

# Degradation of Polyethylene Terephthalate (PET) and Polyamide (PA)

by  
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## **Author's Declaration**

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

## **Statement of Contributions**

The second chapter of this thesis was co-authored with the following researchers who provided valuable guidance for project design and outcomes, as well as review and editing; Stephanie Slowinski, Dr. John Honek, Dr. Rodney Smith, Dr. Fereidoun Rezanezhad and Dr. Philippe Van Cappellen.

The third chapter involved co-authors, Stephanie Slowinski, Dr. Fereidoun Rezanezhad and Dr. Philippe Van Cappellen who contributed to experimental design, set up, execution, manuscript editing and review.

## Abstract

Microplastics have become an increasing concern to humans and ecosystems as plastic production continues to soar, due to their prevalence in the environment and lifespan. Plastic is cheap and durable making it an ideal industrial and commercial material. However, because of this popularity, it resides in most places on earth, including in human blood, and is difficult to remove due to its small size. These plastics can enter the environment through numerous methods, from landfills and dumps to washing machines and sinks. In recent years, there has been significant investigation in reducing plastic pollution. This a difficult task attributed to the varying size, shape, polymer type, chemical properties and location plastic can be found. It's critical to understand the rate of degradation and the factors that influence it for two main reasons; it provides accurate timelines of degradation and techniques that may increase degradation need a starting point.

In Chapter 2, I investigate the degradation rate of laboratory grade polyethylene terephthalate (PET) using a model enzyme (*Huimcola insolens* cutinase) to hydrolyze the plastic. This research aims to characterize the polymers used such that results can be compared and identify the analyses which capture degradation and characterize the polymer best. Environmental factors controlling enzymatic plastic degradation are not well studied and this experiment aimed to study the effect of incubation temperature, exposure to freeze-thaw cycles (FTCs) and extreme temperatures on the degradability of laboratory-grade PET. In addition, we also assessed the degradability of consumer-grade PET, sourced from plastic bottles, for comparison to the laboratory-grade PET. The first test was under variable temperatures, where plastic was incubated at 25 °C, 40 °C and 55 °C. The results show increased temperatures increase the rate of polymer degradation. The second set of tests were conducted under different pretreatments; treatments the plastic would undergo before incubation at 40 °C. Plastic was exposed to a series of freeze-thaw cycles (FTCs) or extreme

temperatures (-70 °C or +55 °C). It was found any type of pretreatment increased the rate of degradation compared to plastics that did not undergo any pretreatment. The final condition tested was plastic type, where PET water bottles were obtained and incubated at 55 °C to determine the differences in degradability between laboratory-grade PET and consumer-grade PET. Consumer-grade PET was found to not have any significant degradation after 10 weeks of enzyme exposure, raising serious concerns regarding its degradability and lifespan. This result suggests that modifications to the consumer-grade PET during the fabrication process, such as heat treatments, are altering its chemistry and its degradation kinetics. Analyses for degradation and characterizing the polymers included: Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and tensile strength measurements. Analysis of the crystallinity, tensile strength, SEM images and FTIR spectra measured indicate that PET's physical and chemical properties were modified when degraded. Overall, the PET's tensile strength decreased and the crystallinity increased with increasing hydrolytic degradation. FTIR spectral changes were seen early on, with peaks of interest at 1237 cm<sup>-1</sup>, 1016 cm<sup>-1</sup> and 1087 cm<sup>-1</sup>, and finally at 1716 cm<sup>-1</sup>, and the flattening of these peaks increased with increasing hydrolytic degradation. The results highlight that enzymatic degradation rates can be highly variable due to differences in environmental conditions. It also highlights the large difference in the degradability of consumer-grade versus laboratory-grade PET, which has significant implications for *in situ* environmental degradation rates.

In Chapter 3, I investigated the rate of laboratory-grade PET and polyamide (PA) degradation in stormwater pond sediment over a 16 month period in a stormwater pond in Kitchener, Ontario. Microplastic accumulation in the environment, especially in bodies of water and sediment is a well-known problem. Stormwater ponds act as a microplastic sink and draw pollutants from urban

and industrial wastewater before it enters oceans or lakes. This results in high levels of microplastics remaining in stormwater pond sediment. Stormwater ponds are an excellent site to determine realistic plastic degradation in the environment, in a contained area where high concentrations of plastic is known to be present. To date, no long-term polymer degradation studies have been conducted in a stormwater pond despite the rising popularity of these ponds. For this study, 8 pore water samplers (peepers) were packed with pond sediment and plastic pieces were inserted into each cell of the peeper. An additional 8 peepers filled with water, such that pore water chemistry could be collected. The peepers were inserted into the pond sediment and sacrificed periodically over the course of 16 months. For the first 8 months both PET and PA plastic increased in mass as they absorbed water. After 16 months of field incubation, PA had degraded by 0.42% and PET was still net positive (higher mass than before the incubation) however it was close to its original weight. The obtained results highlight the lack of degradation to plastics in stormwater pond sediment and suggest lifespans are longer than previously estimated. Based on previous degradation studies under sediment conditions, this study suggests that stormwater pond sediment is the least effective at degradation polymers, which may be attributed to the pond water chemistry and microbial communities present. Microplastics are known to accumulate in stormwater pond sediment but they are found to degrade at slower rates than other sediment profiles. The laboratory experiment results in Chapter 2 show under ideal conditions laboratory-grade PET degrades minimally at low temperatures. Additionally, the lack of degradation seen with the consumer-grade PET in Chapter 2 suggests that under environmental conditions, the polymer would take even longer than the laboratory-grade polymers to degrade. The combination of Chapter 2 and 3 demonstrate the difference between ideal and environmental conditions for polymer degradation. This research provides evidence to strongly advocate for the removal of microplastics before they

enter the environment as I have proven they take considerable lengths of time to degrade under various conditions. I encourage this research to be used by any future researchers who hope to develop methods for plastic pollution reductions.

