Analysis of the Effects of PFAS and Metallic Co-pollutants on Bacterial Respiration

A Major Qualifying Project

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ABSTRACT

Per- and polyfluoroalkyl substances are stable, man-made compounds that persist in the environment. We investigated perfluorooctanoic acid's impact on bacterial respiration in activated sludge and potential co-toxicity with zinc and hexavalent chromium. During experimentation, the concentrations of heavy metals were varied on a gradient with a fixed concentration of PFOA to analyze patterns of growth or inhibition. We analyzed the mass of oxygen consumed over time to draw conclusions about the effects of PFOA, co-toxicity, and age of activated sludge. Our data generally supported the hypothesis of a negative correlation between pollutant levels and bacterial consumption of oxygen.

EXECUTIVE SUMMARY

Problem: PFAS, Activated Sludge, and Co-pollutants

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made compounds known as “forever chemicals” because they do not break down in normal environmental conditions. These organic pollutants are present in minute background concentrations and are detected worldwide in humans, wildlife, and water sources on a scale of parts per trillion. PFAS such as perfluorooctanoic acid (PFOA) have been made as surfactants used in a wide variety of products such as cleaners, fire retardants, and water-resistant coatings. The same properties that make them favorable for manufacturing certain goods also cause them to bioaccumulate and persist as environmental contaminants (Ghisi et al., 2019). PFAS pose health and safety hazards as they have been proven to cause diseases and birth defects in humans. The serious threat of these forever chemicals has led to government regulations on their production in industry, however their perpetual bioaccumulation in nature remains a significant problem (United States Environmental Protection Agency, 2018b).

PFAS move through the environment primarily in water phases and can percolate through soil to enter groundwater systems. They are typically more prevalent in the surface waters of urban areas. PFAS also may interact with other materials in the environment such as metals, and research has shown there can be combined effects on exposed microorganisms. Hexavalent chromium and zinc are two industrially produced materials that contribute to environmental pollution. Zinc can be found naturally or as a result of manufacturing while Cr(VI) is man-made and is acutely more toxic. The combination of PFAS and metals such as these can cause additive toxicity, which increases the threat posed to bacterial activity (Ottoboni, 1991).

Through transport mechanisms, high concentrations of toxic chemicals such as PFAS are found in locations where microorganisms are relied upon for biodegradation such as in wastewater treatment plants (Fitzgerald et al., 2018). This is concerning since PFAS can damage bacteria on a cellular level, and the activated sludge in wastewater plants must be kept at specific conditions so they work most efficiently in the treatment process. The continuous transport and accumulation of PFAS requires an understanding on how they can affect the growth of activated sludge, which contains the bacteria used in wastewater treatment to break down organic matter. It is also necessary to study how bacterial respiration may change when other compounds are present in the water.

Objectives

To determine how the pollutants PFOA, hexavalent chromium and zinc interact and impact the respiration of bacteria found in activated sludge, we devised three main objectives:

1. Explore how Perfluorooctanoic Acid (PFOA) affects bacterial growth and water quality using a respirometer.
2. Analyze how co-exposure with zinc (Zn) and hexavalent chromium (Cr(VI)) affects bacterial growth and oxygen uptake.

3. Determine if our results show that bacteria in our wastewater sludge could be used as an agent for bioremediation.

Approach

To fulfill our objectives, we used respirometry to perform toxicity tests on the three compounds in water seeded with activated sludge. We used the aerobic prompt in QuickScan v.16 software, which collected data in terms of mass (milligrams) of oxygen consumed over time. We conducted trial sets for our sludge alone, for each of the three contaminants individually, and for the PFOA and each metal simultaneously. Each trial set consisted of four samples, including one control. For the single pollutant trials, this control contained only sludge along with the standard BOD solutions and nutrients, while the dual-pollutant trial controls included PFOA at a concentration of 25 mg/L and metal concentrations varied on a gradient. While our concentration of PFOA used was much higher than environmental values, we attempted to vary the metal concentration in a range that would be expected in industrial wastewater. Data collection for each trial set lasted for five days, and new sludge was brought to the laboratory for testing from the Upper Blackstone water treatment plant on November 13th, January 11th, and March 10th.

Findings

For all of our data, the consumption of oxygen over time tended to follow a relatively logarithmic path, as expected. However, we attribute a significant amount of error in the data collection to false readings or missed readings by the respirometer sensors, which proved to be faulty. Comparing total oxygen uptake values, we deduced that the two pollutants in tandem had compounded toxicity, compared to each individually. Unfortunately, we were unable to collect reliable data for the effects of zinc alone, therefore, analysis regarding the compounded effects of zinc and PFOA was limited. Contrary to our hypothesis that estimated a negative correlation between oxygen consumption and concentration of contaminant, our data often reflected that intermediate concentrations of a pollutant yielded the highest oxygen consumption. Reflecting this, simultaneous co-toxicity of PFOA and Cr(VI) demonstrated additive toxicity only at an intermediate concentration of Cr(VI), between 0.002 and 0.050 mg/L.

Aside from experimental data, comparisons of sludge age in our control trials yielded opposite results depending on the presence or absence of PFOA in the sample. Over a period of fifteen days, for sludge aged 40, 45 and 50 days, the older sludge demonstrated higher oxygen uptake and a more steady growth pattern, while younger sludge, namely the 45 day-old sample, consumed less oxygen and had a more erratic growth pattern. In the presence of PFOA, however, and using sludge at 15 and 50 days old, the younger sludge demonstrated higher oxygen consumption than the older sludge, while showing an erratic growth pattern.

Recommendations

Future research on PFAS and wastewater interactions has a multitude of possibilities, as there are over 4700 different forms of PFAS and companies continue to produce new compounds. If using respirometry with activated sludge, we would recommend performing a toxicity analysis on zinc in order to observe an antagonistic, additive or synergistic effect on the oxygen uptake when compared to the PFOA and zinc trial. It was our plan to analyze how zinc alone affects respiration, however we were impeded by equipment malfunctions. Another potential avenue of study would be to evaluate the effect of another PFAS named "GenX" on bacterial growth. GenX was produced as a safer replacement for PFOA, so performing a toxicity analysis would allow verification of this on a microbiological scale. Furthermore, we would recommend using manual methods to measure dissolved oxygen and oxygen uptake instead of a respirometer. The respirometer we used experienced many issues and we had limited resources for troubleshooting and equipment maintenance.

All of these recommendations would not only improve the confidence with which conclusions are made about the effects PFOA and metal co-toxicity on activated sludge, but it would also build upon our body of research and assist in answering the question posed by our third objective. Based only on our results, we could not make definitive claims on activated sludge bacteria's potential as a bioremediator for PFAS-contaminated substances. Furthering the depth of research on the effects of PFOA and bacteria on each other would get us closer to answering this objective.

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1.0 INTRODUCTION

Per- and polyfluoroalkyl substances, generally known as PFAS, are fluorinated organic polymers in the natural environment. These compounds are primarily used to make water-resistant coatings, oil repellents, and fire fighting foams. They accumulate in water systems, soil, and organisms that inhabit these areas (Ghisi et al., 2019).

There are currently 6 PFAS compounds regulated by the United States Environmental Protection Agency, US EPA (United States Environmental Protection Agency, 2018b). The one we focus on is perfluorooctanoic acid, or PFOA. This compound is most commonly generated by the textile and food packaging industries. From these sources, PFOA easily enters soil and drinking water through waste (Ghisi et al., 2019). Humans are most likely to be exposed to PFOA through drinking water. PFOA is detrimental to human health and has been shown to cause harmful diseases including kidney disease, cancer, and birth defects (*PFAS Federal Legislation in the 116th Congress*, 2020).

Several PFAS-related-disease legal settlements put pressure on the government to regulate the presence of PFAS in the environment. However, in many places, this regulation is ill enforced (*Plainfield charter township v. Wolverine world wide, inc.*, 2020). The industries that benefit from loose PFAS regulation include the textile, food packaging industries, as well as the military and fire training facilities.

PFAS are introduced into waterways through point-source emissions from chemical facilities. PFAS deposits onto soil are eventually transported into groundwater. PFOA is found in the highest concentrations in urban surface waters, detected at levels of 0.2-1630.2 ng/L (Kunacheva et al., 2012). In addition, because PFAS are removed from municipal water in standard wastewater treatment plants, the “waste” from these facilities is returned to rivers and contains higher concentrations of PFAS (Becker et al., 2008).

Since wastewater treatment plants have high PFAS concentrations, we used treatment plant sludge as our source of aerobic bacteria. Activated sludge plays an integral role in wastewater treatment; its aerobic bacteria accelerate the natural treatment processes and break down organic matter food sources into energy (*Bacteria and Microorganisms Involved in Water Treatment*, 2020).

The rate of bacterial growth at a specific location indicates the level of contamination (Hermans et al., 2016). We measured bacteria growth using a respirometer that records oxygen consumption levels. PFAS can stimulate the growth of some species while inhibiting others, making the contaminant detrimental to the role of bacteria in bioremediation. PFOA has been shown to hinder bacteria growth in activated sludge (Yu et al., 2018). PFAS are typically resistant to biodegradation in both anaerobic and aerobic conditions, but some studies have shown microorganisms to successfully degrade PFOS (Kwon et al., 2014). In 2019, scientists found a strain of bacteria (*Acidimicrobium* sp. strain A6) capable of “chewing” through PFOS and PFOA when supplied with iron, Fe(III), in the presence of an electron donor (Princeton University, Engineering School, 2019).

One area of research that has been sparsely developed regarding PFAS is their interactions with other environmental contaminants. We were driven to investigate interactions with positively charged heavy metal ions because of the highly fluorinated nature of PFAS. Different toxicological patterns can arise when multiple pollutants interact: compounded toxicity (when the individual effects sum) and antagonistic interactions (when one pollutant nullifies or diminishes the other's effects). We investigated zinc and hexavalent chromium.

Zinc is regarded as a non-toxic health supplement, but despite its frequent use, there is little known about the bioaccumulation of naturally occurring zinc in ecosystems or its combined effects with pollutants like PFAS. Zinc tends to settle in water, and while concentrations of zinc in water never surpass 50 μg/L, they can reach up to 50 mg/L in surface and groundwater areas impacted by mining and industry ("Potential for Human Exposure," 2005). Dissolved Zn^{2+} ions have variable effects on the survival of different species of bacteria, decreasing the survival of some populations and little impacting others (Babich & Stotzky, 1978; Bong et al., 2010).

Hexavalent chromium can cause severe harm to organisms on a cellular level in minute concentrations ("Potential for Human Exposure," 2011). It is a carcinogenic metal found in abundance in wastewater sludge and industrial waste. Cr(VI) is one of the most stable forms of chromium and difficult to remediate. Levels of Cr(VI) in drinking water rarely exceed 10 $\mu g/m^3$, and it is found in larger concentrations in soil and sediment than air and water. Co-exposure of hexavalent chromium and PFAS can lead to cell damage in bacterial communities. For example, when soil bacteria are exposed to both hexavalent chromium and PFOS, cell growth decreases by around half (Li et al., 2020).

In this work, we aim to explore how Perfluorooctanoic Acid (PFOA) affects bacterial growth and water quality using a respirometer, analyze how co-exposure with other contaminants, zinc (Zn) and hexavalent chromium (Cr(VI)), affects bacterial growth and oxygen uptake, and determine if our results show that bacteria in our wastewater sludge could be used as an agent for bioremediation. We performed our experiments using a respirometer and analyzed our findings using traditional respirometry analysis methods.

2.0 BACKGROUND

In order to understand the effects that per- and polyfluoroalkyl substances, also known as PFAS, have on our environment, it's necessary to first understand the characteristics that make them unique. This chapter will examine these characteristics and dive deeper into one type of PFAS called PFOA, and the health hazards that it may cause. Next, the environmental impact of this compound will be evaluated. Specific components and concepts relating to our experimentation including activated sludge, oxygen uptake, and co-toxicity of heavy metals will be explained. By identifying this information, we achieved a thorough understanding of how PFAS impacts the environment, impacts bacterial growth, and the effects of heavy metal co-pollutants.

2.1 Per- and Polyfluoroalkyl Substances (PFAS)

PFAS are highly fluorinated, organic polymers that persist in the natural environment as a result of human activity. These man-made compounds are primarily used in the production and use of water-resistant coatings, oil repellents, and fire-fighting foams, and ultimately accumulate in water systems, soil, and organisms (Ghisi et al., 2019).

