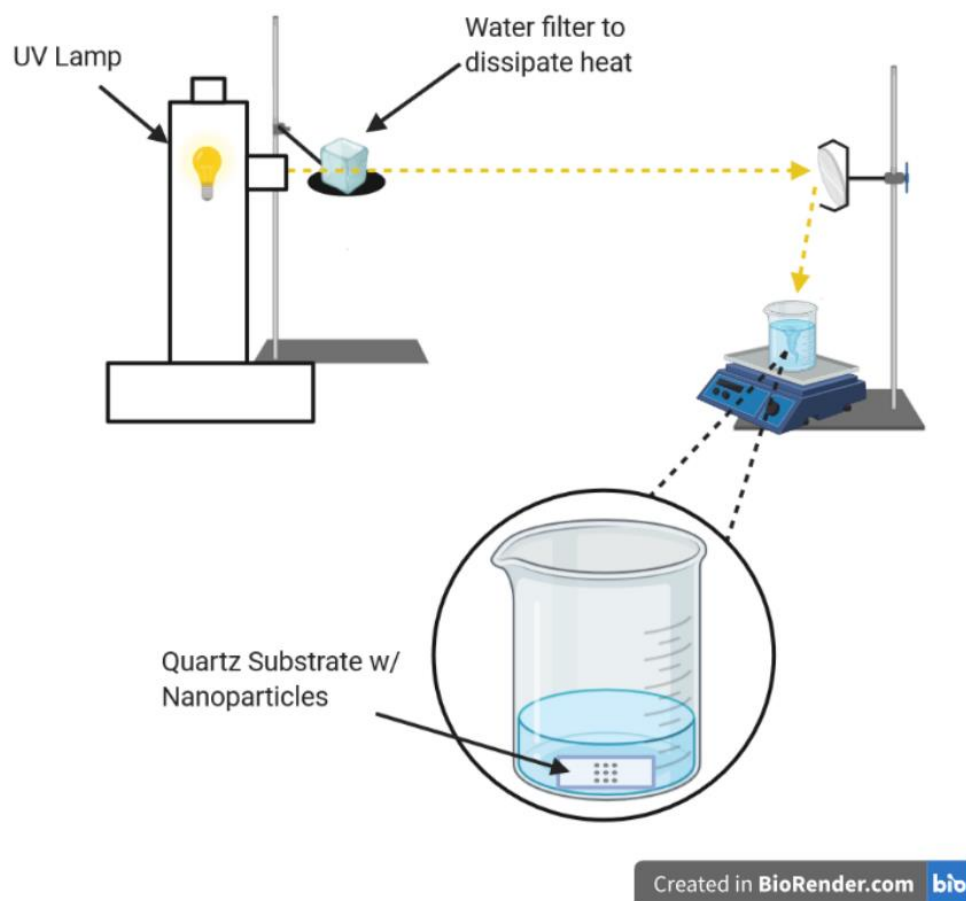


Figure 2.3. NP experimental setup. The UV lamp, water filter, mirror, beaker with PFOA solution, and the aluminum NPs on the quartz slide are all depicted in the image. Figure was created by Emma Smith (16) and made using BioRender.com (13).

#### 2.2.4 PFAS Analysis

Veolia, formerly SUEZ Water Technologies & Solutions, measured the PFAS analytes using the Environmental Protection Agency Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC-MS-MS) (19). The LC-MS-MS

method was used, however no extraction protocol including the solid phase extraction, was used to prepare the samples. Veolia provided the PFOA data in units of ng/L. The ng/L concentrations were adjusted to mg/L or  $\mu\text{g/L}$  PFOA. The concentration units were adjusted to visualize the data more easily, which made it simpler to check my calculations and notice potential mistakes or outliers more conveniently. The data was separated into two groups based on the pH of the working solution, pH = 7 or pH = 10. To account for evaporation, the mass of the samples was measured before and after UV exposure. Adjusted concentration = (mass final\*concentration final)/ mass initial (Equation 1). From this point on, only the adjusted values were analyzed. Each working solution was measured just like the experimental samples exposed to UV light with or without NPs. Evaporation was not a factor affecting the working solution variability because the working solution was never exposed to UV light or any other conditions that would cause evaporation. Rather, the working solution was transferred directly from the container into a 2 mL tube for sampling. An average of all the possible ratios of [adjusted PFOA final] / [adjusted PFOA initial] for each condition, i.e., the negative control without NPs, the 75 nm NP, and 100 nm NP, at each time point were calculated. To estimate the probable variability of the data, the 95% confidence interval was calculated from the mean and standard deviation of the values of all possible ratios for a given parameter. The alpha value was 0.05. The sample size and standard deviation varied depending on the number of replicates and the experimental conditions.

## 2.3 Results

### 2.3.1 Determining the Effect of PFOA Transformation in the Presence of NPs at pH = 7 and pH = 10.

To determine if pH influences PFOA transformation by NP (75 nm and 100 nm) and UV light, the samples were run in the same experimental setup at a pH of 7 and a pH of 10. At neutral pH, in the control (no NP) and in the presence of 75-nm NPs concentration decreased by ~18% over the course of 60 minutes (Figure 2.4A). However, PFOA concentration in samples exposed to 75 nm NPs decreased by more than 99% within the first 15 minutes of the experiment and did not change at the 60 minute interval (Figure 2.4A). In contrast, PFOA solution that was exposed to UV light for 60 minutes and 75 nm NP only had a 10% decrease in PFOA (Figure 2.4A). The 100 nm NP transformed PFOA by a maximum of 23% over the course of an hour and by 7% in the 15 minute experiments (Figure 2.4A).

The pH = 10 solution and larger NP size appeared to play a role in NP transformation of PFOA. The 100 nm NP caused a decrease in PFOA concentration of 65% over the course of 60 minute experiments, the largest decrease in PFOA concentration (Figure 2.4B). In comparison, over the course of 60 minutes, the control was transformed by only 15%. The PFOA exposed to 75 nm NP was decreased by 12% over 15 minutes but no further decreases were observed during the full 60 minute incubation (Figure 2.4B). In pH = 10 solution, although the control had an initial decrease in PFOA concentration in the first 15 minutes of 99% (Figure 2.4B) at the 60 minute time point PFOA concentrations were similar to concentrations at the beginning of the experiment. We believe that the PFOA concentration in the 15 minute control at pH = 10 is likely an artifact.

Although the higher pH solution may enhance NP-mediated attenuation of PFOA, the large variation in the data, especially in the pH = 10 PFOA control, suggests the possibility of artifacts associated with the experimental methods. For example, the apparent decrease in PFOA concentration of 99% in the control after 15 minutes is not supported by the 60 minute experiments (Figure 2.4A). Additionally, the large error bars of the PFOA concentration at the 60 minute time point of the 100 nm NP experiment (Figure 2.4B) could have been because the initial concentration of 50 mg/L PFOA (Figure 2.4B) exceeded the range of concentrations in the standard curve. The method that Veolia used can only measure PFAS concentrations by LC-MS-MS in the 0.125-25 ng/mL range without diluting the samples. Therefore, the staff at Veolia diluted these samples 2,000 times to be within the measurable range for that instrument. This dilution step could have contributed to error in the results and increased variability in the plotted values. As a result, the solutions in subsequent experiments were prepared at concentrations closer to the measurable range in order to align with the standard curves of the method and thus decrease the possibility of errors during dilution. In addition, previous analysis had yet to test the effect of NPs on PFOS, so the next experiment included PFOS as the sole PFAS source.