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## List of Abbreviations

NH <sub>4</sub>	Ammonium
BHET	Bis(hydroxyethyl)terephthalate
C	Carbon
Cl	Chloride
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
EG	Ethylene glycol
FTIR	Fourier-transform infrared
FTC(s)	Freeze-thaw cycle(s)
hcPET	High crystallinity PET
HDPE	High Density Polyethylene
HiC	<i>Humicola insolens</i> cutinase
HCl	Hydrochloric acid
Fe <sup>2+</sup>	Iron (II)
lcPET	Low crystallinity PET
LDPE	Low Density Polyethylene
MDL	Method detection limit
MQ	Milli-Q
MHET	Mono(hydroxyethyl)terephthalate
NO <sub>3</sub> <sup>-</sup>	Nitrate
O	Oxygen
p-NPB	<i>P-nitrophenyl butyrate</i>
PA	Polyamide
PC	Polycarbonate
PE	Polyethylene
PET/PETE	Polyethylene terephthalate
PLA	Polylactic acid
PP	Polypropylene
PS	Polystyrene
pH	Potential of hydrogen
RPM	Revolutions per minute
SEM	Scanning electron microscopy
SBM	Stretch blow molding
SO <sub>4</sub> <sup>2-</sup>	Sulfate
SA	Surface area
TPA	Terephthalic acid
TGA	Thermogravimetric analysis

TOC

Total organic carbon

## List of Symbols

$E_a$	Activation energy ( $\text{kJ mol}^{-1}$ )
$R^2$	Coefficient of determination
$\Delta H_{cc}$	Cold crystallization peak
$\Delta H_m^*$	Crystallization enthalpy
$\rho$	Density
$T_g$	Glass transition temperature
$t_{\frac{1}{2}}$	Half life
$N_o$	Initial quantity of the polymer that decays
$r$	Mass loss rate ( $\text{year}^{-1}$ )
$T_m$	Melting point
$\Delta H_m$	Melting point peak
$A$	Pre-exponential factor
$T$	Temperature ( $^{\circ}\text{C}$ or $\text{K}$ )
$R$	Universal gas constant

# 1. Introduction

Since the beginning of mass-producing plastics which started during the second world war, plastic production has increased exponentially (Thompson et al., 2009), with over 400 million metric tons produced in 2022 (PLASTICS EUROPE, 2023). Plastic was first created in 1862 (Feldman, 2008), as animal-derived materials became scarce, and consumers looked for cheaper alternatives. During the early years, these plastics were used in place of ivory for piano keys and turtle shells for combs (Plastics le Mag, 2022; Hainschwang and Leggio, 2006). However, it was during the 1940's when plastic began to be mass produced (Zhang and Zhang 2021; Ziani et al., 2023), as many precious natural materials were used in the war efforts (Thompson et al., 2009). This marks the existence of single use and disposable plastics. Plastic products, such as synthetic silk, were used in parachutes or rope, Teflon for nuclear interests, and, synthetic rubber and silicone for other needs. After conflict ended, mass production to consumers significantly increased with the popularity of polyester for clothing and polyethylene for containers (Porta, 2021; Kedzierski et al., 2020).

Most plastic is made of carbon and hydrogen atoms bound into hydrocarbons with several other elements that result in different plastic types (Kaushal et al., 2021). Most modern plastics are produced using materials that are by-products of the fossil fuel industry (Tilsted et al., 2023; Geyer et al. 2017). Comparatively, plastics in the 19<sup>th</sup> century were made using natural materials, such as ground wood and gelatin to make purses and brooches, (Boyd, 2011; ACS, 1993) or rubber and sulphur for jewelry and fake teeth (Malik). As a result of using natural materials, plastics made before the 1940's were significantly more degradable and likely bear better resemblance to bioplastics than the traditional petroleum-based plastic seen today (Fredri and Dorigato, 2021; Ahsan et al., 2023).

Microplastics are defined as plastic pieces smaller than 5 mm in size. Their origin can be broken down into primary or secondary microplastics (Campanale et al., 2020). Primary microplastics are produced as microplastics, meaning they measure under 5 mm at time of manufacturing. Secondary microplastics are formed when larger pieces of plastic are degraded through mechanical breakdown or alternative routes, creating smaller plastic pieces (Zhang and Zhang, 2021). Secondary plastics make up the majority of microplastics, with particles derived from water bottles, containers and textiles falling into this category (Usman et al., 2022). As for primary microplastics, many countries have taken the initiative to ban products containing primary microplastics (Mitrano and Wohlleben, 2020; Ghosh et al. 2023). They were commonly found in sunscreen, cosmetics, toothpaste and detergents. Many of these products directly entered wastewater via sinks, washing machines and showers, ending up in the ocean (McDevitt et al., 2017). Comparatively, secondary plastics usually arrive in the ocean, river and lakes in good condition, only to be broken down into microplastics through physical, biological or chemical means (Kye et al., 2023).

As plastic products became disposable and more common, issues regarding storage of plastic waste arose. These concerns are as old as plastic production, increasing every year with increased amounts of plastic found in the environment (Kibria et al., 2023). For a long time, the method of recycling was thought to be a significant strategy to reduce plastic pollution and re-use existing plastic materials (Center for Climate Integrity, 2024; Plastic Wars, 2021; Sullivan, 2020; Sullivan 2022). At peak recycling percentages, only 9% is effectively recycled and re-used (OECD, 2022). Often when plastics are recycled, the quality is reduced each time, until the plastic is deemed unfit for reuse and sent to landfill. No plastic exists in a closed loop system where it can be recycled indefinitely (Hocevar 2020; Noor 2024). Since 2019, waste management systems and the recycling

industry has collapsed leading to significantly lower rates of recycling than previously reported (Ganguly and Chakraborty, 2024; OECD 2022, 3.3.2; Brock 2020; Issifu et al. 2021). In addition, the recycling process is more time, cost and resource demanding (including higher rates of carbon emissions) than the production of new, virgin plastic. In effect, recycling is an inadequate approach to reducing plastic production or pollution despite it being heavily endorsed by plastic manufacturing companies as a solution for decades.

For most plastics, their end of life is found in the environment, either through landfill or illegal dumping (EIA, 2021). Both have drawbacks, as landfills are known to have little biological activity which stalls any degradation of plastic. There are equality concerns, as more wealthy countries routinely send trash to less industrialized countries, where waste disposal laws are less strict or non-existent. This does employ some locals as trash pickers (Franklin-Wallis, 2023) however, these garbage dumps leach toxic chemicals and gas, and decrease air quality (Siddiqua et al., 2022; El-Saadony et al. 2023). A host of negative health effects are seen in populations living close to these unmanaged sites (Njoku et al., 2019).