2.1.1 Characteristics of PFAS

There are currently six different PFAS compounds that are regulated by the United States Environmental Protection Agency (US EPA): perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), and perfluorobutanesulfonic acid (PFBS) (United States Environmental Protection Agency, 2018b).

The common characteristic of all types of PFAS is the long chain of fluorinated carbons. The carbon-fluorine bond within these chains makes PFAS very stable, therefore they are extremely difficult to break down naturally. PFOA and PFNA are similar in structure, with PFNA having just one more fluorinated carbon. Both molecules contain carboxyl groups, a combination of carbonyl and hydroxyl groups, which defines them as perfluorinated carboxylic acids (PFCA's). The carboxylic functional group is considered a preferred leaving group for substitution reactions and is the most vulnerable part of the PFAS structure for biotransformation and chemical decomposition (Ghisi et al., 2019). Out of these six compounds, the focus of this research is on PFOA.

2.3 Perfluorooctanoic Acid (PFOA)

The textile and food packaging industries are the most common source of PFOA contamination in the environment. From these sources, PFOA easily enters soil and drinking water. It is nonpolar and insoluble in water and some oils (Ghisi et al., 2019). PFOA is highly toxic to humans and is a known carcinogen (United States Environmental Protection Agency,

2018a). PFOA has a molecular weight of 414.07 g/mol and is represented by the chemical formula $C_8HF_{15}O_2$. The structure of PFOA is shown in Figure 1 below.

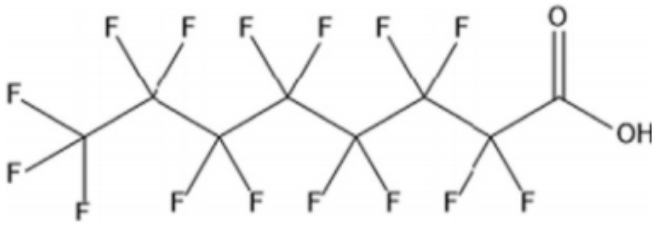

Figure 1: Structure of a PFOA molecule

2.3.1 Health Hazards of PFOA

PFOA can significantly impact human health. The human population is most commonly exposed to PFOA through contaminated drinking water. Additional PFAS may come from contaminated foods, food packaging or cookware, or polluted indoor environments (Domingo & Nadal, 2019). Once PFAS are consumed, they can enter the human bloodstream, which can cause many problems in the body. PFAS including PFOA have been linked to harmful diseases in humans, including kidney disease, cancer, and birth defects (*PFAS Federal Legislation in the 116th Congress*, 2020). It is proposed that in small concentrations, PFOA is present in the blood of every human on earth (New York State Department of Health, 2016).

2.4 PFAS in the Environment

Patterns of PFAS-contaminated water and related health issues in several areas around the United States have spurred community-organized legal action against the facilities responsible. In turn, legal settlements and increasing occurrence of PFAS-related illness has put pressure on federal and state governments to include PFAS regulation in environmental policy. Several pending statutes surrounding PFAS regulation in the environment would serve to amend the Safe Drinking Water Act enacted in 1974. However, the enforcement of these specific laws would be up to the discretion of individual state governments, and PFAS in general have yet to be federally regulated (United States Environmental Protection Agency, 2018b). Individual state governments typically follow and adhere to federal laws (at a minimum), which can cause more issues if the federal laws are not in place. In the past year alone, several regulatory organizations and processing companies, including the US EPA and DuPont, have been the recipients of legal settlements regarding the release of PFAS on the behalf of the unaware surrounding communities (*Plainfield charter township v. Wolverine world wide, inc.*, 2020). The industries that benefit from a lack of PFAS regulation include the textile, food packaging industries, as well as the military and fire training facilities.

PFAS are introduced into waterways through point-source emissions from chemical facilities. Non-point-source deposits onto soil and subsequent percolation transports these chemicals into groundwater. Because PFAS are removed from municipal water in standard wastewater treatment plants, the “waste” from these facilities is returned to rivers and contains

higher concentrations of PFAS (Becker et al., 2008). PFAS are also detected in seawater and have been found in the tissues of ocean-dwelling organisms; the ocean is the final destination or "terminal sink" for these chemicals. This is likely due to their negligible rate of redistribution into freshwater or terrestrial systems (Zhang et al., 2019).

PFOA are found in higher concentrations in urban surface waters. According to a study with a sample size of 539 sites across 41 international cities, PFOA concentrations in urban rivers ranged from detectable levels of 0.2-1630.2 ng/L, with an average concentration of 70.1 ng/L (Kunacheva et al., 2012). Through rainfall or gradual mobilization, PFAS in the soil can transport into deeper layers of soil and contribute to groundwater contamination. Soil accumulates PFAS through runoff, deliberate land disposal, and through the use of PFAS products. For example, the land surrounding military bases and other facilities that use surfactants or firefighting foams often have higher levels of contaminants. The most common PFAS in the soil are PFOS and PFOA; even in the most remote areas on Earth, they occur in "background concentrations" in the soil of 0.05 and 0.007 μg/kg, respectively (Brusseau et al., 2020). However, this data cannot necessarily conclude that the PFAS are naturally occurring in the soil, but rather that they may be highly mobile through soil and groundwater.

2.5 Wastewater Treatment and Activated Sludge

Through the environmental transport mechanisms described above, a high concentration of toxic chemicals such as PFAS are found in locations where microorganisms are relied upon for nutrient and contaminant degradation (Fitzgerald et al., 2018). This is due to the bioaccumulation tendencies of "forever chemicals" that are resistant to decomposition and that persist in the environment over long periods of time. Wastewater treatment plants are a primary site of high PFAS concentrations. These plants receive both municipal and industrial wastewater to process and release into the environment. Microorganisms are critical in wastewater treatment, specifically in the secondary treatment processes after the larger solids have been removed. The stages of a typical treatment facility consist of an aeration basin and a clarifier. The aeration basin contains a high concentration of bacterial colonies, called "flocs." The flocs form after microorganisms in the wastewater are settled out in the secondary clarifier and are then recycled back to the aeration basin. The material returned to the basin is called "activated sludge" (Blacoh, 2015).

Activated sludge is an integral part of wastewater treatment plants because it significantly accelerates the natural treatment processes by breaking down organic matter as a source of food and energy (*Bacteria and Microorganisms Involved in Water Treatment*, 2020). Returning the sludge from the secondary clarifier "activates" it, giving it a purpose within the treatment process (Blacoh, 2015). The bacteria concentration in the aeration basin increases thirty-fold.

The main component of activated sludge is aerobic bacteria that live and grow when there is oxygen present (Glymp, 2015). These bacteria consume materials in the wastewater by adsorption and absorption. When the organic matter and food particles are large, bacteria use

adsorption to break them down by releasing enzymes. This enables the activated sludge to absorb the smaller particles that pass through the cell membrane.

Because bacteria are essential for wastewater treatment, aeration basins and clarifiers must be kept at specific conditions so that the microbiota in sludge can thrive and work most effectively. Wastewater treatment plants are designed so water quality conditions are favorable for the billions of bacteria needed to remove pollutants from the water so it can be safely channeled back into rivers and streams (Glymp, 2015). Specifically, the flocs require oxygen, neutral pH, a moderate temperature, and a reasonable food to microorganism (F/M) ratio (Innerebner, 2015).

2.6 Water Quality and Toxicity Testing

One way to quantify the viability and performance of activated sludge is by measuring its oxygen consumption. The oxygen uptake rate is calculated for toxicity testing so researchers can see how certain chemicals affect bacterial respiration; oxygen consumption is directly correlated to the bacterial activity in the sludge (Environmental Business Specialists, 2010). Wastewater treatment plants would typically measure a baseline oxygen uptake rate for them to compare when performing routine testing of the water quality. This measurement is critical for quality control. A higher oxygen consumption than the baseline indicates proliferation and increased biomass in the activated sludge, while a lower oxygen consumption shows the presence of toxins (Rumbaugh, 2017).

Another common measurement of water quality is biochemical oxygen demand (BOD). The BOD of a sample of water represents the amount of dissolved oxygen (DO) required for microorganisms to consume and break down the organic matter present in the water (*Biological Oxygen Demand (BOD) and Water*, 2020). Bacteria in activated sludge work under aerobic conditions, so they require oxygen to respirate and function. Dissolved oxygen is crucial for aquatic ecosystems and there must be enough to supply all of the organisms in the water. When there are pollutants and organic matter in the water, the microorganisms require more oxygen to oxidize and decompose these materials, which can deplete the DO. A high BOD signifies poor water quality since the bacteria require more DO than there may be available.

The relationship between organic matter and oxygen consumption is represented by the following equation:

$$\mathbf{C_5H_7NO_2\ (\textit{biomass})} + \mathbf{5O_2} \rightarrow \mathbf{4CO_2 + H_2O + NH_4HCO_3\ (\textit{Ammonium bicarbonate})}$$

As described by this decomposition reaction, when the amount of biomass in water increases, the oxygen consumed in the water stoichiometrically increases because there is more respiration. In the presence of nitrifying bacteria, the ammonium bicarbonate product chemically reacts to form nitrate, water, and a proton, so the water becomes more acidic (Ovivo, 2016). In a lab setting, a buffer is required to ensure that the pH remains at a stable level when measuring the oxygen consumption.

2.7 PFAS and Bacteria

PFAS can cause negative health effects in mammals, so it is important to study how these chemicals affect the microorganisms that partake in important environmental functions. The rate of bacterial growth at a specific location can indicate the level of contamination and the effects of certain compounds on the stability of the environment and all of its components (Hermans et al., 2016). Bacteria can experience oxidative damage, DNA damage, general cell lesions, and membrane damage when exposed to toxic chemicals (Liu et al., 2016).

PFAS can stimulate the growth of some species while inhibiting others. Compounds with longer carbon chains—like PFAS—are more harmful to bacteria colonies than their short-chain counterparts (Fitzgerald et al., 2018). Longer carbon chains allow for higher concentrations of these compounds to permeate bacterial membranes and accumulate within an organism. Bioaccumulation of trace amounts of PFAS can amplify the harmful effects.

Since PFAS can be detrimental to bacterial growth, they would disturb the important role of bacteria in bioremediation. PFOA has been shown to restrain the growth of bacteria in activated sludge, however certain species are tolerant and proliferate after the others die. After a long exposure period, the biodiversity of microbial communities in sludge can shift, affecting the efficiency of the water treatment (Yu et al., 2018).

Typically, PFAS are resistant to degradation in both aerobic and anaerobic conditions because of their stability and the strength of the carbon-fluorine bond. However, in 2013, it was discovered that the bacteria *Pseudomonas aeruginosa* strain HJ4 isolated from wastewater sludge can successfully degrade PFOS (Kwon et al., 2014). In only 48 hours, the microorganisms removed around 67% of the initial concentrations of these chemicals, which is remarkable since the half-life of PFOS in water is around 41 years (United States Environmental Protection Agency, 2012). This strain of bacteria was acquired from a preexisting sample of activated sludge from a wastewater treatment plant, so a relatively common microbe can be used to biodegrade PFAS relatively successfully.

In 2019, scientists were able to determine that another strain of bacteria was capable of "chewing" through PFOS and PFOA when supplied with iron, Fe(III), in the presence of an electron donor (Princeton University, Engineering School, 2019). This experiment focused on developing a bioremediation strategy that cleaves the carbon-fluorine bond to "defluorinate" the perfluoroalkyl substances and break them down into smaller, safer, and more manageable products. The microorganism *Acidimicrobium* sp. strain A6 naturally participates in a Feammox reaction, the oxidation of ammonia by iron in conditions where little oxygen is present in soil (Huang & Jaffe, 2019). It was determined that the *Acidimicrobium* bacteria were able to simultaneously oxidize ammonia and remove the PFAS contaminants from soil under Feammox conditions. Approximately 60% of the chemicals were biologically degraded over 100 days of incubation, with resulting fluoride products showing that the C-F bond was successfully targeted in this process (Princeton University, Engineering School, 2019).

In these previous studies, the chosen bacteria were grown on a controlled medium using the perfluoroalkyl compound as a substrate that the organisms react to or consume (Kwon et al.,

2014). Additionally, glucose was often used to support growth along with several other vitamins and minerals. When performing each study, it was found that the soil bacteria targeting PFAS grew best in a pH range from 5-7 and around 25-35 degrees Celsius.