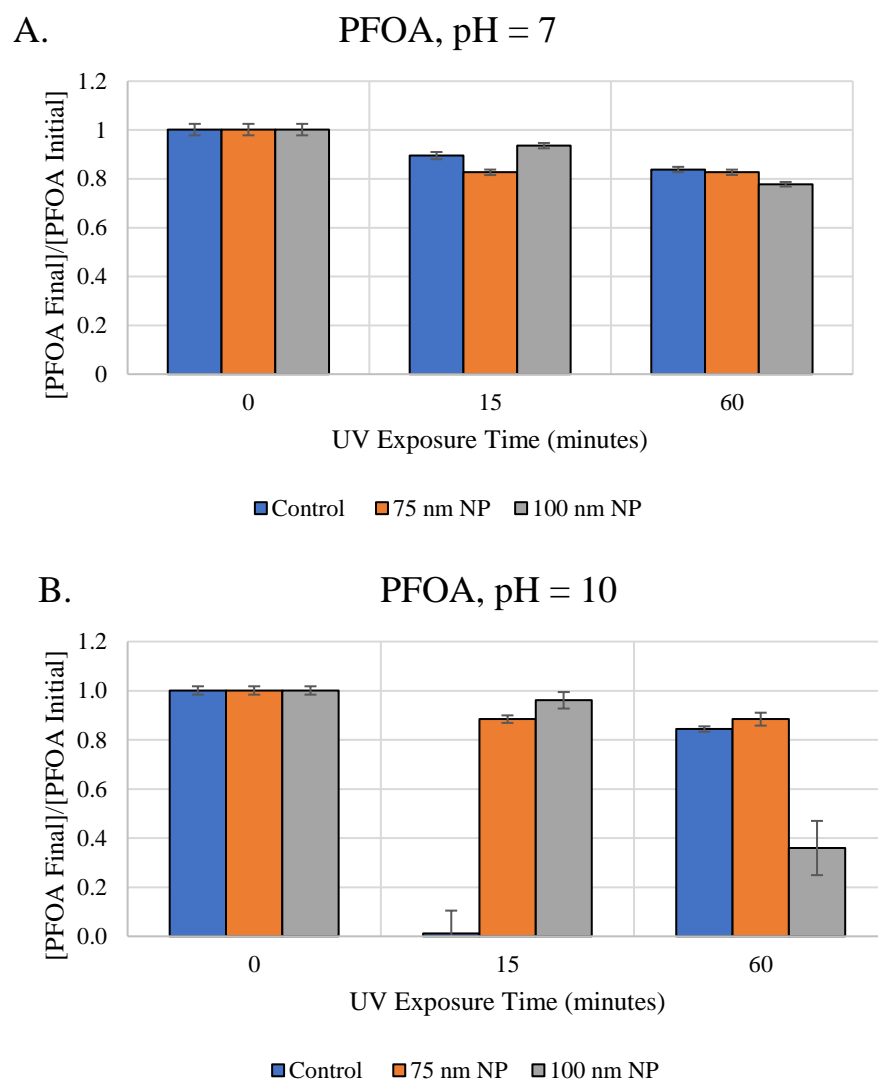


Figure 2.4: The effect of UV light and NPs on 50 mg/L PFOA solution. The error bars represent the 95% confidence interval. The control (red) was never exposed to NPs but was exposed to UV light. 75 and 100 nm NP = exposed to NPs and UV light. A. At pH = 7, the control and 75 nm NPs transformed PFOA by 18% over the course of 60 minutes. The 100 nm NPs transformed PFOA by a maximum of 23% over the course of an hour. B. In pH = 10 solution, PFOA exposed to 75 nm NPs was transformed by 12% over 15 minutes. In contrast, 100 nm NP caused a decrease in PFOA concentration of 65% over the course of 60 minutes, which is the largest decrease in PFOA concentration observed in this project.

### **2.3.2 Identifying the Effect of NPs on the Transformation of 100 µg/L PFOS.**

At pH = 7 and pH = 10, the effects of NPs and UV light on the transformation of 100 µg/L PFOS were investigated. In pH = 7 PFOS solution, the control, the 75 nm NP, and the 100 nm NP did not lead to PFOS transformation over the course of 15 or 60 minutes (Figure 2.5A). In fact, a higher concentration of PFOS was observed at the end of all the experiments (Figure 2.5A). In pH = 10 solution, the control, 75 nm NP, and the 100 nm did have a measurable decrease in PFOS concentration through time (Figure 2.5B).

Regardless of pH, the PFOS concentration after exposure to UV light and NPs was higher than the initial PFOS concentration in all experiments. However, the UV light, the NPs, and the solution pH are likely not causing the measured increase in PFOS concentration. The experimental design made identifying sources of variability more difficult to identify. As a result, future experiments were designed using JMP's Design of Experiments protocol. The lack of PFOS transformation could be because PFOS has a longer carbon-fluorine chain and is therefore more likely to be stable. In addition, PFOS has two more carbon-fluorine bonds than PFOA. As a result, subsequent experimental designs were made using JMP's Design of Experiments software, which planned experiments to decrease the effects of random error, and 20 µg/L PFOA was the substrate tested.