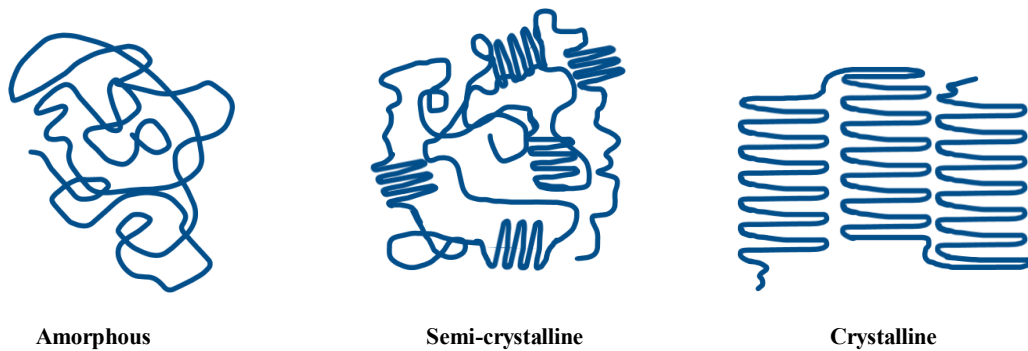
Even removed from landfills, plastic can pose serious health and ecological negative impact. Chemicals that leach from consumer plastic (such as water bottles) (Sharma et al., 2021) can cause endocrine disruption, cancer and changes to hormones leading to reproductive and growth disruption in humans (Ullah et al., 2023; Campanale et al., 2020). Inhalation of micro or nanoplastics in the air can cause cardiovascular issues and reduce lung capacity (Le et al., 2023). For animals, larger plastic particles can cause blockages in the intestine (Sá et al., 2023), while microplastics can cause declined feeding and fertility, and alter growth and development (Jewett et al., 2022; Zhang and Zhang, 2021). Leaching of plastics in the environment can contaminate surrounding soil, plants, and water, including groundwater (de Souza Machado et al., 2018). It's

estimated that 1.7 million tones of plastic enter the ocean each year, mainly through rivers (Ritchie and Roser, 2023) so this is not a dwindling problem.

Plastics have varying lifespans, dependant on plastic type (Chamas et al., 2020), manufacturing processes (Wu et al., 2023), chemical properties (Chamas et al., 2020) and characteristics of the polymer's environment (Colwell et al., 2023). Plastic type has a substantial influence on the length of time degradation will take (Chamas et al., 2020). For example, polypropylene (PP) has a longer estimated lifespan than polystyrene (PS) due to its chemical properties, molecular weight and configuration- which in this case, refers to the manufacturing processes (Mohanani et al., 2020). Manufacturing processes can alter the lifespan by changing the molecular properties. For instance, biaxially orientated processes stretch plastic in both transversal and longitudinal directions, resulting in a molecular chain orientated in two directions which can influence how susceptible a polymer is to enzymes or mechanical degradation (Calafut, 1998). Another common process involves a second heating of the plastic which creates a more durable and thus more degradation-resistant product (Thomas, 2023). General chemical properties such as molecular weight (Madras et al. 1997; Sudduth, 1996), crystallinity (Kumar et al., 2021) and melting point ( $T_m$ ) (Tokiwa and Calabria, 2015) can have a large impact on the degradation rate of a polymer. Decreases in molecular weight lead to increased mobility of the polymer chain and a declined glass transition temperature ( $T_g$ ) (Thomas et al., 2023; Asgreen et al. 2020). The  $T_g$  is the temperature needed for the polymer to change from a solid/glassy state to soft state (Buschow, 2001; E Alfredo Campo, 2008). The  $T_g$  directly affects the polymer's strength, durability and, thus, degradation resistance (Kinloch and Young, 1995; Kobayashi et al., 2021). Crystallinity is a factor that largely controls the  $T_g$  of a polymer (Askadskii et al. 2013). Based on this host of factors, it becomes clear that not all plastic is made equally and in fact, even plastic of the same type, such as PET, differs vastly in

its chemical properties and characteristics. This issue is largely a result of manufacturing companies continuously updating polymer formulas and production methods to create better consumer plastics. As a result, consumer and laboratory-grade plastic used in research have very different makeup and production methods. Additionally, there has been minimal research on how these updated formulas and methods alter degradation rates of plastics or how they compare to laboratory-grade polymers.

Plastics are made of amorphous and crystalline regions, similar to building blocks. Crystalline regions contain molecular chains that are organized and arranged in an ordered way. Amorphous regions have molecular chains held together loosely with more space between them, whose order is not structured, rather it is random and unorganized (Han et al., 2013; Kinloch and Young, 1995). As a result, amorphous regions are much easier to degrade compared to crystalline regions, whose chains are closer and better ordered (Yoo and Im, 1999; Kumar et al., 2021).



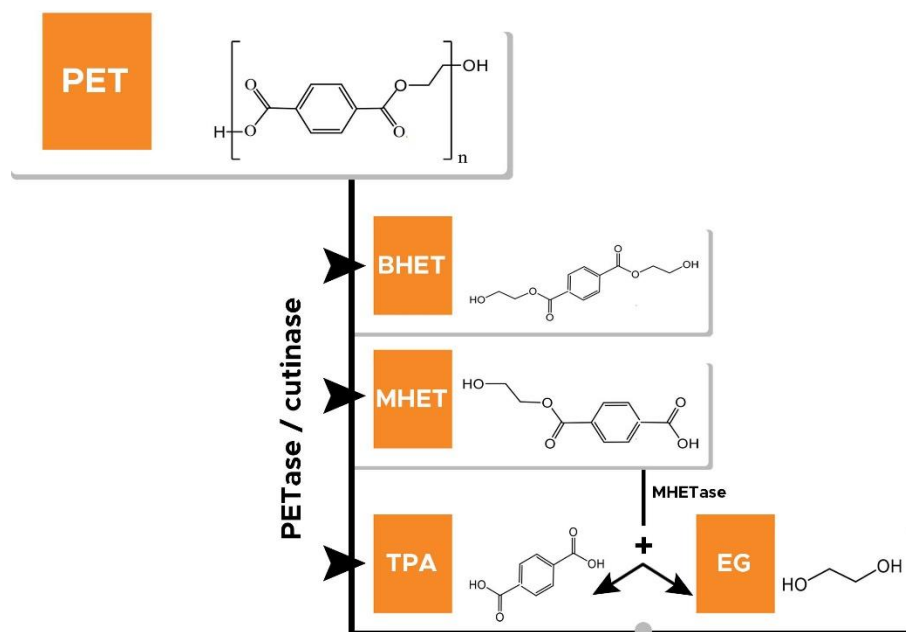
**Figure 1-1.** Polymer chain structure of amorphous, semi-crystalline and crystalline plastics.