2.8 Heavy Metals and Co-toxicity

One area of research that has been sparsely developed regarding PFAS is their interactions with other environmental contaminants. In order to analyze the effects of heavy metals on the toxicity of PFAS, it is important to define toxicological patterns that occur when two or more pollutants interact. Additive toxicity is the simple superposition of the effects of each pollutant, where the combined toxicity is neatly equal to the toxicity of one pollutant added to the toxicity of the other (Ottoboni, 1991). When one of the pollutants nullifies or diminishes the effects of the other, the interaction is antagonistic. If a non-toxic compound in the presence of a known toxin increases the effect of the known toxin, the mechanism of toxicity is called potentiation. Finally, when the combined effects of two toxins are greater than the sum of the single-toxin effects, the two substances have a synergistic effect.

Due to the heavily-fluorinated nature of PFAS, we were driven to investigate their interactions with positively charged heavy metal ions. The relationship between a metal ion and the fluorinated carbon bond (C-F) is categorized as a cation-dipole interaction, indicating electrostatic attraction between the two (Takemura et al., 2001). Our team decided to investigate two metals on either end of a hazard spectrum: Zinc, regarded mostly as a non-toxic health supplement, and hexavalent chromium (Cr(VI)), which at minute concentrations can cause severe harm to organisms on a cellular level. The different oxidation states of these two contaminants in an aqueous environment, +2 for Zn and +6 for hexavalent chromium, is also a notable subject of comparison between the two metals.

2.8.1 Zinc (Zn)

Zinc, a naturally occurring element, also exists in industrial byproduct streams and municipal waste streams. Cloquet et al. evaluated the significance of zinc as an air pollutant, in rain and wastewater, in soil, and tracked the bioaccumulation in plants. The study concluded that the mineral undergoes structural, isotopic changes, called fractionation, as it shifts through the environment and is absorbed by plants (Cloquet et al., 2007). Despite its frequent use as a health supplement, there is little known about the bioaccumulation of naturally occurring zinc in ecosystems, nor about its synergistic effects with other metals or pollutants like PFAS.

According to the Agency for Toxic Substances and Diseases Registry (ATSDR), environmental levels of zinc vary depending on the population density and surrounding industries of a site (“Potential for Human Exposure,” 2005). In the atmosphere, zinc is present in low levels that range from 0.0033 $\mu g/m^3$ in remote regions up to 0.67 $\mu g/m^3$ in urban environments. Indoor concentrations of zinc tend to be almost negligible, with zinc often settling as heavy particulate matter. Elevated levels of zinc often occur in proximity to smelting facilities, where they can reach ambient concentrations of up to 5 $\mu g/m^3$.

Zinc tends to settle in water, so it is often found in river or lakebed sediments compared to dissolved water ("Potential for Human Exposure," 2005). Natural and background concentrations of zinc in water never surpass 50 µg/L, but reach up to 50 mg/L in surface and groundwater around areas impacted by mining and industry. Concentrations of zinc in undisturbed areas of the ocean are on the order of nanograms per liter. Even in contaminated areas such as the Gulf of Mexico, concentrations do not exceed 10 µg/L.

In drinking water, elevated zinc levels have been found to an average value of 0.144 mg/L due to the materials used in municipal water distribution systems ("Potential for Human Exposure," 2005). In soils, zinc was found in cultivated and uncultivated media at average concentrations of 36 and 51 mg/kg, respectively. Soil and sediment near highways, landfills, and municipal waste deposits are much higher than uncontaminated soils, with levels around 1 g/kg of zinc being detected in these areas.

Research has determined that dissolved Zn^{2+} ions have variable effects on the survival of different species of bacteria, notably that it, "decreased the survival of *Escherichia coli*; enhanced the survival of *Bacillus cereus*; did not significantly affect the survival of *Pseudomonas aeruginosa*, *Norcardia corallina*, and T1, T7, P1, and phi80 coliphages" (Babich & Stotzky, 1978). Another study exploring the exposure of marine bacteria to high concentrations of Zn demonstrated that the element in surplus amounts halts the enzymatic activity of the microbes, but did not stunt the viability of the population (Bong et al., 2010). Specifically, the surplus Zn effectively inhibited the functioning of aminopeptidases, a category of zinc-dependent regulatory proteins.

2.8.2 Hexavalent Chromium (Cr(VI))

Hexavalent chromium is a carcinogenic metal found in abundance in wastewater sludge and waste from certain industries. This chromium isotope enters natural ecosystems due to some lenient water regulation enforcement, which polluting agencies take advantage of, allowing the metal to contaminate municipal wastewater lines and rivers. Hexavalent chromium is one of the most stable forms of chromium and is therefore persistent in the environment and difficult to remediate (Mishra & Bharagava, 2015). Once the metal isotope enters the human body, it causes damage on the molecular level in cells and proteins, and can lead to several different types of cancers. The industries that primarily use and dispose of chromium in the environment are metallurgical, preservative manufacturing, and energy.

According to the ATSDR report on chromium toxicity, this industrial pollutant persists through many different phases and media in the environment including in air, water, and soil ("Potential for Human Exposure," 2011). Generally, chromium is found in ambient air at levels ranging from 5 to 525 ng/m^3 in remote to urban environments. In indoor settings, these concentrations can be anywhere from 10-400 times higher than ambient presence depending on the smoking habits of residents. Mean values of chromium (VI) indoors between two studies ranged from 0.2 and 1.2 ng/m^3. Estimates have concluded that approximately one-third of all atmospheric chromium is hexavalent chromium, which is removed from the atmosphere

gradually through fallout or precipitation. Chromium concentrations in rainwater, either dissolved or as suspended particles within the droplets, range from 0.2-1 $\mu g/m^3$.

Hexavalent chromium in aqueous sub-surface environments is eventually reduced to another soluble state, trivalent chromium, Cr(III) ("Potential for Human Exposure," 2011). These two states of chromium account for almost all soluble chromium in aquatic environments, with all other forms being present only as suspended solids or deposited onto clay or sediment. Levels of Cr(VI) in drinking water rarely exceed 10 $\mu g/m^3$. Generally, chromium is found at much larger concentrations in soil and sediment when compared to levels in air and water. Chromium (VI) that has been reduced to Cr(III) in acidic soil is highly immobile; however, in the presence of oxidizing agents, Cr(VI) in the form of CrO_2^{-2} or $HCrO_2^-$ is much more mobile through the soil. In one study, the level of Cr(VI) on the side of a busy road was measured to be 64 mg/kg, on average two to four times higher than the background concentrations of Cr(VI) in the surrounding forest.

The endurance of hexavalent chromium in the environment is a serious issue, and co-exposure to other pollutants such as PFAS can lead to further cell damage in bacterial communities. For example, when soil bacteria are exposed to both hexavalent chromium and PFOS, cell growth decreases by around half (Li et al., 2020). This is primarily due to oxidative damage and increased membrane permeability, and certain enzyme functions are inhibited by the presence of PFOS. Hexavalent chromium that has been reduced to its trivalent form Cr(III), even without the co-toxicity of a PFAS, has similar oxidative effects on the membrane proteins of *B. subtilis* and *E. coli* (Fathima & Rao, 2018). The effects of co-exposure may be common with similar environmental pollutants such as the other PFAS used in our study, PFOA, which has not been studied in tandem with Cr(VI) or Cr(III) in the past.

3.0 METHODOLOGY

Our goal for this project was to determine the effects PFAS have on bacterial growth. In addition, we wanted to explore the co-toxicity effects of heavy metal pollutants that might occur in the environment. We chose to analyze these effects using a respirometer, the Challenge Technology™ Quickscan BOD Analyzer model. Our specific objectives are outlined below.

Objectives:

1. Explore how **Perfluorooctanoic Acid (PFOA)** affects bacterial growth and water quality using a respirometer.
2. Determine how co-exposure with heavy metals **zinc (Zn)** and **hexavalent chromium (Cr(VI))** affects bacterial growth and oxygen uptake.
3. Determine if our results show that bacteria in our wastewater sludge could be used as an agent for possible **bioremediation**.

3.1 Materials

The PFAS compound studied in this work was PFOA. It was purchased from Sigma Aldrich, along with additional co-pollutants that were chosen to be analyzed. The necessary chemical information can be found in Table 1.

Table 1: Key Information for PFAS compounds and co-pollutants used for BOD analysis.

Chemical Name	*Abbreviation*	*Formula*	*Manufacturer*	*Product Number*
Perfluorooctanoic Acid	PFOA	$C_8HF_{15}O_2$	Sigma-Aldrich	171468
Hexavalent Chromium	Cr(VI)	Cr(VI)	Sigma-Aldrich	266299
Zinc	Zn	Zn	Sigma-Aldrich	209988

3.1.1 Wastewater Sludge

Liquid wastewater sludge samples were obtained from the Upper Blackstone Wastewater Treatment facility, with an approximate bacteria concentration of 2500 mg/L. These samples were taken from the output of the bioreactors at the plant (secondary treatment). The samples were kept in a 4℃ refrigerator until use. The wastewater sludge was added to the solution of nutrients, buffers, and contaminants in a 500 mL respirometer reactor bottle for testing.

3.2 Respirometry Stock Solutions

In order to run respirometry tests, certain solutions must be added to the testing bottle to correctly measure oxygen demand. These solutions are outlined in Table 2 below. These stocks

were made in larger quantities to be kept on-hand for multiple trial sets. All of these solutions were made using purified water as the solvent. The phosphate buffer and glucose-glutamic acid solution were stored in the refrigerator (4℃) when not in use. The concentrations of these solutions were chosen based on typical concentrations used in past BOD studies (University of Wisconsin Board of Regents, 2006).

Table 2: Necessary stocks solutions for BOD testing

Solution Name	*Solute Chemical Formula(s)*
22.5 g/L Magnesium Sulfate	$MgSO_4 \bullet H_2O$
27.5 g/L Calcium Chloride	$CaCl_2$
0.25 g/L Ferric Chloride	$FeCl_3 \bullet 6H_2O$
Phosphate Buffer	KH_2PO_4, K_2HPO_4, $Na_2HPO_4 \bullet 7H_2O$,NH_4Cl
0.15 g/L Glucose-Glutamic Acid	$C_6H_{12}O_6$, $C_5H_9NO_4$

To maintain consistency throughout our trials, the reactor bottles were always filled in the same order. First, the stock solutions listed in Table 2 above were added, then the water, then the contaminants (PFOA, Zn or Cr(VI)), and lastly the sludge solution. The wastewater sludge was also stored in the refrigerator. Additional information about solution prep can be found in Appendix A.

3.3 Operating the Quickscan BOD Analyzer

The Challenge Technology ™ Quickscan BOD Analyzer (respirometer) was used to conduct our experiments. This respirometer consisted of four low-pressure chambers connected in parallel between an oxygen cylinder and a vacuum bottle, as shown in Figure 2. The instrument collected data by detecting oxygen bubbles that were pulled through the chambers due to the demand of the bacteria, and passed through a light sensor within each chamber. The diameter of each oxygen bubble was used to calculate the volume of oxygen pulled into the sample, which was then displayed on the computer. Oxygen volume and consumption rate data were displayed using Quickscan v.16 software. The respirometer measured the total oxygen consumption over the chosen time period. Data for each trial set were collected in 15-minute iterations over 5 days.

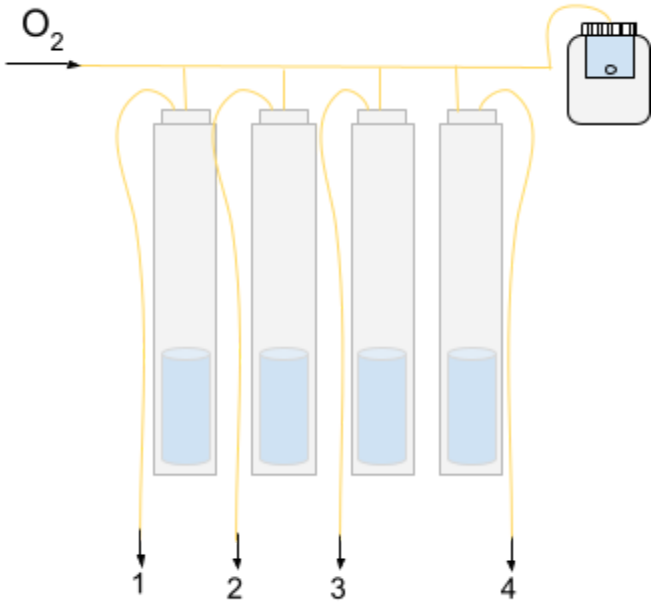

Figure 2: A diagram of the chamber and tubing arrangement of the Challenge Technology™ Quickscan BOD Analyzer.