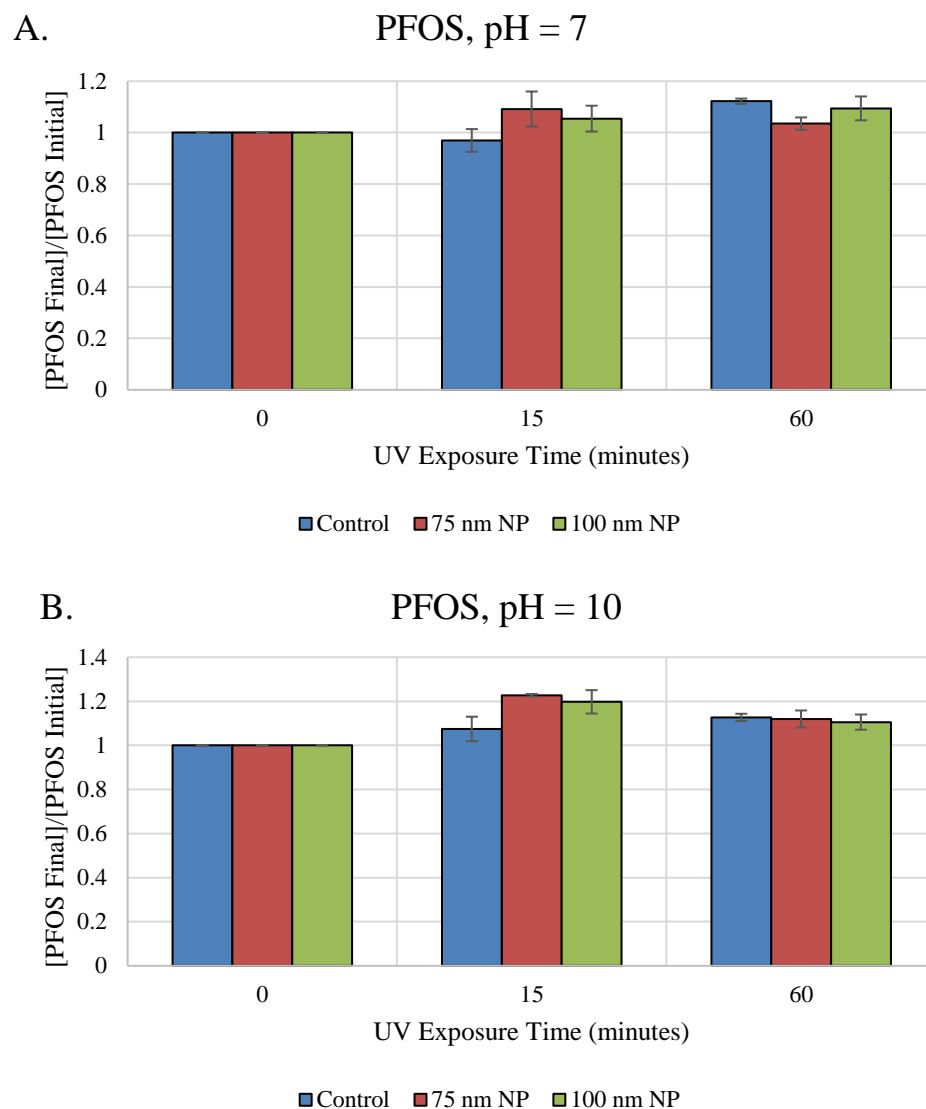


Figure 2.5: The effect of UV light and NPs on 100  $\mu\text{g/L}$  PFOS solution. The error bars represent the 95% confidence interval. The 15 and 60 minute time points had 4 replicates for each parameter. Control 0 time point for the pH = 7 had 2 replicates and the control for the pH = 10 had 2 replicates. Red = control was never exposed to NPs and only exposed to UV light + a quartz slide. 75 and 100 nm NP = exposed to NP. Both A. and B. No change in PFOS concentration was measured through time.

### **2.3.3 Determining the Effect of NPs on the Transformation of 20 µg/L PFOA at pH = 7 and pH = 10.**

To determine if pH influences 20 µg/L PFOA transformation in conditions with NP (75 nm and 100 nm) and UV light, side by side experiments were run at a pH of 7 and a pH of 10. In neutral pH PFOA solution without active NPs, the PFOA concentration in the 2 hour experiments did not change from the 0 time point, while 4 hour experiments had a 70% higher PFOA concentration than the initial time point (Figure 2.6A). Neutral pH PFOA solution with 75 nm NP had a 21% higher PFOA concentration at the 2 hour time point and 33% higher PFOA concentration at the 4 hour time point than the 0 time point (Figure 2.6A). In neutral pH PFOA solution with active 100 nm NP, the PFOA concentration did not change throughout the 2 or 4 hour experiment (Figure 2.6A).

Although the final PFOA concentration was higher than the initial PFOA concentration, the high PFOA concentration was likely not due to PFOA synthesis by the NPs or the UV light. Both UV light and NPs break down chemicals rather than synthesize the chemicals (20).

In contrast to the neutral pH PFOA solution, the pH =10, PFOA solution had no significant change in PFOA concentration (Figure 2.6B). Both the control and the 75 nm NP after 4 hours of UV exposure had a decrease in PFOA concentration of 0.5% (Figure 2.6B). PFOA concentration in the 100 nm NP experiment decreased by 10% over the course of 2 hours (Figure 2.6B). According to the 95% confidence interval, the maximum PFOA transformation that potentially occurred was 10.1% in the solution exposed to pH = 10, 100 nm NP (Figure 2.6B). Thus, PFOA transformation is potentially possible using UV light + 100 nm NP at the pH = 10.

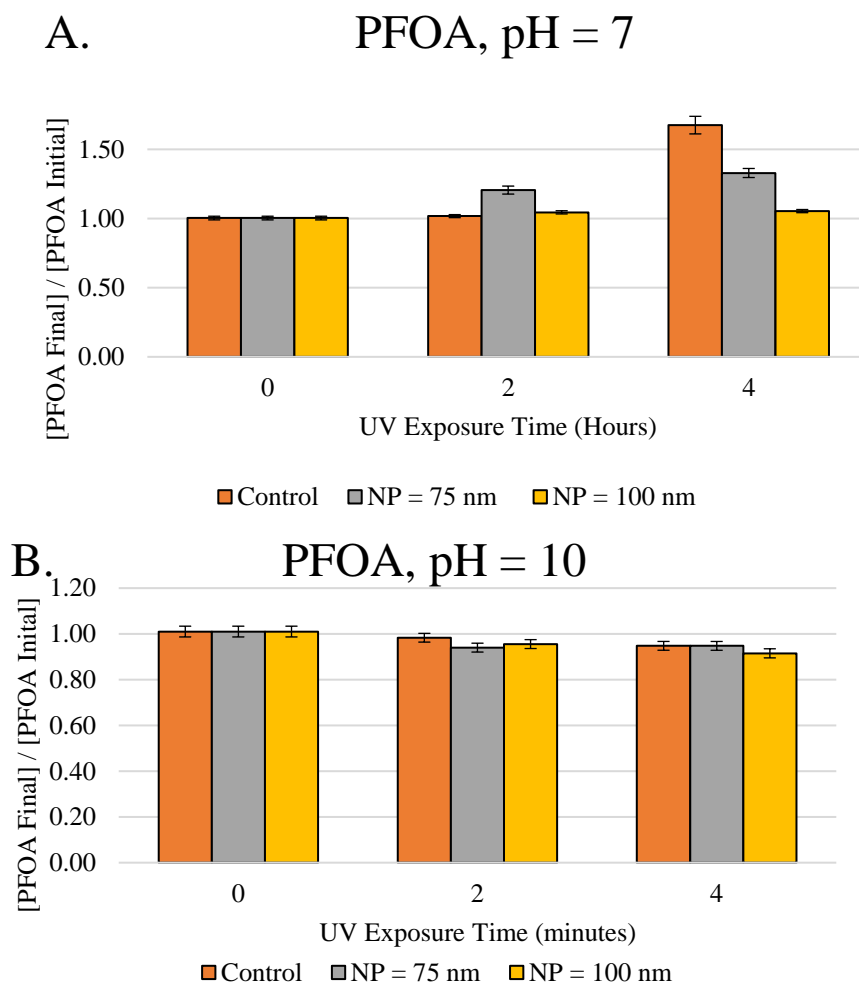


Figure 2.6: The effect of UV light and NPs on 20  $\mu\text{g/L}$  PFOA solution. The error bars represent the 95% confidence interval. The 15 and 60 minute time points had 4 replicates for each parameter. Control, 0 time point for the pH = 7 had 12 replicates and the control for the pH = 10 had 12 replicates. Red = control was never exposed to NPs and only exposed to UV light + a quartz slide. A. In neutral pH PFOA solution, a measured increase in PFOA concentration was observed. B. In contrast to the neutral pH PFOA solution, the pH = 10, PFOA solution, saw no significant change in PFOA concentration.