Plastics can be sorted into two categories: amorphous or semi-crystalline polymers. Crystalline polymers contain amorphous areas, resulting in the name of semi-crystalline (Sysel, 2016). Common amorphous plastics include polystyrene (PS) and polycarbonate (PC) while common



semi-crystalline plastics can be polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET). The plastic type will dictate the degree (or rather the percent) of crystallinity (Lin et al., 2020). Regardless of plastic type, a fully crystalline plastics do not exist. High crystallinity makes a plastic stronger but more brittle, as such, amorphous regions are needed for the plastic to bend (Yuan and Xu, 2023). Semi-crystalline polymers possess a melting point ( $T_m$ ) where above that temperature, the arrangement of chains transition from an organized to disorganized state. Contrary to amorphous plastics, which have a  $T_g$ .

This study distinctly focuses on two commonly produced plastics; polyethylene terephthalate (PET) and polyamide (PA). Both are semi-crystalline polymers, with varying crystallinity percentages. PET has an estimated lifespan of 450 years in the environment with crystallinity levels of most PET polymers ranging from 0-50% (Kaushal et al., 2021) compared to PA, which has a shorter estimated lifespan (Bakošová, 2020) due to lower crystallinity percentages. PET when degraded via hydrolysis, breaks down into bis(hydroxyethyl)terephthalate (BHET), mono(hydroxyethyl)terephthalate (MHET), terephthalic acid (TPA), and ethylene glycol (EG) (Maurya et al., 2020). Many enzymes have been detected to degrade plastics, for example, enzymes that can hydrolyze PET include PETase, MHETase, cutinase (cutinase HiC from *Humicola insolens* and lipase CALB from *Candida antarctica*) (Mohan et al., 2020). Degradation occurs when the enzyme hydrolyzes the ester bond of PET (Yang et al., 2023). *Humicola insolens* enzyme (HiC) is a model enzyme due to its Ser-His-Asp catalytic triad (customary for the  $\alpha/\beta$  hydrolase superfamily) and is commonly produced by the thermophilic fungus in the environment (Arya and Cohen, 2022). This enzyme was chosen based on its previously known ability to degrade PET in literature and ease of acquiring the enzyme.

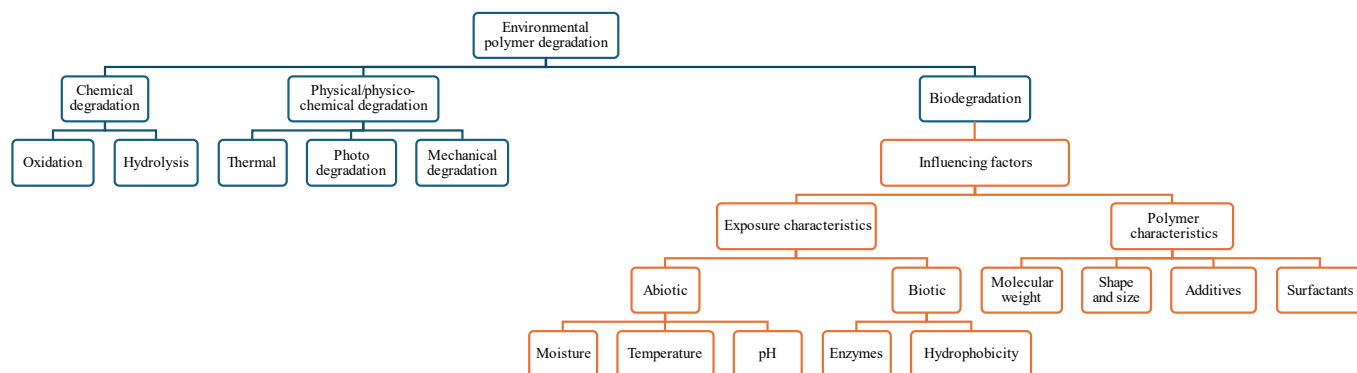


**Figure 1-2.** Chemical structure of polyethylene terephthalate (PET), and breakdown products of PET including into bis(hydroxyethyl)terephthalate (BHET), mono(hydroxyethyl)terephthalate (MHET), terephthalic acid (TPA), and ethylene glycol (EG) (Maurya et al., 2020).

The degradation of polymers can occur through three different pathways: chemical (oxidation, hydrolysis), physical (thermal, photo, mechanical) or biodegradation (Findrik Balogová et al., 2021). The presence of sunlight (photodegradation) or lack of UV light can alone alter the rate of degradation. A study using high density polyethylene investigated the rate of degradation in direct sunlight compared to plastic buried under soil and found a mass loss of 22.7% in direct sunlight after 30 days compared to less than a 1% mass loss for plastic buried in soil for the same duration (Muthukumar et al., 2010). Mechanical degradation often occurs in the ocean, where plastics collide with rocks and are agitated by waves, resulting in plastic breaking down into smaller pieces (Wayman and Niemann, 2021). Other environmental conditions, such as pH and temperature, which fall under exposure characteristics, can also play a role in polymer degradation (Kaushal et

al., 2021; Mušič and Škapin, 2023). Because of many routes of degradation, estimated lifespans for plastics are strongly associated with environmental conditions and thus degradation rates presented in literature may not be relevant.

Biodegradation includes biotic characteristics, such as enzymes and microbes, some of which can degrade polymers (Kaushal et al., 2021). A significant amount of research has been conducted on identifying microbes that can use plastic as a carbon (energy) source. Many species of microbes have been identified, most found in the *Pseudomonas*, *Escherichia*, and *Bacillus* genera (Cai et al., 2023). Most microbes are specific to the type of plastic they can degrade, similar to enzymes in that sense. The microbes present in an environment are dependant on location and other elements and contaminants present. In this thesis, a study was conducted to determine the rate of polymer degradation in stormwater pond sediment as these stormwater ponds are becoming a more popular method of managing wastewater. Previous research has shown that microplastics accumulate in the sediment and these ponds are a contained area to test polymer degradation under environmentally relevant conditions.