The first trial set performed was of control trials, during which data was collected to see the bacterial growth uninhibited by any introduced PFOA or heavy metals, with varying levels of nutrients. To test the viability of the sludge over time, control samples were used within each trial set. Each trial set consisted of 4 samples, including one control, and 3 experimental samples. Data collection happened simultaneously for all samples during each trial.

The concentration of PFOA was chosen to ensure that it would not completely kill the bacteria sample while still showing a significant deviation in respirometry data compared to the control. After determining the ideal PFOA concentration, the PFOA concentration was held constant while the concentration of the heavy metal varied from a low to a high environmental concentration. The control sample for trial sets using heavy metal co-pollutants contained only our set concentration of PFOA.

After sample preparation (see Appendix A), the reactor bottles were filled with solutions, contaminants, and sludge were prepared for data collection:

1. A large (2 inch) stir bar was added to each of the sample bottles.
2. 4 mL of 30% KOH solution were dispensed into four 10 mL cuvettes.
3. Plastic holsters were positioned onto the cuvettes such that the holes on either side of the bolster are exposed through, and so each cuvette sat at the bottom of the holster.
4. A cuvette and holster were inserted into the top of each sample bottle and sealed using the septum cap.
5. Each sample was placed on the magnetic mixer according to the sample number (see Figure 3 below)

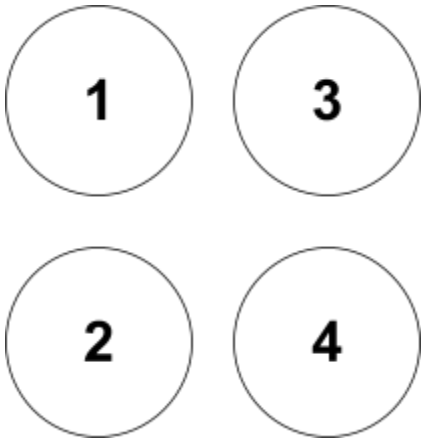

Figure 3: The arrangement of samples in the magnetic mixing plate. Sample 1: The control. Sample 2: Lowest contaminant concentration. Sample 3: Medium sample concentration. Sample 4: Highest sample concentration.

Respirometer Start-up:

1. The respirometer was turned on using a switch and the back of the machine (sensors illuminate red when the machine is on).
2. The vacuum bottle was filled with deionized water to the dotted line (appx. 75 mL), with water barely covering the hole in the center cylinder.
3. The cylindrical valve of the oxygen tank was opened and the pressure valve was adjusted until a slow stream of bubbles was seen in the vacuum bottle, but not in any of the sample chambers.
4. The magnetic stir plate was set to 700 rpm.

Software Start-up:

1. Quickscan v.16 software was opened and the prompts were followed to name each sample if necessary.
2. Option "1" was selected to confirm the use of the aerobic analysis. After this prompt, labeled numerical data is shown on the left and a graphical representation to the right.
3. To the far left, the interval was set to the lowest possible value (15 minutes).

Experiment Start-up:

1. Using a clean 20-gauge needle, each sample was vented to equalize pressure by quickly puncturing each septum.
2. The needle caps were removed from the needles attached to each respirometer chamber and the septum cap of each sample was carefully punctured at a 45° angle.
3. Using a syringe affixed with a 20-gauge needle, 20 mL of headspace was removed from each sample by withdrawing gas until one or two numerical counts were shown on the computer.

    a. Note: You will know that the headspace is successfully removed when the meniscus of oil in the back of the chamber is lowered below the weir and is visible from the front of the device.
4. The experiment was run undisturbed for 5 days. The vacuum bottle was periodically refilled to the 75 mL line with more water using a syringe *only if* significant evaporation occurred.

4.0 RESULTS & DISCUSSION

This chapter covers the results of the three objectives and presents respiration data for the toxicity assessments of perfluorooctanoic acid, zinc, and hexavalent chromium (detailed in the Methodology chapter). There were many inconsistencies in the data collection due to equipment failure that influenced the results. Appendix B describes each problem that occurred and how our team approached troubleshooting. Because of discrepancies in the experiments, the data may have significant error; however, each data set was interpreted without correction factors. Specific sources of error are described below for each data set, as documented in the lab records that we maintained throughout the course of our experimentation.

4.1 Varied Levels of PFOA

In this trial set we tested the toxicity of PFOA. The respiration of each sample was evaluated to measure how the chemical affects bacterial growth. We used PFOA concentrations of 5 mg/L, 25 mg/L, and 50 mg/L PFOA and a control with 0 mg/L. These concentrations are 10^9 times higher than they would be found in the environment or in a wastewater treatment plant. We wanted to start with high concentrations to determine the ideal concentration that causes inhibition without total sample death. The oxygen uptake trends are shown in Figure 4 below.

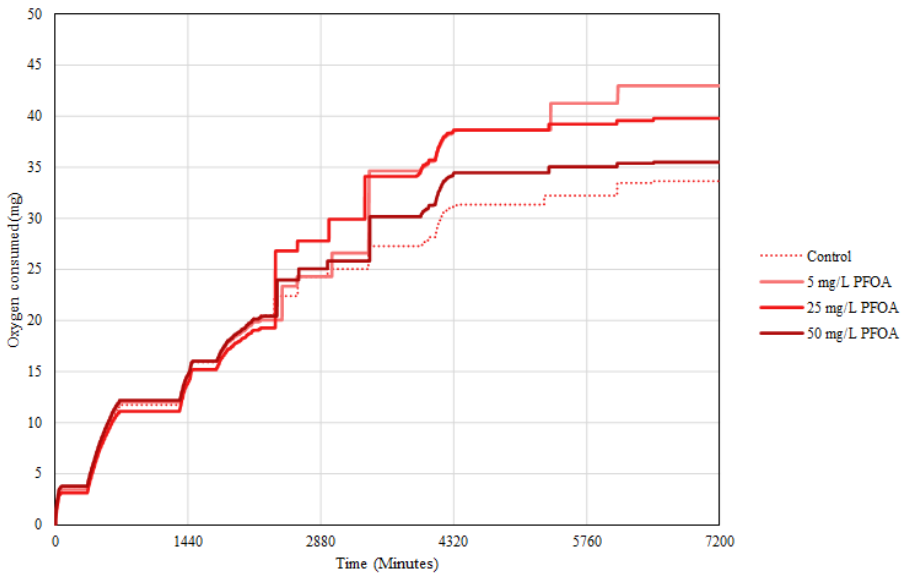

Figure 4: Varied levels of perfluorooctanoic acid with activated sludge over a period of 5 days. Note that 1440 minutes is equal to one day, so each vertical major gridline separates a period of 24 hours.

The sludge for this trial set was 50 days old at the start of the trial, meaning it had aged significantly in the storage refrigerator. Because it was one of trials with the oldest sludge, this data may have uncertainties influenced by time. The retention time for sludge in the wastewater treatment process should be as short as possible and best within a span of a few days, but our personal access to sludge was limited.

The oxygen consumed for the control in this trial was high compared to the controls of the experiments that used newer activated sludge. The bacteria in the water proliferated over the 50 days, which increased the oxygen uptake. Each of the samples containing PFOA were in the 30-45 mg O_2 range at the end of the five days. The respiration of the samples decreased with higher concentrations as predicted; however, the control did not follow this trend.

For the first day and a half, every sample had steady growth and they all followed the same respiration trend as shown by the overlapping curves. They began to diverge just before the second day. The sample with 25 mg/L had a rapid increase of oxygen uptake while the others remained around the same for almost a full day. The samples with 5 mg/L and 50 mg/L then increased, with the former eventually surpassing the total oxygen consumption of the 25 mg/L sample.

The vessel with 5 mg/L PFOA had the highest oxygen consumption at the end of the 5 days although they were all close in value. We expected the oxygen consumption to increase with a lower concentration of PFOA, and this was supported by this data with the exception of the control which had the lowest respiration rate. It was in support of our hypothesis that the low concentration PFOA sample was the least contaminated, so the bacteria could grow and respirate more to consume the most oxygen.

Overall, the PFOA concentrations used in this trial set did not drastically inhibit the growth of the bacteria in the activated sludge. Each curve grew steadily and there were no unexpected spikes or flatlines. They were all within a 10 mg O_2 range, so repeat trials would be needed to verify the toxicity assessment of PFOA. If the test was continued longer than five days, it is possible that the control would surpass the PFOA samples, assuming the chemical takes longer to limit the growth of the bacteria. If higher toxicant concentrations were used, we would expect the oxygen consumption of the samples to decrease, while the control would proliferate and have the highest respiration rate and consumption of oxygen.

Because the sludge in this trial was old, these numbers may not be representative of the growth patterns of the bacteria in the presence of PFOA. Changes to the bacterial population over time would change the ratio of the chemical to bacteria, causing inconsistencies with the other trials that used newer sludge.

4.2 PFOA and Hexavalent Chromium

In this experiment, we examined the co-toxicity of PFOA and chromium. Both are environmental pollutants, and it is possible that the effects of these pollutants might change when they are both present at the same time compared to when only one is present.

4.2.1 Varied Levels of Chromium (without presence of PFOA)

First, we examined the effects of hexavalent chromium on the activated sludge without PFOA present. We used concentrations of 0.002 mg/L, 0.015 mg/L, and 0.050 mg/L. We selected these concentrations in an attempt to reflect concentrations at which chromium might exist in the environment, while also using a large enough range to see differences between the datasets. The

graph below shows the data received from the trial set using these concentrations of chromium with activated sludge over a period of 5 days. The sludge used in this trial was 40 days old.

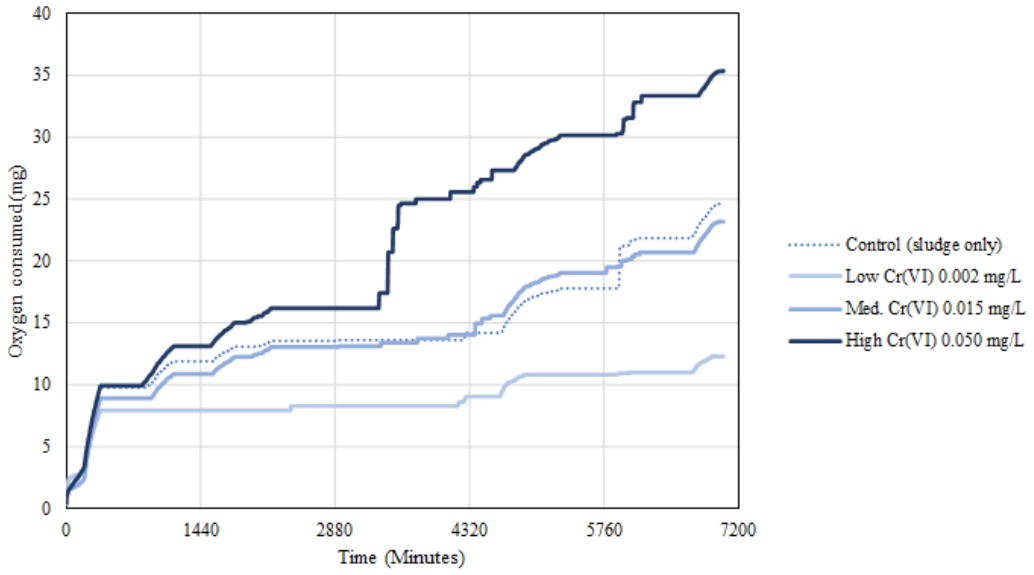

Figure 5: Various levels of hexavalent chromium with activated sludge over a period of 5 days.

Overall, Figure 5 shows the opposite of the expected trends from this experiment: the sample with the lowest amount of chromium has the smallest amount of consumed oxygen, and the sample with the highest chromium concentration has the largest amount of consumed oxygen. This was not what we originally hypothesized about this data, because in respirometry, the lowest amount of pollutants usually have the highest amount of consumed oxygen. This is normally explained by the fact that the lower levels of pollutant inhibit respiration less than higher levels. This could support an alternative hypothesis that the high levels of chromium cause more bacterial death, which cause the living bacteria to work harder and to consume more oxygen during the experiment.

In addition, the difference in the amount of consumed oxygen at the end of the experimental trial between the highest concentration of chromium and the lowest concentration of chromium shows that chromium was significant to the growth of the bacteria in the activated sludge. This difference was larger than the difference between the highest and the lowest samples in the PFOA only trial (shown earlier in Figure 4), demonstrating that the concentration of chromium we tested had a larger effect on the activated sludge than the tested concentrations of PFOA alone.