#### **2.3.4 Evaluating how DMSO: Water Ratio Impacts PFAS Transformation via UV Activated NPs**

To identify whether the ratio of DMSO: water or water constituents influences 12 µg/L PFOA transformation in conditions with NPs (75 nm and 100 nm) and UV light, experiments were run in conditions with milli-Q water and water from natural sources and at ratios of DMSO: water of 8:1, 1:1, 1:8 v/v (Figure 2.7). Regardless of DMSO: water ratio or the type of water used in the experiment, neither affected PFOA concentrations; in fact, a measured increase in PFOA concentration was observed (Figure 2.7). Additionally, variations in working solution were accounted for in the 0 time point measurements and should not have changed because a large batch of solution was made up for each v/v ratio of DMSO: water. Ultimately, many hypotheses for why the increase in PFOA occurred, like sample order, residue on glass the effect of water type, were refuted.

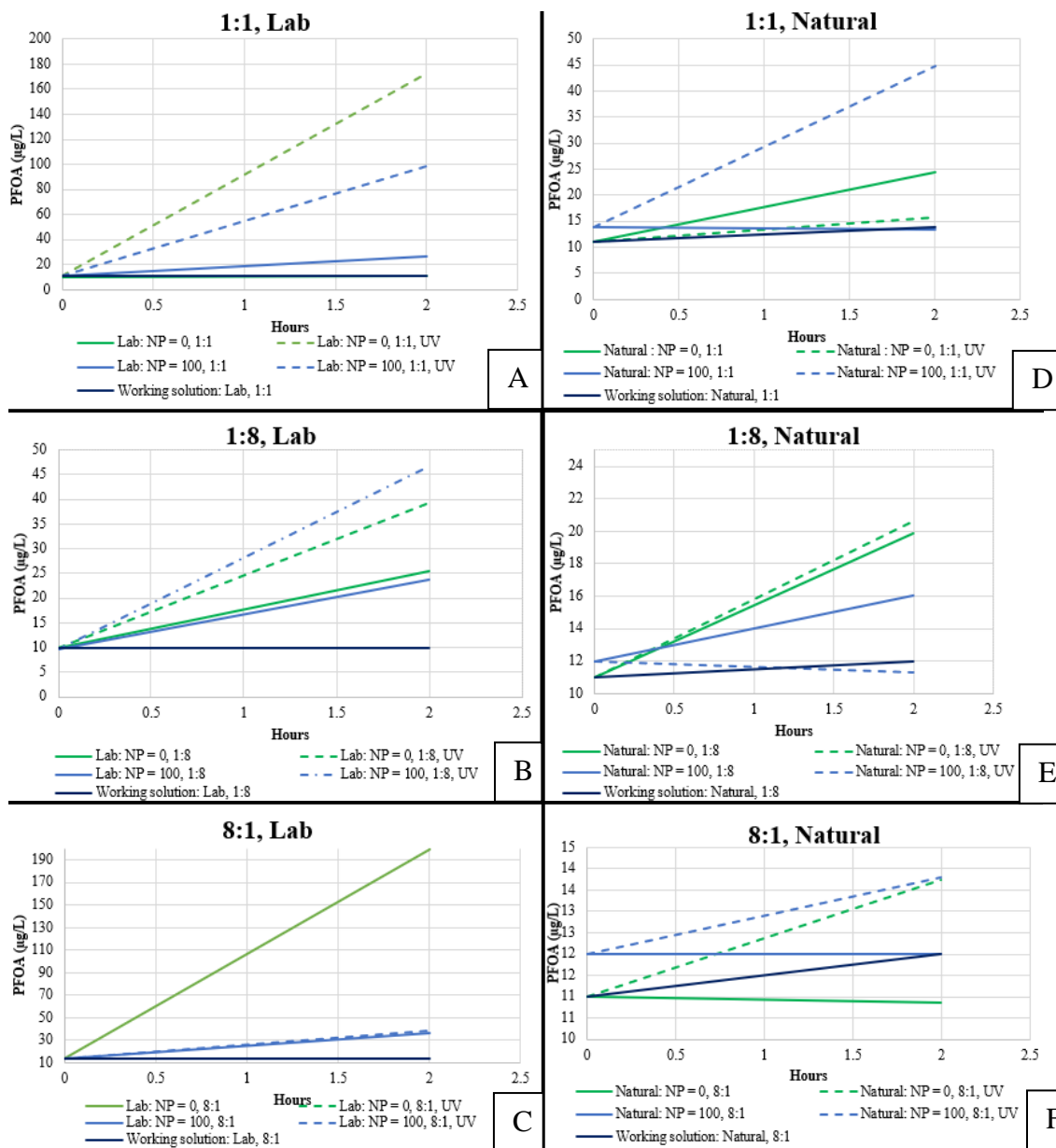


Figure 2.7: The ratio in each figure represents the DMSO: water ratio added to each working solution of 12 µg/L PFOA. Solid lines = no UV exposure. Dashed lines = UV exposure. Lab = water was milli-Q water from the lab. Natural = water with ~ 0.14 µg/L PFOA and ~ 1.8 µg/L PFOS. Replicate number = 1. In every experiment, there is no trend of PFAS transformation. 0 nm NP = control sample.

## **2.4 Discussion**

### **2.4.1 Goal of the Study**

The goal of the NPs experiments was to determine if previous results from the lab suggesting that PFAS can be transformed using UV activated NPs could be improved upon. Here, I tested the effects of solution parameters including pH and solvent type on PFOA transformation by UV-activated aluminum NPs. The results presented here show that a pH ~ 10 and 100 nm NPs may enhance PFOA transformation (Figure 2.4B).

### **2.4.2 Future Directions**

The higher pH leading to increased PFOA transformation agrees with a 2011 study that tested when pH plays a role in the ability of microwave-activated persulfate to transform PFOA. Using both UV activated NPs and microwave-activated persulfate leads to increased PFOA transformation at pH values approaching 10 (21). Microwave-activated persulfate is similar to UV-activated NPs because they both use the concept that excited electrons can break bonds in PFAS molecules. In this experiment, approximately 40% of the initial PFOA remained (Figure 2.4B). When using microwave-activated persulfate, a little more than 40% of the PFOA was transformed in the first hour with 80% of the initial PFOA being transformed within 4 hours (21). Therefore, future experiments should continue to measure PFOA transformation at higher pH.