**Figure 1-3.** Mechanisms of polymer degradation and factors affecting biodegradation.

There is a multitude of environmental conditions which have been previously studied for their effect on polymer degradation, however, these studies differ vastly and have resulted in a patchwork web of research that is difficult to piece together. Some studies do not provide enough information on the polymer, do not list degradation rates or utilize multiple analyses, and many use different polymers. The field of plastic and thus microplastic research have very few standards regarding methods and analyses used. One of the goals of the research presented in this thesis was to present factors which affected the degradation of the polymer. For example, it is well known that higher temperatures result in higher degradation but the increased rate of degradation is unknown for many polymers. Through this research, it was determined that characterizing the traits of the polymers used would be vital for this research to be comparable due to the differences in plastic formula and production methods. Thus, multiple analyses were conducted to identify the features of the polymer and identify which analyses best visualized degradation. As a result, this research can be used to determine which analyses are best suited to capture specific traits of degradation.

## **1.2 Thesis objectives**

The main objective of this thesis was to investigate the degradation rate of two common plastics (PET and PA) and factors which influence their degradability. The combination of Chapters 2 and 3 provide a comprehensive analysis of plastic degradation in environment and laboratory settings. Two additional objectives included: determining the difference between laboratory-grade and consumer-grade PET, and, characterising the polymers used to help create standards within the field of plastic research, such that the results could be accurately compared to previous studies.

This was performed using a series of controlled experiments in Chapter 2 with polyethylene terephthalate (PET) and the use of a cutinase enzyme to determine the influence of temperature and pretreatments on degradation. Several different types of commercial PET were tested to identify if there were any differences between laboratory grade and commercial PET. The overall objective for Chapter 3 was to determine the degradation rate of PET and polyamide (PA) in stormwater pond sediment over 16 months. Stormwater ponds are a great test site because they receive plastic pollution from surrounding areas and are a contained environment. This study was conducted with the use of pore water peepers, and sediment pore water chemistry was collected through the duration of the experiment.

### **1.3 Thesis Outline**

This thesis is comprised of four chapters, including the current chapter that outlines relevant information regarding plastic pollution, and factors affecting degradation. Chapter 2 comprises of a series of controlled experiments determining the factors affecting the degradation of PET, using HiC as a hydrolyzing enzyme. Plastic pieces were incubated with HiC at varying temperatures to determine an activation energy. Pretreatment to the plastic and plastic type were investigated to determine the effects of each. The polymers used in this experiment were well characterized. Chapter 3 consists of an in-situ, 16 months experiment where PET and PA were placed in pore water samplers with pond sediment before installation into stormwater pond sediment. Samplers were sacrificed at a series of timepoints to determine the rate of degradation over 16 months. Pore water chemistry was collected to determine if any abnormal elements or sediment zones were present. Chapter 4 summarizes the research findings, links both laboratory and in field experiments to the current global situation of plastic pollution and suggests future research ventures. This is

followed by references and Appendices I and II that provide additional figures of experimental results that were not included in Chapters 2 and 3.

## **2. Effects of environmentally relevant treatments on the enzymatic degradation of polyethylene terephthalate (PET)**

### **2.1 Introduction**

Reliance and production of plastic have been exponentially expanding in the past several decades leading to increasing amounts of plastic pollution found in the environment (Thompson et al., 2009; Morrison et al., 2022). Polyethylene terephthalate (PET) is one of the most produced polymers worldwide (Kalathil et al., 2022). PET is a lightweight, clear, and strong plastic commonly used for food packaging and fibers in clothing. PET has a high temperature resistance resulting in a high resistance to degradation, especially in the environment (Webb et al., 2021; Thomson et al., 2022). Many studies have investigated the influence of commercially produced enzymes under laboratory relevant conditions in efforts to speed up PET degradation as a method for recycling PET. Very little research has been conducted under environmentally relevant conditions. While the results presented in this chapter are considerably more dramatic than results expected in the environment, we present this research to show the high rate of degradation possible at each condition and prove each condition does have an impact on degradation rates.

Several properties of plastics determine their lifespan, or potential degradability. In the case of PET, higher crystallinity reduces the potential for enzymatic degradation because hydrolytic enzymes preferentially degrade amorphous regions of plastic. Most PET typically ranges from 30 to 50% crystallinity (semi-crystalline), increasing its environmental lifespan. Other plastics such as polystyrene (PS) and polyvinyl chloride (PVC) have 0% crystallinity, resulting in much shorter lifespans (Mohanani et al., 2020). Plastic manufacturers create plastics of the same polymer type

using different methods to increase their lifespans, resulting in plastics of the same polymer type with different crystallinity, configuration, molecular weight and additives and therefore with a different degradability potential (Calafut, 1998; De Vries, 2016).

One of the major environmental variables controlling enzymatic degradation rates is temperature (Zandieh et al., 2023). Fluctuations in temperature can impact enzymatic plastic degradation rates in two ways – (1) via the impact of incubation temperature on enzymatic degradation rates (Maurya et al., 2020), whereby enzyme degradation rates increase exponentially with temperature until some maximum temperature threshold, after which point enzyme activity and degradation rates decrease (Daniel et al., 2007), and (2) via the impact of exposure to temperature fluctuations and/or extreme temperatures on the physical and chemical properties of the plastic itself, and therefore on its degradability. Examples of everyday exposures of plastics to extremes or variations in temperature before they reach the environment include repeated freezing for food packaging, freezing and thawing of recycling bins and other outdoor plastics such as any building materials containing PET or most asphalt, which contains plastic (Niu et al., 2021; Hussain et al., 2023). Once plastics reach the environment, whether as macro-, micro- or nano-plastics, they may also be exposed to fluctuations in freezing and thawing temperatures and other temperature extremes in soils and other environmental compartments (Koutnik et al., 2022). Very few studies investigating the effects of exposure to freeze-thaw cycles have been conducted, with the majority analyzing the effect of exposure on the durability of materials such as PVC water pipes, concrete or clay materials, as these are the materials which are most often exposed to extremes and/or fluctuations in temperature (Gio et al., 2022; Zhand et al., 2022; Ma et al., 2022).