This trial showed a significant change in consumed oxygen right at the beginning of the trial compared to the other datasets. This shows that the bacteria respirated very quickly, perhaps due to the presence of Cr(VI) placing stress on the bacteria's environment. But, this change levels off during day 2 and the graph continues to follow a more typical shape.

Also, we expected the control to show results most similar to the lowest level of chromium. We suspect that this might be due to differences in the sludge sample that can occur

during sample prep. However, when taking into account sludge age, it aligns closely with the control used in the PFOA only trial above.

4.2.2 Co-toxicity of PFOA and Chromium

The next experimental trial explored the simultaneous effects of PFOA and hexavalent chromium on activated sludge. In this experiment, we kept the amount of PFOA constant at 25 mg/L (the middle concentration tested in the PFOA-only trial set) and varied the amount of chromium using the same high, medium, and low amounts as the previous trial. The graph below shows the results of these pollutants combined with activated sludge over a period of 5 days. The sludge in this trial was 55 days old.

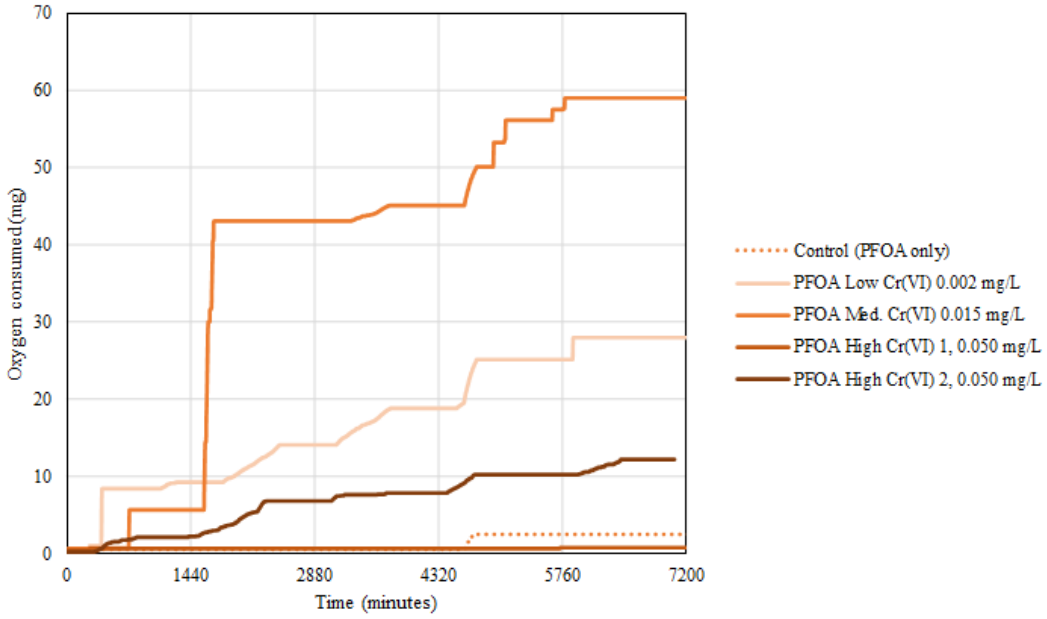

Figure 6: Co-toxicity of PFOA and Cr(VI) with activated sludge over 5 days

During this trial, the PFOA Cr High sensor was not working (named PFOA High (Cr(VI)) 1), so we used the PFOA Cr(VI) High data from an experimental trial performed on a different day for comparison (named PFOA Cr(VI) High 2). The sludge for this sample was 33 days old.

PFOA Cr(VI) High 2 shows low levels of oxygen consumed from the start. This was likely because the pollutants were coupled together and high levels of Cr(VI) inhibited the growth of the bacteria quickly and drastically. Also, the control containing only PFOA showed little change throughout the course of this experiment, which was likely due to equipment failure or sludge age.

The most noticeable change in this graph is the large change of the 0.015 mg/L chromium sample on day 2 compared to the expected trend. This jump may have occurred from the sudden stress on the bacteria, or partial instrument failure. In this trial, and in most future trials, the medium level of the pollutant tested was recorded through chamber 3 of the respirometer. Many

of the medium level solutions show large random jumps in the graphs, indicating that there may be an issue with this chamber of the respirometer.

However, the 0.015 mg/L Cr(VI) sample shows nearly exact additive effects with the PFOA-only and Cr(VI)-only trials. To explain further, the amount of oxygen consumed by the 25 mg/L PFOA trial on day 5 was about 40 mg O_2, and the medium level of chromium by itself on day 5 consumed about 23 mg O_2, which adds up to approximately 63 mg O_2. This value is very close to 60 mg O_2, the amount consumed on day 5 by the simultaneous PFOA and 0.015 mg/L Cr(VI) sample shown in Figure 6. This demonstrates that the effects of the two pollutants compounded when they were both present, and this effect was significant.

4.2.3 Possible Co-toxicity Between PFOA and Chromium

Considering our data for the separate PFOA-only and Cr(VI)-only trial sets, as well as the simultaneous dual-pollutant trial set, we compared oxygen consumption between the three concentrations of Cr(VI). Using this comparison and fit of the two curves for each Cr(VI) concentration, we determined possible types of co-toxicity that were exhibited.

During the course of the trials, data for the 0.015 mg/L Cr(VI) samples had the most similar growth patterns and oxygen consumption between the sum of separate trials and the simultaneous trial. The simultaneous trial curves in orange of the low and high Cr(VI) samples fell below the sum of separate trial curves in red. This pattern signified lower oxygen consumption and higher toxicity of the dual-pollutant trials at low and high Cr(VI) concentrations. However, there was an exponential increase in this simultaneous trial (in Figure 6) after 24 hours, which may be due to respirometer sensor error, and without this increase, this intermediate concentration data would have followed a similar path to the low and high concentration data. In cases such as the 0.015 mg/L Cr(VI) where the two curves were superimposed, additive toxicity regarding respiration was exhibited. In the cases displaying the dual-pollutant (simultaneous) curve below the sum of separate pollutants, oxygen consumption was lower, therefore this trial set exhibits synergistic toxicity regarding respiration.

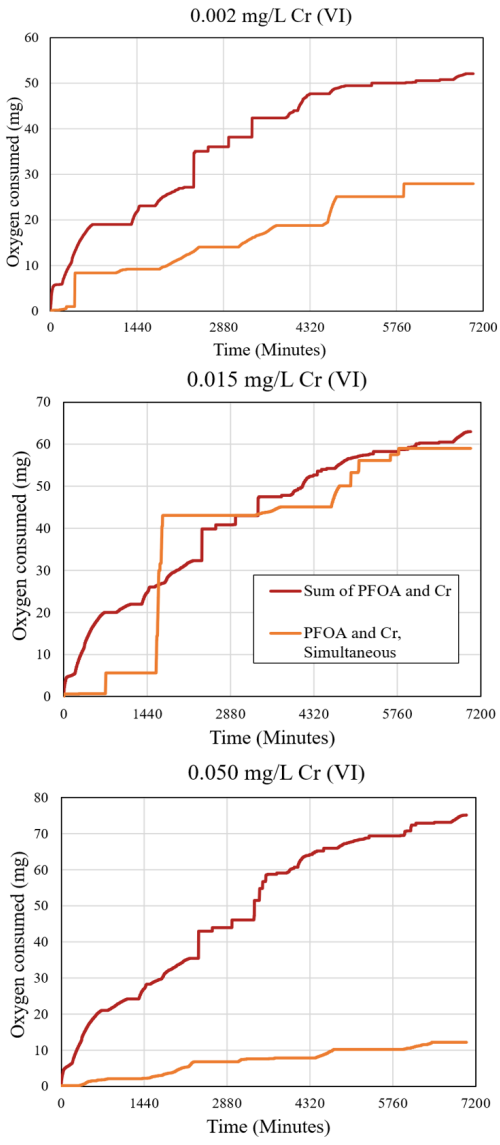

Figure 7. Comparisons between the respiration of the simultaneous PFOA + Cr(VI) trials and the sum of separate PFOA and Cr(VI) trials at different concentrations of Cr(VI).

To more concisely analyze the differences between each graph, we determined the value of the difference between the two curves over time. Consistent with the three graphs in Figure 7, the highest concentration had the highest deviation between the two trial sets, the intermediate concentration data had the lowest deviation, and the low concentration had some deviation. However, each of these three graphs followed the same pattern for the first 12 hours of data collection, likely due to the simultaneous samples' slow initial takeoff. We visually display these deviation differences in Figure 8.

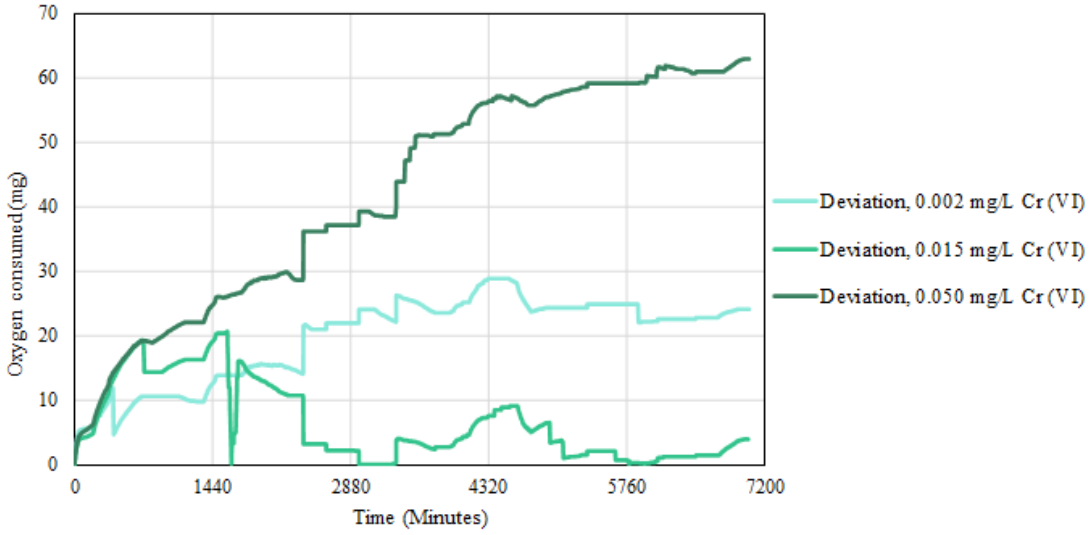

Figure 8: The absolute value difference between the simultaneous PFOA + Cr(VI) respiration and the sum of respiration values for the separate PFOA and Cr(VI) trials.

The low deviation between separate sum data and simultaneous data for the 0.015 mg/L sample suggested additive toxicity between PFOA and Cr(VI). This may be a valid conclusion only for intermediate concentrations of Cr(VI), because the toxicity of the simultaneous trials at low (0.002 mg/L) and high (0.050 mg/L) concentrations of Cr(VI) was higher than the sum of the two separate trials. Based on this evidence, there could be an intermediate minimum of co-toxic potency between Cr(VI) and PFOA at different Cr(VI) concentrations, with an optimal concentration at some value between 0.002 and 0.050 mg/L.

4.3 PFOA and Zinc

The third group of trials were performed using PFOA and zinc, each individually, and then in combination. Failings in this dataset impeded the production of an intricate compounded toxicity analysis like for the PFOA and hexavalent chromium dataset. Zinc levels were varied between 0 and 50 mg/L. In the trials that combined zinc and PFOA, the PFOA levels were kept constant and zinc levels varied. With a few exceptions, these datasets supported our hypothesis that increasing sample contamination decreased oxygen consumption.

4.3.1 Varied Zinc Levels (without the presence of PFOA)

Since zinc is a common pollutant present in industrial byproduct streams and municipal waste streams, places where PFAS are likely to also be present, it was important to test the co-toxicity of the two contaminants and their effects on oxygen consumption levels. To compare to the co-toxicity data, the effect of zinc on oxygen consumption was tested independent of the presence of PFOA. Contaminant levels both below and above those that typically occur in waste streams were tested to obtain a range of data.

Trials varying zinc levels were conducted three different times. The four zinc concentrations tested were 0 mg/L, 2 mg/L, 10 mg/L, and 50 mg/L. None of the three attempted trials resulted in usable data. In the first trial, the data read between 1000 and 3000 mg O_2 after 2 days, an unreasonably high value, so the trial was ended and deemed unusable. For the second trial, only the first and fourth sensors (containing 0 and 50 mg/L Zn, respectively) presented any readings. For the final attempt, only the first sensor (containing the control sample) was reading; again, this data was not usable.