In other studies, the type of water used to dissolve the PFOA matters more than the pH. In pH = 5.5 solution, the PFOA was transformed by 87% in ultrapure water (distilled and deionized), while only 37% of the PFOA was transformed in river water

(22). Therefore, future experiments could test the effect of natural or Milli-Q water at a basic pH on PFOA transformation using UV-activated NPs.

## Chapter 3

### MICROBIAL DEFLUORINATION OF SHORT-CHAIN PFAS CHEMICALS

#### 3.1 Introduction

##### 3.1.1 Why Cultivate Microbes?

The second step in PFAS breakdown involved utilizing microorganisms. If NPs can break the long-chain PFAS into smaller components, then the short-chain PFAS can be transformed by microbes. Short-chain PFAS require less energy input to transform than the long-chain PFAS chemicals (1). Therefore, microbes would benefit more from removing fluorines from carbons because the anabolic or catabolic benefits would be worth the energy input.

##### 3.1.2 Cultivation of Microbes from WWTP

Emma enriched microorganisms from the aeration basin of the Veolia WWTP the Frank E. Van Lare Water Treatment Plant, and King's Landing WWTP (16). Of the several locations in the WWTP, the aeration basins were sampled from because the aeration basin has the highest number of microbial operational taxonomic units (OTU) of any location in WWTPs (23). Emma's experiments had to be archived due to the COVID-19 laboratory closures. The enrichment cultures were archived in 10% glycerol at -80°C for 24 months (16), until I revived them.

The goal of the microbial cultivation experiments was to cultivate microbes that can defluorinate short-chain PFAS, like monofluoroacetate (MFA) or trifluoroacetate (TFA). MFA only has one carbon-fluorine bond, while TFA has three carbon-fluorine bonds. If the microbes can transform short-chain PFAS chemicals and non-PFAS carbon sources, then the microbes could be completely degrading PFAS

after photocatalysis using UV-activated NPs while maintaining the ability to effectively compete in the process called bioaugmentation. Here, I demonstrate that microorganisms can biologically remove fluorines from short-chain PFAS chemicals and release fluoride into solution.

## **3.2 Materials and Methods**

### **3.2.1 Growth Media Preparation**

The basal growth media was made using the recipe from Alexandrino et al. 2018 in which mono-, di-, and tri- fluoroacetate were shown to be transformed by microbes (24). The media was made in two parts, the minimal salts solution, and the trace elements stock (100x). The minimal salts media contained 2.7 g/L sodium phosphate dibasic dihydrate, 1.4 g/L potassium dihydrogen phosphate, 0.5 g/L ammonium sulfate, and 0.2 g/L magnesium sulfate. The trace element 100x stock contained 1.0 g/L calcium chloride, 2.0 g/L ferrous sulfate heptahydrate, 0.4 g/L manganese (II) sulfate, 10.0 g/L sodium sulfate, 12.0 g/L ethylenediaminetetraacetic acid (EDTA) disodium salt, 0.4 g/L zinc sulfate, 0.1 g/L sodium molybdate, 0.1 g/L copper (II) sulfate pentahydrate, 0.5 mL/L sulfuric acid, 2.0 g/L sodium hydroxide. The solid media had the same components, except for the addition of 15 g/L agar.

To make a liter of growth media, components of the minimal salts solution were added to 950 mL of Milli-Q water and the components were mixed together until dissolved. Then, 10 mL of 100x trace element solution was mixed into the solution. The pH was adjusted to 7.0 with HCl and the solution volume was adjusted to 1 L. After the volume was adjusted, the carbon sources, MFA, TFA, and/or acetate, were

added to the media. Because the concentration of the carbon sources varies between experiments, the carbon source concentrations are listed in Figure 3.1. The media was sterilized by filtering through a 0.2-micron filter. 1% yeast extract media plates were made by measuring out 10 g of yeast extract and adding it to 1 L of the basal growth media described above.

### **3.2.2 Enrichment Cultures Cryo-revival**

The microbial culture used in the following experiments originated from the Wilmington WWTP. The initial sampling date was April 9<sup>th</sup>, 2019, and the culture was preserved in the freezer on July 17<sup>th</sup>, 2019, after being cultivated on 2  $\mu$ M MFA for three months. The microbial cultures in the experiments were revived from being frozen in 12% glycerol. Initial revival was done by adding 3 loopfuls of inoculum from the preserved samples to 10 mL liquid basal media with 200  $\mu$ M MFA. The microbes were cultivated in aerobic conditions without shaking. Because most of the revived samples had a green color, which indicated that photosynthetic organisms were present in the samples, the cultures with phototrophs were covered with a box, and allowed to grow for two weeks in the dark, until no green microbes were visible. Isolation of individual strains from the enrichment cultures was not attempted. Cultures with MFA were expanded to up to 50 mL cultures. After more than a month of growth, 20 microliters from the liquid cultures were plated on solid media with 20 mg/L MFA or 1% yeast extract.

### **3.2.3 Fluoride Measurements**

Fluoride measurements were done using the Thermo Fisher Scientific Ion Selective Electrode fluoride meter. A standard curve was made by recording output, millivolts, at known concentrations of sodium fluoride. The concentrations used to make the standard curve were 1, 5, 10, 50, and 100 mg/L respectively inside of glass tubes. The fluoride meter was cleaned by being submerged in milli-Q water before and after each round of measuring. The cultures in the fluoride experiments were cultivated in high density polyethylene 100 mL screw cap containers. When measuring fluoride in microbial cultures, a 10 mL aliquot was removed from the culture and filtered through a 0.2-micron filter prior to fluoride measurements.

Defluorination was evaluated by tracking the production of fluoride during incubation. Defluorination in MFA cultures vs. uninoculated control samples was measured, and there were four replicates per condition. Next, defluorination was measured in cultures with 20 mg/L TFA + 40 mg/L acetate, 50 mg/L MFA + 20 mg/L TFA, 100 mg/L MFA, 40 mg/L TFA and two controls with 40 mg/L TFA and 100 mg/L MFA. Each condition had three replicates. A sample from the 100 mg/L MFA sample was used to inoculate samples with 100 mg/L MFA, 100 mg/L MFA + 40 mg/L acetate, and 100 mg/L MFA + 40 mg/L acetate + 20 mg/L TFA (Figure 3.1). When the initial fluoride concentration varied, the initial fluoride concentration was subtracted from each fluoride measurement.