Environmental pollution caused by plastic mostly consists of consumer-grade plastic and industry-manufacturing waste products (Napper and Thompson, 2020). Consumer-grade plastic is defined



as plastic created and distributed for consumer use, often containing additives such as dyes and stabilizers. These plastics include plastic water bottles, food containers, and plastic bags. We also see plastic pollution from industrial use, such as plastic shavings, or byproducts from plastic items (White and Winchester, 2023). Most of the research conducted on plastic degradation using enzymes or other degradation methods use laboratory-grade plastic provided by manufacturers, as it is considered free of contamination and contains fewer additives. However, minimal laboratory-grade plastic is found in the environment, as it is produced mainly for research purposes. In fact, the majority of studies only state when they use plastics retrieved from the environment, rather than explicitly stating they are using laboratory-grade plastic. This makes it difficult to sort which literature is using consumer and laboratory-grade plastics, creating a gap in research that is largely ignored.

This research aims to address several gaps in literature regarding plastic degradation. The effects of temperature on degradation rates are a well-known mechanism (Maurya et al., 2020) however reported results are often difficult to compare or lacking information about the characteristics of the plastic used. When we investigate pretreatments, such as freeze thaw cycles, we aim to observe how plastics in the environment change after years with changing seasons. And finally, we investigate the differences in laboratory-grade and consumer-grade plastic when degrading, which has large implications on degradation in the environment. These experiments were done under extreme conditions to produce a significant result and prove changes would occur, however under less extreme conditions, which would replicate environmental conditions better, results would be less drastic and would take considerably more time to produce.

## 2.2 Materials and Methods

### 2.2.1 Materials

The enzyme *Humicola insolens* cutinase (HiC) was used as a model enzyme to stimulate enzymatic PET degradation in the experimental incubations. HiC is produced by a thermophilic fungus, *Humicola insolens* (Kawai, 2021), to degrade cellulose and hemicellulose in the environment. Given HiC's high optimal temperature (50-60 °C), wide pH range adaptation and reasonable thermostability, it is used as an industrial enzyme for degrading plastics with hydrolytic bonds such as PET (Xu et al., 2019; Lui et al., 2021). The HiC stock (Novozym® 51032) was purchased from Strem Chemicals (Newburyport, MA, USA). The manufacturer indicates that the enzymatic activity of the stock is 15 kilolipase unit per gram (KLU g<sup>-1</sup>). The phosphate buffer (sodium phosphate dibasic dihydrate) was purchased from Sigma-Aldrich (Oakville, ON, Canada).

Laboratory-grade 0.20 mm thick, colourless PET sheets were purchased from Delta Scientific Laboratory Products Ltd (Mississauga, ON, Canada). The PET sheets were cut into 1 cm x 1 cm squares, rinsed with 70% ethanol and left to air dry before use. Each sample was weighed using an analytical balance (Mettler Toledo XS205 DualRange) having a precision of ± 0.02 mg before the experiment began.

Sacrificial incubation experiments were set up for each experimental condition. In addition to the treatment of PET exposed to HiC in a pH 8 phosphate buffer (PET + HiC), each batch of sacrificial incubations had 2 controls: i) PET in pH 8 phosphate buffer with no HiC added (PET only), and ii) incubations of the pH 8 phosphate buffer and HiC solution with no PET added (HiC only). A phosphate buffer made with disodium hydrogen phosphate dihydrate salt was used to maintain the pH at a value of 8 for all the experiments. Phosphate was chosen as the buffer solution rather than

an organic buffer as organic buffers can create coatings on the plastics and/or inhibit hydrolytic activity (Schmidt et al., 2016). A pH of 8 was chosen as it is within the optimal range for HiC activity (Bisceglie et al., 2022).

The incubations were conducted in 15 mL polypropylene centrifuge tubes. The starting volume of solution in each tube was 3 ml of 1:100 (1.215 - 1.842 mg or 0.405 – 0.614 mg mL<sup>-1</sup> of enzyme protein) HiC in phosphate buffer solution, with additions of 0.3 mL of freshly made 1:10 (4.05 – 6.14 mg mL<sup>-1</sup> protein concentration) HiC in phosphate buffer solution (PET + HiC and HiC only conditions) occurring every 2 days to refresh the incubations with new HiC to maintain hydrolytic activity. Phosphate buffer additions of 0.3 mL were also added every 2 days to the PET incubated in the buffer with no HiC added (PET only) to keep the time outside the incubator and total solution volumes consistent.

All incubations were conducted in temperature-controlled incubators (Percival I-41NL XC9 incubator or MyTemp™ mini digital incubator) to control the temperature at the target temperature of that incubation. Temperature loggers were installed in the incubators to monitor temperature and showed that the temperature did not fluctuate beyond the set temperature.

At each sampling point, for each laboratory experiment, triplicate incubations were sacrificed for each condition. For experiments lasting more than 4 weeks, the tubes were centrifuged at 4400 RPM (1388 g) for 1 hour every second sampling point to prevent the inhibition of enzyme activity by the accumulation of hydrolysis breakdown products in solution. The liquid supernatant was poured off, leaving the PET sample in the tube. Each tube was then topped up with fresh 1:100 HiC in phosphate buffer solution (PET + HiC and HiC only conditions) or buffer solution (PET only). The tubes were then returned to the temperature-controlled incubator. For experiments lasting for 4 weeks or less, no centrifugation was undertaken.

### *2.2.2 Experimental conditions*

To investigate the effect of temperature on HiC-induced PET degradation rates, incubations were done at 3 different temperatures: 25 °C, 40 °C and 55 °C. The 40 °C incubation lasted for 16 weeks with sampling every 2 weeks, 25 °C for 16 weeks with sampling every 3 weeks, and 55 °C for 10 days with sampling every 2 days.

To investigate the effect of exposure of the PET to extreme or fluctuating temperatures on the PET degradation rate, the following pre-treatments were used: exposure of dry PET squares for 6, 16, 26, 75 and 100 freeze-thaw cycles (FTCs) where temperature fluctuated between 40 °C and -12 °C every 6 hours, and exposure of dry PET samples to the extreme temperatures of 55 °C or -70 °C for 1 week. All pretreatments were done under dry conditions (no solution including buffer or HiC was involved in this step). Following the exposure to the pre-treatments, the PET squares were incubated with HiC in a temperature-controlled incubator at 40 °C (following the incubation experiment design described in section 2.2.1) for 4 weeks to assess the impact of the pre-treatments on the PET degradation rate.