Had these trials have resulted in usable datasets, they would be expected to have decreasing levels of mg O_2 consumed with increasing levels of pollutant. These readings would also be lower than those associated with control trials that contained no pollutants. It is unclear whether these O_2 levels would be lower for samples contaminated with only zinc, hexavalent chromium, or PFOA. Since the pollutant levels for experimentation were chosen based on environmental concentrations found in literature, it was not possible to compare the relative severity of the contaminants.

4.3.2 Co-toxicity of PFOA and Zinc

The same zinc levels as the trials above were used (0, 2, 10, and 50 mg/L), with each sample containing also 25 mg/L PFOA. Since PFAS were the primary focus of the project, those contaminant levels were kept constant, while the metal concentration varied to observe the co-toxicity effects on oxygen consumption. Trials for this data were run twice. In the first trial, only sensor 3 (containing the 10 mg/L sample) had any readings on the respirometer, rendering this data essentially unusable. The second attempt was successful until between the second and the third day, when the oxygen tank ran out of gas. However, we were able to analyze the first 48 to 72 hours of the data.

In Figure 9, the oxygen consumption levels over five days of testing are displayed for activated sludge samples with varying levels of zinc and the co-toxicity with PFOA. The sludge for this trial was five days old.

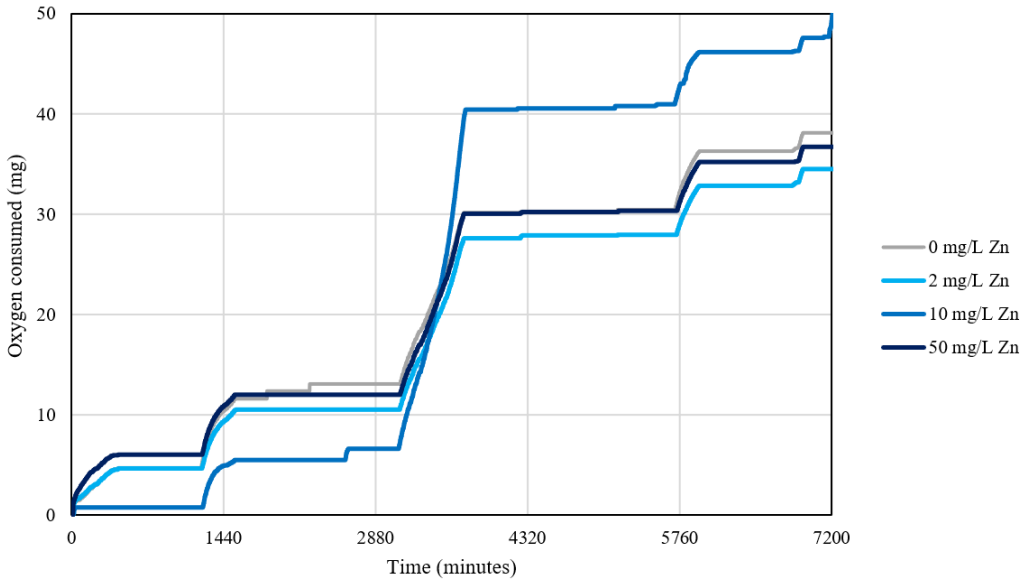

Figure 9: Co-toxicity of PFOA and Zn with activated sludge over 5 days

Based on laboratory log recordings made over the course of this trial, the data collection progressed as to be expected for the first 48 hours. The control solution and the 2 and 50 mg/L samples all maintained similar oxygen levels, with the 10 mg/L sample slightly lower. As the oxygen tank rapidly emptied, there was a corresponding spike in O_2 levels recorded (see dramatic increase on Figure 9 between 3000 and 4000 minutes). After the oxygen influx, the control, 2 mg/L, and 50 mg/L samples still remained close in level, this time with the 10 mg/L sample at higher levels. Also recorded in the lab log was rapid bubbling in the tube corresponding to the 10 mg/L sample as the O_2 tank depleted (sensor 2). Our analysis with respect to this detail is that the relative levels of the 10 mg/L sample throughout the trial could tell us more about the mechanism of the respirometer than the toxicity of pollutants in the sample.

We also predicted that the data being recorded after the oxygen tank was fully exhausted were false positive readings caused by the cell oil level passing back and forth across the sensors. Because of the oxygen tank malfunction, we restricted our analysis to the first two days (approximately 3000 minutes) of data. This dataset is presented in Figure 10.

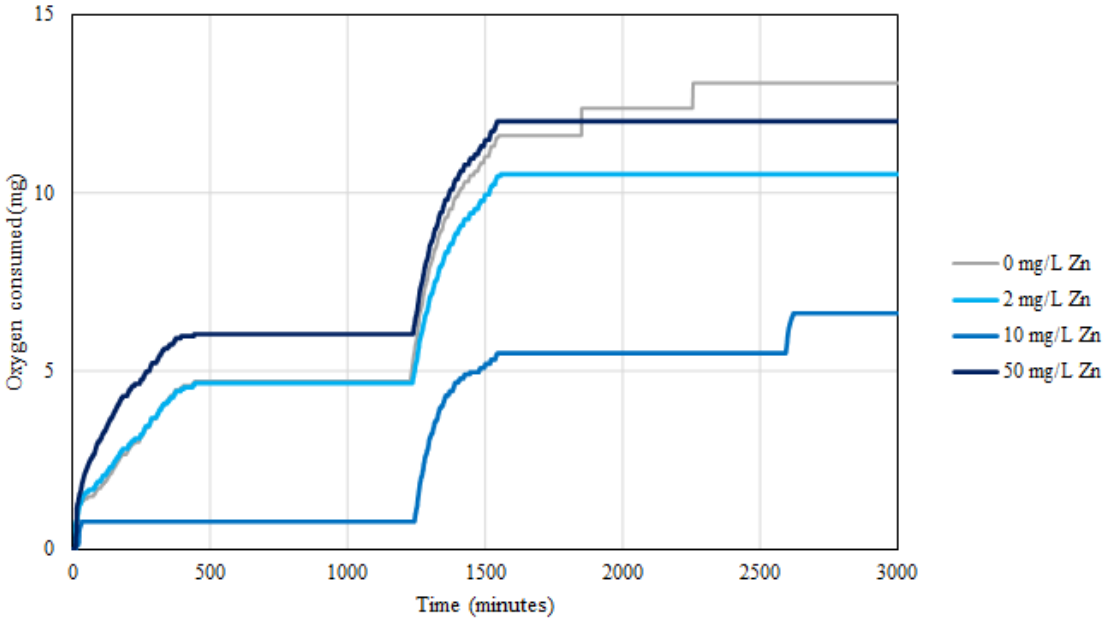

Figure 10: Co-toxicity of PFOA and Zn with activated sludge over 2 days

Excluding the 50 mg/L zinc sample, the relative positions of the 0 mg/L, 2 mg/L, and 10 mg/L zinc samples were consistent with our hypothesis that higher levels of zinc, with and without the presence of PFOA, would lead to decreased oxygen consumption by the sludge bacteria. For the majority of the trial, the 2 mg/L zinc sample remained close to or below the control sample, and the 10 mg/L sample was well below the control for the entirety of the 2 days. Since the 2 and 10 mg/L samples were either very close to or below the control sample throughout the course of the trial, this suggests potentiation between PFOA and zinc. At the same concentration of PFOA, adding a small amount of zinc would decrease the oxygen consumption levels of the solution. With higher concentrations of zinc, the consumed oxygen levels would further decrease.

The 50 mg/L sample initially produced higher oxygen consumption levels than the control sample, contrary to our hypothesis. Later, those levels dipped below the control's, but we would have expected the levels to be below those of the 2 and 10 mg/L samples as well. This occurrence could support our alternative hypothesis that high pollutant levels cause bacteria death, which would provide further organic matter as a food source for the living bacteria. It would be interesting to further explore this theory, but since our samples did not contain an oxidant to assist in the breakdown of dead bacteria, this idea is less certain than our original.

4.4 Comparing Effects of PFOA, Hexavalent Chromium, and Zinc

To compare the results from the PFOA and Cr(VI) experiments and the PFOA and Zn experiments, we graphed both datasets in Figure 11. This data compares the results of these varying contaminant levels affecting the oxygen consumption of activated sludge over a period of 5 days. Both datasets contain the metal in combination with 25 mg/L of PFOA. The PFOA

and hexavalent chromium data was already shown in Figure 6, discussed in section 4.2.2, and the PFOA-zinc data was graphed in Figures 9 and 10, discussed in section 4.3.2. The sludge was 55 days old and 33 days old (0.050 mg/L Cr(VI)) for the PFOA-hexavalent chromium trial and 5 days old for the PFOA-zinc trial.

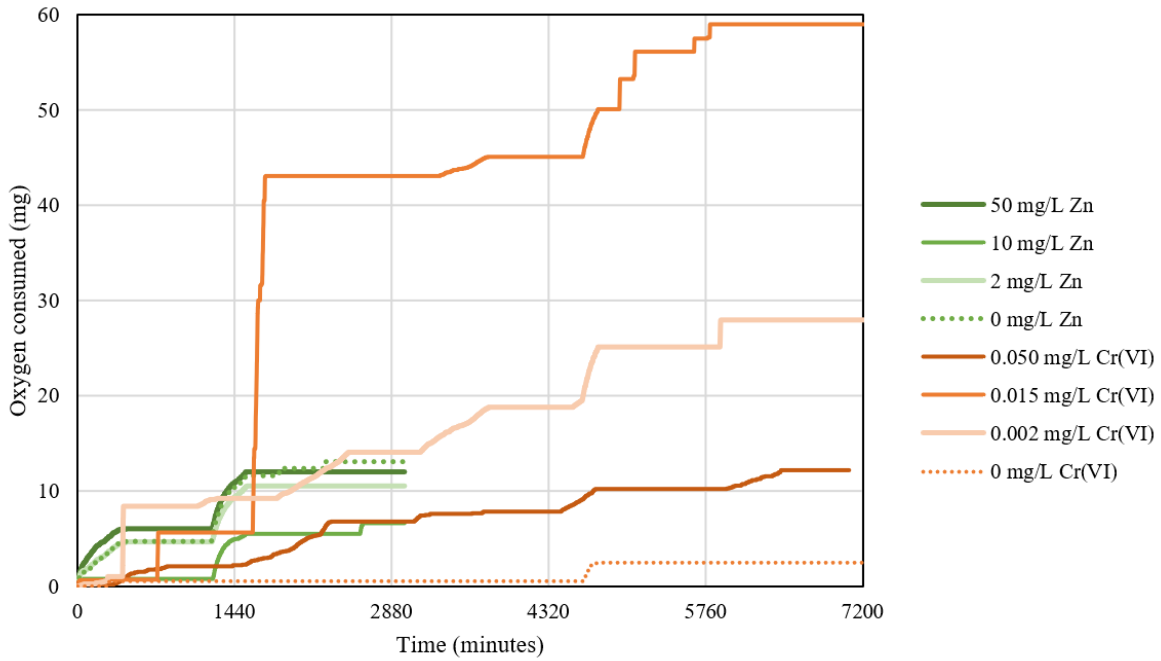

Figure 11: Comparison of the co-toxicity of PFOA with Cr(VI) and with Zn with activated sludge over 5 days

Both control trials, shown with dotted green and orange lines in Figure 11, had the exact same composition, the only difference being the age of the sludge. The PFOA-Zn sludge was new and this control trial had much higher oxygen levels than the PFOA-Cr(VI) sludge control trial. Based on our sludge age analysis later in section 4.5, this is consistent with our other findings. When the control trials have a contaminant (PFOA) present, older sludge has lower oxygen consumption levels than newer sludge.

The PFOA-Zn samples were more closely grouped together than the PFOA-Cr(VI) samples, suggesting that the presence of zinc at the levels tested had less impact on the variability of oxygen consumption levels. However, this disparity between the two groups of samples could be due to sludge age and a difference in bacteria composition between the two batches of sludge collected.

In Figure 11, the entire PFOA-Zn dataset and the 0.002 and 0.050 mg/L Cr(VI) samples support our hypothesis that control trials would have the highest oxygen consumption levels, and that these levels would decrease with increased concentration of contaminant. The suspiciously low values for the control trial with old sludge as well as the extremely sharp spike early in day two for the 0.015 mg/L Cr(VI) sample suggest possible malfunctions with the respirometer. The elimination of these data points would present promising data to support our hypotheses about the negative correlation between pollutant concentration and oxygen consumption.