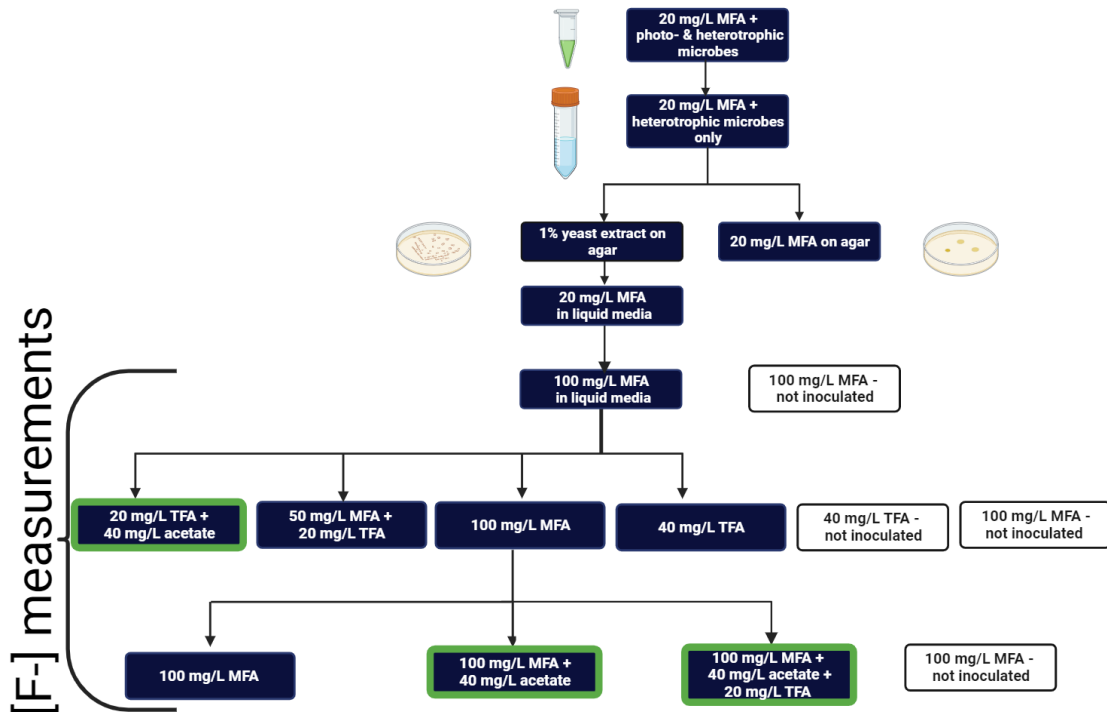


Figure 3.1: Diagram of microbial transfers. MFA = MFA, TFA = TFA. Figure was made using BioRender.com (13).

### 3.3 Results

#### 3.3.1 Carbon Source Affects the Diversity of Microbes That Can Be Cultivated on Solid Media.

After reviving the Wilmington wastewater enrichment cultures and selecting against phototrophs in basal media with 0.2 mM MFA provided as the sole carbon and energy source, the microbial density in the cultures was significantly decreased based on visual comparison of cultures with and without phototrophs. To increase biomass in liquid cultures, an aliquot of the 0.2 mM MFA cultures from the Wilmington WWTP were plated on solid media with two different carbon sources. One culture only had

0.2 mM MFA while the other culture had 1% yeast extract. Visible colonies took 4 weeks to appear on medium with 0.2 mM MFA and only one colony type grew (Figure 3.2A). When the same inoculum was plated on 1% yeast extract, the microbial colonies grew faster, ~2 weeks, and had more colony types. The MFA culture likely had identical diversity when being plated. Fewer colony types were present in cultures grown on MFA than cultures grown on 1% yeast extract. There was a total of four colony types on the 1% yeast extract plate. One colony was medium size, beige, and circular. Another colony was orange and circular. Another colony type was medium-sized, beige without a defined shape. The final colony type was clear, small, and circular. The colonies range in appearance from yellow orange within a well-defined circle to white and beige and bright orange with less defined shape (Figure 3.2B). One representative of each colony type on the yeast extract medium was picked and transferred into a mixed culture for subsequent defluorination experiments. The new culture was referred to as “simplified microbial culture.”

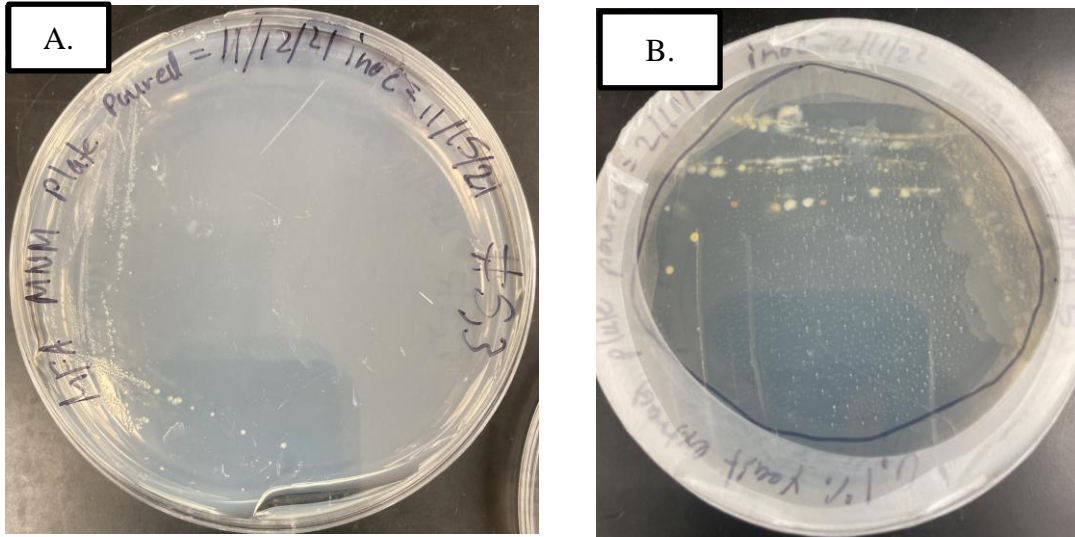


Figure 3.2: Carbon source affects the apparent diversity of microbes that can be cultivated on solid medium. A. After 4 weeks, only one colony type was observed on solid medium with 0.2 mM MFA. B. After two weeks, four colony types were observed on solid medium with 1% yeast extract.

### 3.3.2 Simplified Microbial Culture from WWTP Defluorinates MFA When MFA is the Sole Carbon Source.

To accurately measure defluorination with the fluoride meter, the MFA concentration was increased from 20 mg/L to 100 mg/L to measure small amounts of fluoride in solution (Figure 3.3). Each data point is the average of three data points, and the error bars are the standard deviation (Figure 3.3). The inoculum was 1% of the culture volume (Figure 3.3). In cultures with cells, microbes defluorinated MFA and produced fluoride (Figure 3.3).