To compare the degradability of the laboratory-grade PET used for the temperature and pre-treatment experiments with that of consumer-grade PET, two consumer-grade PET water bottle products were cut into squares and incubated with HiC at 55 °C following the incubation experiment design described in section 2.2.1. The two water bottles used are differentiated by their colour – blue and clear. The blue bottle PET was obtained from 4 L water bottles. The bottle label indicated that this PET was made from 100% recycled plastic. The water was drained from the bottle and flat pieces of the bottle were cut into 1 cm by 1 cm squares for this experiment. The plastic triplicates were cleaned with 70% ethanol and air dried overnight before being incubated with HiC. Plastic samples were immersed in a small beaker of ethanol at room temperature for

approximately 10 seconds, swirling the beaker before flat head tweezers were used to remove the plastic. The clear bottle PET was obtained from 1.5 L clear transparent water bottles. The bottle label gave no indication of whether it was recycled plastic, and a PETE 1 recycle symbol was found on the bottom of the bottle. The same process was used to prepare these PET pieces for incubation with HiC.

Before any experiments were conducted, several chemical washes including Tween 0.01%, ethanol 70%, MQ and combinations of each were tested on laboratory-grade PET to determine if they altered the untreated plastic. We observed minimal changes to the plastic surface using Fourier-transform infrared spectroscopy (FTIR) and literature review showed many previous studies have used ethanol or Tween to wash plastics without any changes to the plastic morphology (Zandieh et al., 2023). As such, all sacrificed PET samples were washed with 0.01% Tween surfactant and then MQ water after being sacrificed. The Tween surfactant was used to ensure that the HiC was washed off the PET pieces. After the washing procedure, the samples were left to air dry before being analyzed.

## 2.3 Analyses

Once the triplicates of PET squares were air dried, they were weighed using a balance (Mettler Toledo XS205 DualRange, precision:  $\pm 0.02$  mg) to determine changes in PET square weights over time. Each data point represents the average of the triplicates for said timepoint.

Mass loss at each time point was calculated by dividing the difference in mass by the initial mass:

$$\text{Mass loss (\%)} = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} * 100 \quad (\text{Eq. 1})$$

Mass-normalized mass loss rates ( $\text{year}^{-1}$ ) were calculated using Eq. 2:

$$\text{Mass loss rate (year}^{-1}\text{)} = \frac{\frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}}}{\text{Length of incubation (years)}} \quad (\text{Eq. 2})$$

In addition, surface area-normalized mass loss rates were calculated using Eq. 3:

$$\text{Mass loss rate } (\mu\text{m year}^{-1}\text{)} = \frac{\frac{\text{Initial mass} - \text{Final mass}}{\rho \cdot \text{SA}}}{\text{Length of incubation (years)}} \quad (\text{Eq. 3})$$

Where  $\rho$  is the density of the PET ( $1.2 \text{ g cm}^{-3}$ ) and SA is the surface area of the PET ( $2.1 \text{ cm}^2$ ).

Dissolved organic carbon (DOC) was measured to determine the buildup of degradation products in solution using the nonpurgeable organic carbon method on a total organic analyzer (Shimadzu TOC-LCPH/CPN, method detection limit = 0.03 mM).

Additionally, HiC activity was monitored via enzyme assays using *p*-nitrophenyl butyrate (*p*-NPB) throughout the temperature experiments. A rapid assay of HiC activity was performed using *p*-NPB, as PET can act upon it (Hong et al. 2019; Pfeffer, 2019). The buffer and the solution for the incubated tubes containing PET were pipetted into a standard clear plastic 96-well plate and the substrate was added. Each well received 140  $\mu\text{L}$  of MQ, 30  $\mu\text{L}$  of solution from the incubated tube (PET + HiC and HiC only conditions contained the enzyme) and 30  $\mu\text{L}$  of the substrate *p*-NPB. The concentration of *p*-NPB stock was 14.2 mM in ethanol. Control wells had an additional 30  $\mu\text{L}$  of MQ added, as they did not receive solution from the incubated PET tubes. The pH for each assay was approximately 8, with some variation of  $\pm 0.2$  pH units. Please reference Pfeffer (2019) for an estimated extinction coefficient. Absorbance measurements of the substrate *p*-NPB at 405 nm was used to measure the HiC activity using a microplate reader (FlexStation 3 Multi-Mode Microplate Reader, Molecular Devices).

Fourier transform infrared spectroscopy (FTIR) was performed on the PET samples with instruments found in Rodney Smith's laboratory, Chemistry Department, University of Waterloo. Spectra were recorded using the Thermo Fischer Nicolet 6700 FTIR spectrometer. Parameters included a resolution of  $4\text{ cm}^{-1}$  for 64 scans.

Scanning electron microscope (SEM) images of plastic samples were taken of select samples to view surface morphology differences caused by HiC degrading amorphous regions of the plastic. These images were produced from the Canadian Centre for Electron Microscopy (CCEM) at McMaster University, under the supervision of Christopher Butcher. For SEM imaging, samples were dried with a vacuum oven (VWR 1370-FM) for 1 week at  $40\text{ }^{\circ}\text{C}$  before analysis. SEM imaging was done with a FEI Magellan 400 at varying magnification.

Tensile strength testing was performed using an Instron 5548 micro tester at 70 psi for all the laboratory-grade PET samples. This was done at the University of Waterloo, Department of Mechanical and Mechatronics, by Kaiping Zhang. A pressure of 90 psi was used for analyzing the consumer-grade PET as the consumer-grade PET was thinner than the laboratory-grade PET. The 1 cm by 1 cm PET squares used in the incubations were cut into rectangular strips measuring 5 mm by 10 mm. A 3 mm length was used for testing tensile strength.

Thermogravimetric analysis (TGA) was used to measure the thermal stability of the PET samples before they could be analyzed using the DSC protocol. This was performed with nitrogen gas and blanks were heated to  $350\text{ }^{\circ}\text{C}$  employing a TA instruments Q500 calorimeter. Samples for this test were dried in a vacuum oven for 1 day at  $70\text{ }^{\circ}\text{C}$  before analysis.