4.5 Comparison of Sludge Age

As mentioned in our methodology and earlier results, each trial set was run simultaneously with a control sample. For the trial sets testing only PFOA, the control sample contained only sludge, nutrients, and chemical buffers. For trial sets testing for co-toxicity between PFOA and a metal, the control was only contaminated with PFOA at the same concentration as the experimental bottles (25 mg/L). The purpose of these controls, aside from having a baseline reference sample with which we would compare experimental samples, was to observe how the activity of the sludge changed over time, whether it was exposed to PFOA or free of any pollutants.

4.5.1 Comparing Uncontaminated Sludge Control Samples Over Time

Using control trials that contained only sludge, nutrients, and buffers, we evaluated the changes in capacity for oxygen consumption over time. This analysis created a baseline for our experimental data and helped to explain some phenomena and differences between trials as the sludge used matured in storage. Figure 12 shows control samples using sludge from the same batch, with trials ending February 20th (40 days old), February 25th (45 days old), and March 2nd (50 days old).

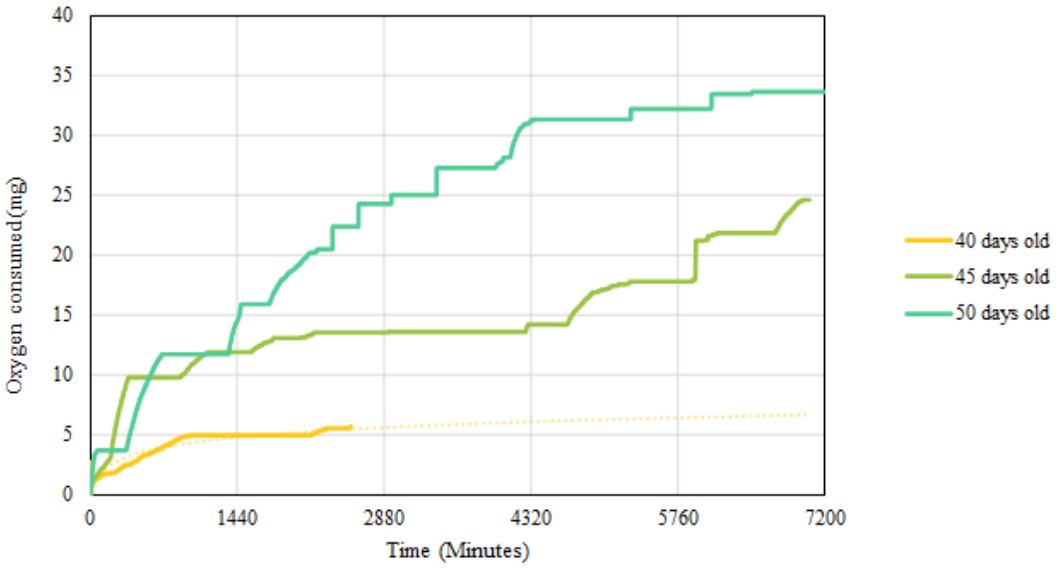

Figure 12: Comparison of uncontaminated control samples over time. The samples shown above were all from the same bottle of sludge, retrieved on 1/11/21.

During the first two days of activity, the consumption of oxygen by the control sample has a positive correlation with the sludge age. After two days of activity, only assuming the 40 day-old sludge followed a roughly logarithmic path of oxygen consumption, this pattern remained.

While not much can be gathered from the specific patterns of the newest (40 day old) sludge growth and respiration after 2 days, the character of the curves representing 45 and 50

day-old sludge respiration varied. While the oldest sludge followed a fairly logarithmic path, flatlining around 3 days, the 45 day old sludge flatlines and then had a noticeable increase at the end of the trial period. These flatlines sometimes occurred in the midsection of the data, followed by an increase, like we see with the 45 day old sludge. The 50 day old sludge experienced stepwise-like increases in oxygen consumption, as well as fewer significant flatline periods. These periods could be rest periods of bacterial growth rather than an inability to respirate due to resource constraints or bacterial death. However, the age range of only 5 days between these two trial sets may not be a significant amount of time to observe major consistent differences in sludge bacteria activity.

4.5.2 Comparing Contaminated Sludge Control Samples Over Time

We also analyzed the differences between new and old sludge contaminated with PFOA. In addition to learning from this analysis, we also compared the respective analyses of contaminated and uncontaminated sludge at varying ages. However, the age range used for the PFOA-contaminated-sludge was much wider (15 to 50 days) than the sludge age range analyzed for uncontaminated sludge controls (40 to 50 days). The data in Figure 13 below was from two trialsets, one ending January 26th (15 days old) and one ending March 2nd (50 days old), both of which had sludge from the same wastewater collection bottle.

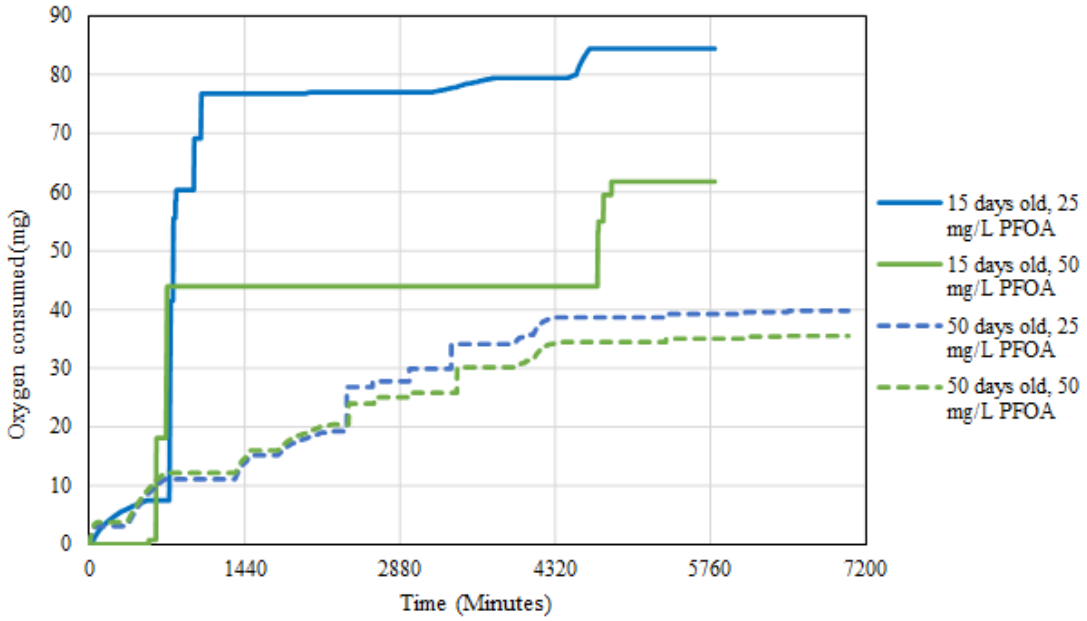

Figure 13: Comparison of control samples exposed to PFOA over time. Both the 15 and 50 day old sludge was from the bottle retrieved on 1/11/21.

Contrary to the data for the uncontaminated control samples, samples contaminated with 25 and 50 mg/L of PFOA demonstrated lower oxygen consumption as the sludge matured. The newer sludge also has much sharper, sudden increases in data values.

The lower oxygen consumption values for older samples demonstrated that older activated sludge bacteria colonies were more sensitive to PFOA than newer sludge. As the sludge matured in the storage refrigerator, it exhausted the dissolved oxygen in the container. Therefore, it was likely that there was a higher concentration of dead bacteria in the older samples. Newer sludge contained bacteria that were less likely to have already exhausted the oxygen in the closed container, so they were more viable in samples. Thus, the newer, higher populations were more robust against the presence of PFOA.

In the first 12 hours of data collection, both 50 day old samples and the 15 day old 25 mg/L sample had regular, steady increases in oxygen consumption, while the 15 day old 50 mg/L sample had no activity during this time. However, about halfway through the first day of testing, both 15 day old samples experienced a rapid spike in O_2 consumption. The 50 day old samples are nearly superimposed graphs, with the one exception being the spike in oxygen consumption at about 2300 minutes, where the 25 mg/L sample rose above the 50 mg/L trial.

Qualitatively, we noticed that for the contaminated sludge dataset, the newer sludge had more significant spikes and longer rest periods, the opposite of our observation for the uncontaminated sludge age analysis. The older contaminated sludge controls discussed in section 4.5.1 consumed less oxygen than their newer counterparts. Overall, we discovered evidence for a positive correlation between sludge age and oxygen consumption without PFOA, and a negative correlation between sludge age and oxygen consumption in the presence of PFOA. These discoveries exemplify why monitoring the viability of the sludge over the course of experimentation is vital for the integrity of our data.

5.0 CONCLUSIONS & RECOMMENDATIONS

This work demonstrated that PFOA, hexavalent chromium, and zinc all significantly impacted the growth of bacteria in activated sludge. In most cases, lower amounts of these pollutants led to higher levels of oxygen consumption, showing that lower pollutant levels corresponded with lower inhibition of bacterial growth. We also discovered that, in general, when the bacteria in dual-pollutant samples, the effects of each individual pollutant were compounded and multiple pollutants inhibited growth more than when present individually. This compounded toxicity demonstrated potentiation between PFOA and the metals. In addition, we did not see any decrease in the amount of consumed oxygen, indicating that these bacteria are likely not a valid method of bioremediation.

5.1 Recommendations

Based on our findings and lab experience, we have several recommendations for researchers pursuing the topics of respirometry and PFAS. For future MQPs involving oxygen consumption and bioassay toxicity testing with activated sludge, we suggest performing manual BOD bottle tests instead of using the respirometer. This would allow for more control over experimental variables. There was little information about the Challenge Technology™ respirometer we used online due to the device's age; when we were experiencing problems and researching solutions, the troubleshooting help was sparse. However, there were sufficient resources for manually measuring consumed oxygen of activated sludge. Furthermore, this method would not require an oxygen tank, saving time and money. When performing bottle testing, the data is recorded manually, eliminating the need for the quick-scan computer program. This method would also provide information about the initial conditions and content of the sludge, and the data would be simplified for interpretation.

Dissolved oxygen uptake rate (DOUR) or BOD bottle testing would eliminate problems we had with the respirometer, however it presents its own limitations as well. Since the procedure is manual, there is a higher risk of quality control. BOD tests can still be imperfect and unreliable due to human error or calibration missteps. They also take longer to set up and must be more consistently monitored than the respirometer. When deciding which method to use to measure oxygen consumption, future researchers must decide what to prioritize in terms of time, accessibility, dependability, cost, and patience.

If using the respirometer to quantify the oxygen consumption of activated sludge, we recommend procedural steps for more more consistent results. One aspect of quality control that greatly impacted our results was the age of the activated sludge. Our trials used sludge varying between zero and over 50 days old, and the oxygen consumption significantly increased over time to show the bacterial growth during prolonged storage. This led to inconsistencies in our data, so for future studies we recommend getting new sludge every two weeks. The retention time of activated sludge in wastewater treatment should be as short as possible, so it would be best to mimic realistic sludge conditions by only using fresh sludge samples. We also

recommend replacing the needles on the respirometer tubing frequently to avoid pieces of rubber getting stuck in the needles and blocking the oxygen flow. Over the course of our experimentation, there were instances when there were leaks present in the tubing, so it is important to check the tubing connections consistently if there are noticeable drops in the oxygen tank. To check these connections, pour diluted dish soap over them and see if bubbles form where leaks may be present.

There is abundant additional research that can be investigated on the interactions between PFAS and activated sludge. One potential direction for future research would be to include the study of how GenX affects activated sludge compared to PFOA. GenX is manufactured as a safer "replacement" for PFOA. Measuring the respiration of activated sludge exposed to GenX could be used to verify the relative environmental toxicity of this allegedly safer alternative. We outline background information about GenX in Appendix C.

We experienced difficulties when collecting data for the zinc trials without PFOA, so it would be important to measure the effect of zinc alone on sludge for compounded toxicity; trends could then be observed between PFOA and zinc trials. Our team would also recommend using smaller concentrations of the pollutants to better align with realistic values of these chemicals in the environment. We chose high concentrations for easier measurement with the tools available to us. If we had time, it would have been valuable to replicate more of our findings and verify the oxygen consumption trends.

Lastly, we would recommend plating and incubating the activated sludge and subsequently performing zone of inhibition tests on prominent microbial isolates. This would allow for characterisation of the bacteria species in the sludge using microscopy or gram staining techniques, as well as a better understanding of the baseline conditions. Plating the isolates of interest on media inoculated with PFOA, GenX, zinc, and Cr(VI) could give clues about the behavior of growth of particular species when exposed to these chemicals.