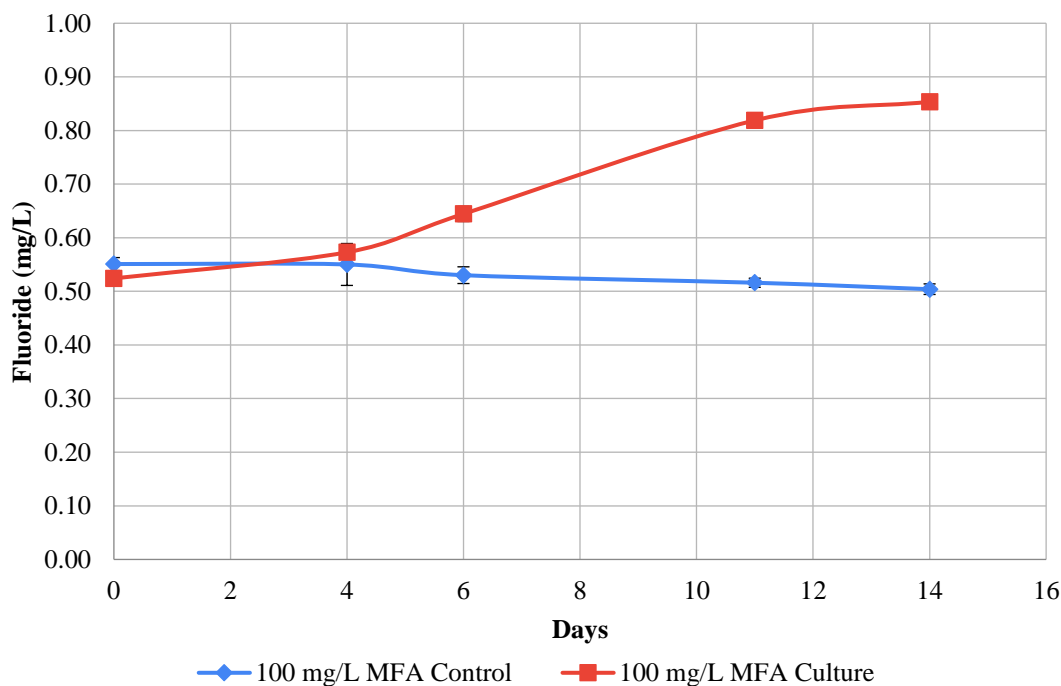


Figure 3.3: Microbes from wastewater defluorinate MFA. Blue line = uninoculated control, Red line = inoculated sample. Error bars represent the standard deviation between replicates. Replicate number per condition = 4. Over time, the more fluoride is released into cultures with cells than in media without cells.

### 3.3.3 Measured Defluorination of Microbes with a Combination of MFA, TFA, and Acetate

When provided TFA and MFA, along with acetate, the defluorination rates vary depending on which substrates are provided to the microbes. Defluorination in 100 mg/L MFA is higher in conditions with cells than without cells in the control (Figure 3.4). The 40 mg/L TFA culture also had more measurable microbial defluorination than the control (Figure 3.4). The combination of 50 mg/L MFA and 20 mg/L TFA also had defluorination (Figure 3.4). However, when the microbes were provided with 20 mg/L TFA and 40 mg/L acetate, no fluoride was released during the

experiment (Figure 3.4). Fluoride release was greatest when the simplified culture was grown on MFA and for this reason cultures grown on MFA were used as inoculum in subsequent experiments.

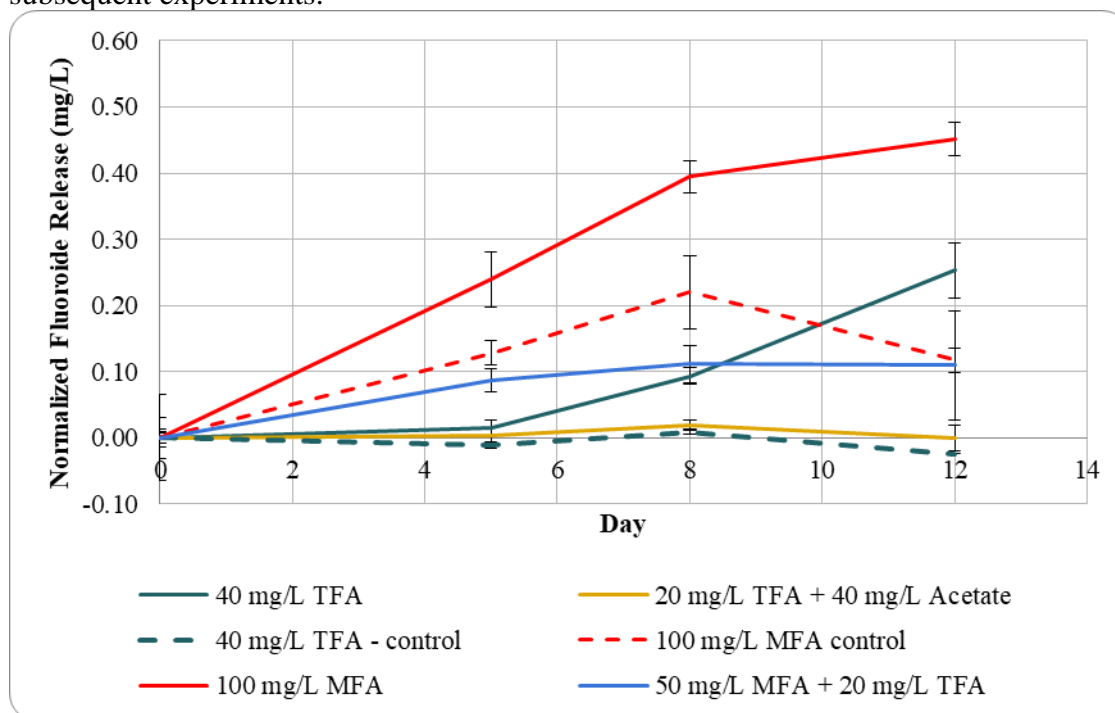


Figure 3.4: Microbes defluorinated MFA and TFA. To normalize the data the initial concentration was subtracted from each time point. MFA = MFA. TFA = TFA. Dashed lines = controls. Solid lines = with cells, Teal lines = 40 mg/L TFA, Red lines = 100 mg/L MFA, Yellow line = 20 mg/L TFA + 40 mg/L acetate, Blue line = 50 mg/L MFA + 20 mg/L TFA. Replicate number per condition = 3. Defluorination in 40 mg/L TFA and 100 mg/L MFA with cells was higher than the control. The 20 mg/L TFA + 40 mg/L acetate culture did not see any fluoride release during the experiment.

To measure microbial defluorination when cultivated with multiple substrates, the culture that was grown solely on MFA was added to media with 100 mg/L MFA, or with 100 mg/L MFA + 40 mg/L acetate, or with 100 mg/L MFA + 40 mg/L acetate

+ TFA. The fluoride release was measured over the course of 13 weeks (Figure 3.5). When MFA + TFA + and acetate were all provided for the microbes, the fluoride release was less than with MFA alone and with MFA + acetate (Figure 3.5). However, the presence of TFA did not inhibit defluorination. In the presence of MFA alone, the microbial cultures defluorinated 22.1% of the MFA (Figure 3.5). In the presence of both MFA and acetate, 30.1% of the MFA was defluorinated (Figure 3.5). The addition of a non-PFAS carbon source, acetate, increased the PFAS defluorination of MFA more than MFA alone.