There are a few successful steps we took in our experimentation that we also would recommend for future studies. We kept a shared lab log in Google Drive as a lab notebook, cataloging daily work performed in the lab. We documented the dates, team members who went in, tasks completed, and observations made. We also checked the oxygen tank level and gas flow rate multiple times per day to make sure the vacuum seal chamber was at the right level. The respirometry stock solutions detailed in the Methodology also worked well, and we recommend preparing them ahead of time before the start of each new trial to save time. The 5-day test also was a favorable length of time to observe the respiration rates of the samples.

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APPENDICES

Appendix A: Solution Details

The table below outlines our planning for the experimental trials that we performed.

Table A1: Solution details

Solution Name	PFOA (mg/L)	Cr(VI) (mg/L)	Zn (mg/L)
Control Set	0	0	0
Control	0	0	0
PFOA low	5	0	0
PFOA med.	25	0	0
PFOA high	50	0	0
Control (PFOA)	25	0	0
PFOA, Zn low	25	0	2
PFOA, Zn med.	25	0	10
PFOA, Zn high	25	0	50
Control	0	0	0
Zn low	0	0	2
Zn medium	0	0	10
Zn high	0	0	50
Control (PFOA)	25	0	0
PFOA, Cr low	25	0.002	0
PFOA, Cr med.	25	0.015	0
PFOA, Cr high	25	0.050	0
Control	0	0	0
Cr low	0	0.002	0
Cr medium	0	0.015	0
Cr high	0	0.050	0

Appendix B: Failures

Plans versus Reality

In this Appendix, we outline and briefly discuss some of the factors that contributed to us not accomplishing all of the highest hopes for this project. We initially planned to test both PFOA and GenX in combination with Zinc and Chromium. Ultimately, we were unable to perform trials with GenX due to project delays and the constraints of the academic timeline. We mention in our recommendation section in Chapter 5 that future student groups examine co-toxicity between GenX and metal pollutants and the effect on bacteria. For their convenience, we have catalogued the background information we gathered on GenX in Appendix C. We also planned to plate sludge samples and use microscopy and gram-staining techniques to see what types of bacteria were present in our samples. This also did not happen, and while it was less integral to our final conclusions, all four of us were interested in learning more from this process.

While previous MQP student groups worked with PFAS, they used NMR technology in their analysis. We further explored PFAS, but with a novel project idea, making our MQP the

first iteration of the project. Based on our advisers' experience, first iterations of projects do not tend to produce a tremendous amount of valid or accurate results and conclusions, and they face many challenges. Despite knowing this, it seemed we still overestimated the success we would have this year.

In the following we discuss complications we faced with sludge irregularity, respirometer failings, road bumps with the oxygen tank, and additional challenges due to Covid 19.

Complications with the sludge

For this MQP we dealt with wastewater sludge from Upper Blackstone Clean Water. We picked up sludge at three different dates: November 13, 2020; January 11, 2021; and March 10, 2021. Thus, we used sludge to do experimentation that was varying in age. During the trials process, we did not quantify the differences in data based on sludge age, but later, in our data analysis phase, we observed that sludge age impacted the data. The frequency with which we would need to obtain new batches of sludge to eliminate this variability was not conducive to our academic and general MQP schedule, so we had to deal with this variability.

Since we did not know the general composition of material in the sludge, nor did we plate sludge samples to see what types of bacteria we present, we did not know if there was variability between batches. Was the batch collected in November more or less dense in pollutants or bacteria? We may never know, and this had the potential to impact our results.

Respirometer failures

One of the most common challenges we faced in this MQP was the respirometer failing. The equipment was old, and no longer being made, so there were limited online resources available to us for troubleshooting the machine. Our main resource was Mark from Challenge Technology, the manufacturer of the respirometer. He was able to provide some guidance for us, but communication with him was usually delayed, so we had to solve all our urgent problems independent of him.

Another impact of the device's age was a dissonance between the sensors and the software readings, which we eventually discerned was an issue with the sensor alignment. When we removed headspace from our samples with a syringe, we at first did so only until the meniscus in the back of the chamber was pulled down to ease the passage of O_2 past the sensors. However, we noticed differences in the stability and continuity of the chambers' ability to produce readings when a full 20mL of O_2 was removed from the headspace. Whether or not one chamber would produce readings even when bubbles were observed passing the sensor was one of the most persistent inconsistencies throughout our experiments. Most of the time, chamber 1 would read while chamber 2 was the chamber with the most frequent absence of readings, followed by chamber 4.

Troubleshooting:
To troubleshoot the sensors, we would carefully push down on the misread chamber (most often chamber 2) to realign its sensors. This proved to be a very temporary fix. A lack of data collection was sometimes a software issue, but other times, we observed no bubbles passing the sensors. In this case, we would test for leaks and clogs in the tubing. To expel any potential obstacles in the tubing, we reattached all of the tubing together to bypass the chambers, and passed oxygen through the tubing at a very high pressure.

Chamber 3 was the most impacted by another peculiar issue—the presence of a second liquid in the chambers and piping that was observed about half-way through our experimentation. When a bubble would pass by the sensor, a globular mass of the oil would fall back down, sometimes past the sensor again, yielding a false positive. This occurred because as the bubble of oxygen passed through and was enveloped by the oil layer, some oil was carried through the boundary between the oil and water layers. As the bubble was released into the headspace above the chamber, some oil would fall back down. We deduced that this second liquid was likely water, and entered the closed chamber system due to a sudden increase in pressure when a lab member was refilling the vacuum container.

Maintenance of the vacuum containers proved to be a meticulous task that demanded attention at odd hours of the day. If the O_2 settings were such that the vacuum container was bubbling vigorously, the container, which had a pressure relief hole at the top, would have its water more readily evaporated. If the water evaporated leaving the oxygen-expelling orifice exposed to atmospheric pressure, the oxygen tank would rapidly empty. When refilling the vacuum container, we had to be considerate of the sensitive pressure within the system. At first, we were usually taking the container apart and quickly filling it and re-assembling, but then were advised to use a spray bottle with reagent-grade water to fill the container through the top pressure relief hole. However, this strategy would effectively plug the pressure relief hole, causing the water at the oxygen-expelling orifice boundary to be pulled into the tubing, resulting in the aforementioned dual-liquid (oil and water) issue.

Troubleshooting:
After discovering that the spray bottle strategy was the culprit of the system contamination, the chambers were thoroughly cleaned, dried and refilled with oil. Moving forward, we used a thin syringe to refill the vacuum container with water, because the syringe was thin enough not to completely cover the pressure relief orifice and disturb the system pressure. To test the regulator and connective tubing for leaks, we would use a spray bottle with water and dish soap and observe whether or not bubbles formed. Wherever bubbles form, we assumed there was a leak.

We also dealt with general mechanics issues with the machine. Since we were poking needles through the rubber bottle caps to allow oxygen to flow, the sharpness (newness) of the needle had an effect on the seal of the hole through which the needle protruded. We did not catalogue exact, regular instances of needle replacement, which could have impacted our results.

Oxygen tank supply failures

Failures, malfunctions, and general goonery with the oxygen tanks were some of our primary issues with this entire experimentation process. We started trials in B-term with a nearly empty oxygen tank. Replacing it was a delayed process due to shipping and supply complications related to the pandemic, and twice we were delivered an oxygen tank that leaked and was almost empty when we tried using it. We did not immediately notice the emptiness of the tanks because, based on the pressure gauge, the tank reads full for approximately the first 75% of fullness, and the gauge reading rapidly decreases for the last 25% of gas. Thus, we would attach the tank, deem it to be full, and return to check on the respirometer the following day to find its reading malfunctioning.

Using oxygen tanks and re-ordering them was an expected part of our project, but delay time and delivered tank issues set us back significantly. Further, we would often have to wait an additional day to start trials once a new tank arrived because we needed Wenwen's help to switch the connection to the respirometer from the old tank to the new one. In addition to the general inconvenience of needing a new tank, when the tank rapidly emptied the last 25% of the gas, it would usually blow powerfully through the respirometer, often blowing out our samples and disrupting the oil in the back tubing of the machine. Topping all of these mechanical errors, we struggled at the beginning of the year because random lab-users would turn off the oxygen to the respirometer for seemingly no reason, until we put up a sign.

Complications as a result of the Covid-19 pandemic

We began the academic year with the understanding that lab access could be restricted or terminated at any time depending on the state of the pandemic and/or WPI's preparedness for socially distanced work. In the initial phases of this MQP, we had plans to fall back on an extensive literature review, should the Covid 19 numbers become out of control. Thankfully, for the most part, we were able to retain access to the Environmental Lab through the course of the year.

The first specific complication we faced was general delay in the ordering and shipping of materials. In the fall, when we started experimentation, the US was in a phase of re-opening, and many things (including oxygen tanks) were back-ordered. Delayed access to materials pushed back the start date for experimentation into B-term.

In addition, Covid 19 restrictions required us to get special visitor's permission to obtain sludge samples from Upper Blackstone Clean Water treatment facility. If these restrictions were not in place, we likely would have been able to obtain sludge more frequently, and likely would have seen fewer discrepancies caused by sludge age in our results.

Due to the extended winter break that WPI scheduled, we did not have access to the environmental lab for the latter half of December and the beginning of January. In January, Deanna, Olivia, and Jen were able to re-integrate to the WPI bubble by starting their weekly Covid 19 tests, so for the remainder of January, only three of our four members could work on trials. At the start of B-term, lab occupancy was reduced to 50%, and we had to choose 2

members only to continue experimentation, further reducing our efficiency and in-person troubleshooting abilities.

When poor lab access and slow delivery/response time coincided, our progress was further hindered. This was in addition to the general uncertainty we all felt at the looming idea that we might lose in-person access to the environmental lab at any point.

Appendix C: GenX Background Information

GenX is another type of PFAS that was first used to eliminate the use of PFOA. The formal name for GenX is 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy) propanoic acid (chemical formula $C_6HF_{11}O_3$) ("PubChem Compound Summary for CID 114481," 2020). When GenX comes into contact with water, it breaks down and releases an ammonium group and the molecule becomes HFPO-DA. This new molecule is a strong acid which has the tendency to deprotonate. The conjugate base can be easily detected in water (United States Environmental Protection Agency, 2018c).

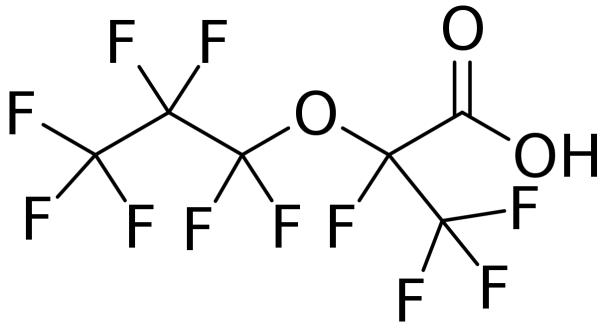

Figure A1: Sketch of a GenX molecule with a molecular weight of 330.05.

GenX is used in food packaging and non-stick coatings and polymers like Teflon. The goal of using GenX instead of PFOA was to eliminate the toxic and carcinogenic consequences of using PFOA with an alternative that is healthier for humans (Hogue, 2018). However, over time studies have been conducted that have found that GenX has the same toxicity effects as PFOA (Lerner, 2016). The effects of GenX include impacts on the kidneys, blood, immune system, and liver, including cancer.

Like PFOA, GenX are found in higher concentrations in urban surface waters. Although GenX is a fairly new industrial chemical, there have been several case studies that can provide clues about GenX's ambient concentration in water. On the border between Germany and the Netherlands, concentrations of GenX in the Rhine river downstream of a fluorochemical processing facility were found to be initially in the range of 0.5-0.75 ng/L (Gebbink & van Leeuwen, 2020). This result aligned with the results from another study that included samples from upstream surface water from all over the world and indicated the background concentrations of GenX in surface water to be between 0.21 and 2.02 ng/L.

Through rainfall or gradual mobilization, PFAS in the soil can transport into deeper layers of soil and contribute to groundwater contamination. Soil accumulates PFAS through

runoff, deliberate land disposal, and through the use of PFAS products. At sampling sites near point sources of a contaminant release, GenX concentrations in soil were found to range from 0.18 to 4.7 ng/g dry weight, and PFOA concentrations ranged from 9 to 84 ng/g dry weight (Gebbink & van Leeuwen, 2020).