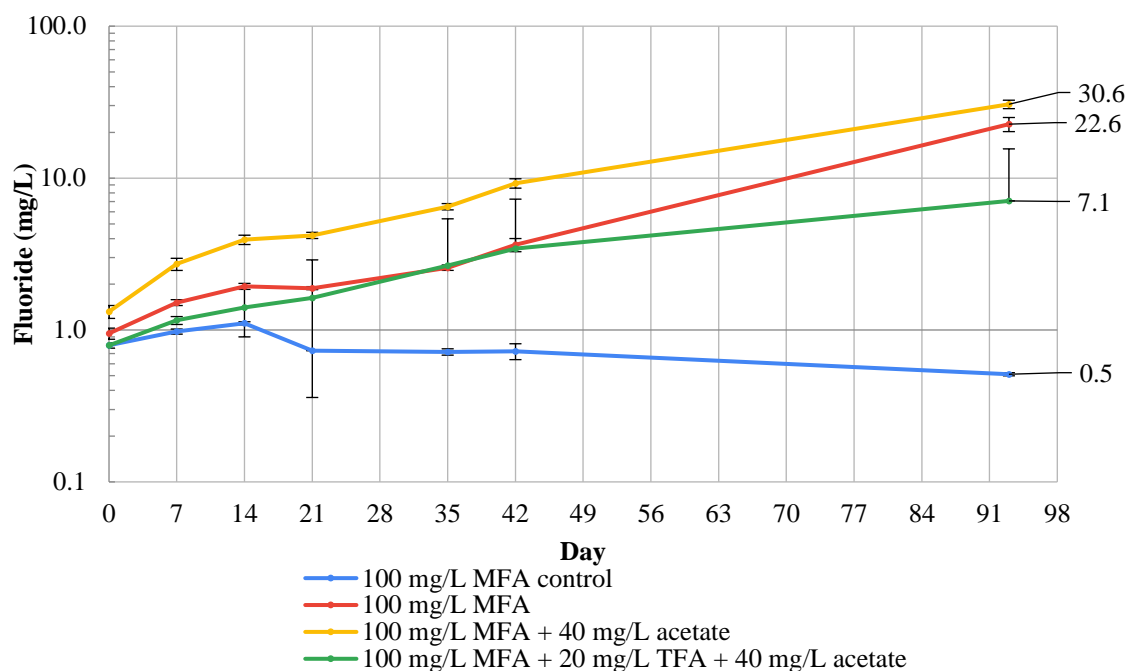


Figure 3.5: Long-term fluoride release with multiple PFAS and one non-PFAS carbon source. The PFAS carbon sources were the MFA and TFA. The non-PFAS carbon source was acetate. Inoculum dilution = 1:10. Replicate number per condition = 3. Cultures with MFA and acetate had the highest rates of fluoride release through time. On day 42, nearly 9% of the initial MFA was defluorinated, while only 3.5% of the initial MFA was defluorinated in cultures with only MFA and the culture with only MFA. No fluoride release was observed in the MFA control.

### **3.4 Discussion**

#### **3.4.1 The Goal of the Study**

The aim of the microbial cultivation experiments was to determine if microorganisms cultivated from WWTP are capable of defluorinating small fluoro-organic molecules using MFA and TFA as model compounds. Here we measured microbial defluorination of MFA in media with only MFA and MFA + acetate. In addition, we demonstrated that TFA + MFA + acetate does not completely inhibit microbial defluorination. However, the presence of TFA appears to decrease the defluorination rate. However, acetate does appear to inhibit defluorination of TFA. The WWTP microbes can remove fluorine from carbon molecules and release fluoride as a product, but how do the cultures in the defluorination experiments compare to research on microbes from soil of MFA producing plants?

#### **3.4.2 How Microbial Cultures in These Experiments Compare to Other Research**

The microbes cultivated from WWTP defluorinated MFA at a much different rate and lower yield than microbes cultivated from soil surrounding MFA producing plants. The WWTP microorganisms cultivated in 100 mg/L MFA required approximately 13 weeks to reach a maximum defluorination of 30%. In contrast, microbes cultivated from the soil surrounding MFA producing plants are capable of degrading 100% of 2,000 mg/L MFA within 40 hours (25). The microorganisms in this work transform MFA at slower rates than microbes cultivated from soil with MFA because WWTP have longer-PFAS chemicals, >6 carbon chain, rather than short chain PFAS chemicals, like MFA (26).

Interestingly, defluorination of TFA was able to occur in conditions with only TFA. However, TFA defluorination did not occur with only TFA and acetate. In the culture with 20 mg/L TFA and 40 mg/L acetate, the acetate was higher in concentration than the TFA culture. Thus, the selection for microbes to defluorinate TFA was decreased because the microbes theoretically had enough acetate to survive at least over the 12-day experimental period. This result agrees with studies that show that TFA defluorination is possible if the acetate concentration is lower than TFA concentration (24). To defluorinate PFAS chemicals with multiple carbon-fluorine bonds, the microbial populations need strong selective pressure to utilize the PFAS chemicals for energy.

### **3.4.3 Future Directions**

One of the main reasons why MFA and TFA were the PFAS source in the microbial cultivation experiments was because the chemicals could be potential byproducts of long-chain PFAS transformation via UV activated NP. The microbes' role would be to complete the breakdown process of the PFAS chemicals into innocuous chemicals, like fluoride, carbon dioxide, or biomass. Are MFA and TFA potential chemicals produced in the breakdown of long-chain PFAS chemicals? When PFOA and PFOS are transformed using plasma-based water treatment, TFA is a byproduct (27). However, chemicals like perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA) were produced twice as much as TFA (27). Therefore, future microbial cultivation experiments could use longer, more complex PFAS chemicals as those chemicals may be more representative of PFAS breakdown products than mono- and TFA.

## CONCLUSIONS

PFAS chemicals, the “forever chemicals,” can be transformed. PFAS chemical transformation is essential in beginning the process of PFAS degradation and eventually PFAS mineralization. Methods like photocatalysis using UV-activated NPs and microorganisms were shown to chemically transform PFAS chemicals with relatively low energy demand. In addition, both methods are potentially scalable and could be used to treat larger volumes of PFAS contaminated waters if the processes are optimized.

From the results in this thesis, one can conclude that the long-chain PFAS chemical, PFOA, is transformed when exposed to 100 nm NP in the presence of UV light in a solution with a pH ~ 10. In addition, microbial defluorination of MFA and TFA is best under conditions with higher concentrations of perfluorinated chemicals than alternative organic carbon sources. In addition, microbes from WWTP can defluorinate MFA and TFA when provided either as the sole carbon source. Future research will expand the substrates to include PFAS chemicals with more carbon-fluorine bonds, like PFPeA, PFH<sub>x</sub>A, and PFHpA.

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