Differential scanning calorimetry (DSC) was performed on both control PET squares not exposed to HiC but exposed to the given incubation temperature (PET only) and those exposed to HiC

(PET + HiC). DSC was conducted under nitrogen gas. The heating and cooling protocol for the DSC analysis was set to 300 °C, cool to 30 °C and heat to 300 °C. Samples were dried at 70 °C with a vacuum oven for 1 day before DSC analysis (TA instruments Q2000 with RCS90). Sample weights ranged from 3-10 mg due to sample availability and stage of degradation. Both TGA and DSC were done in the Department of Chemical Engineering, at the University of Waterloo, under the supervision of Charles Dal Castel.

The crystallinity percentage for laboratory-grade plastic samples was calculated using the melting point peak ( $\Delta H_m$ ) and the cold crystallization peak ( $\Delta H_{cc}$ ) in equation 4 (Doumeng et al., 2021). The crystallization enthalpy ( $\Delta H_m^*$ ) for an ideal 100% crystalline PET sample is 140 J g<sup>-1</sup> (Blaine).

$$\% \text{ crystallinity} = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^*} \quad (\text{Eq. 4})$$

Both of the consumer-grade PET types (blue and clear water bottle PET) did not have a cold crystallization peak. Hence, the crystallinity percentage was calculated using the hot crystallization peak instead.

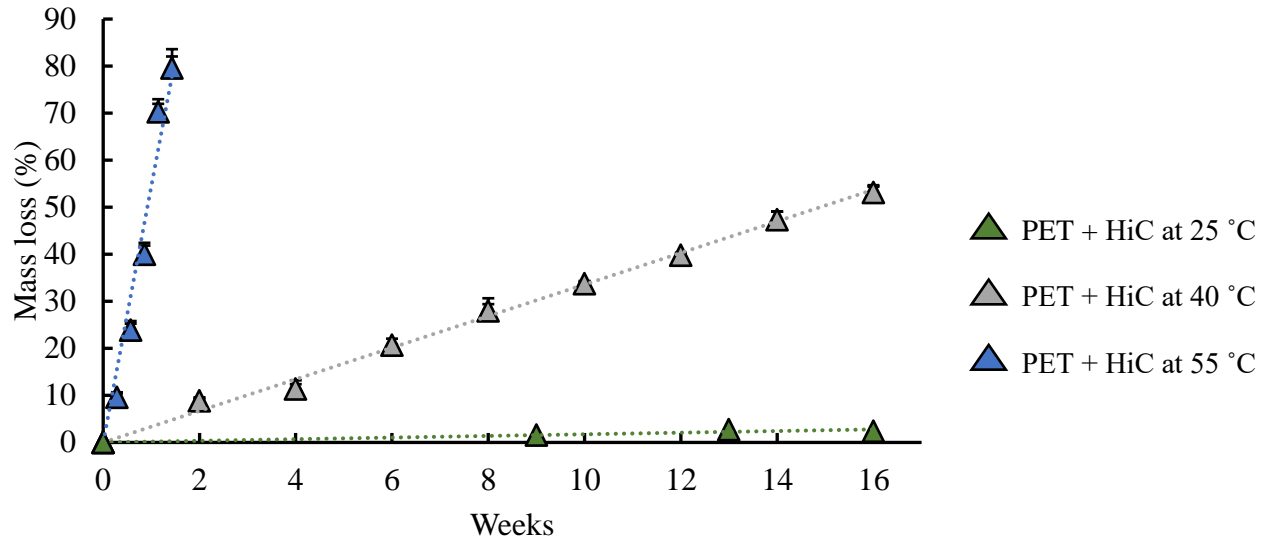
## 2.4 Results

### 2.4.1 Mass loss rates: effect of temperature

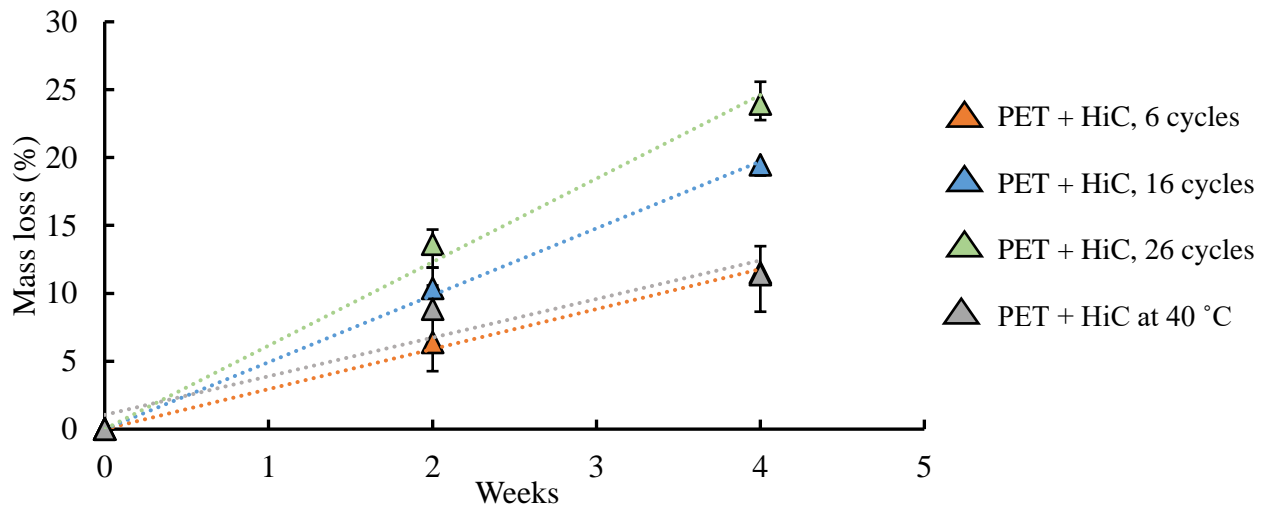
Mass loss for temperature experiments was found to have a large variation depending on incubation temperature. The PET-only condition was used as a control for the PET + HiC treatment, results listed here have been averaged from the triplicates sacrificed at each timepoint. For the 25 °C PET-only incubation, a slight weight gain of around 1% was observed in later weeks, around week 12.



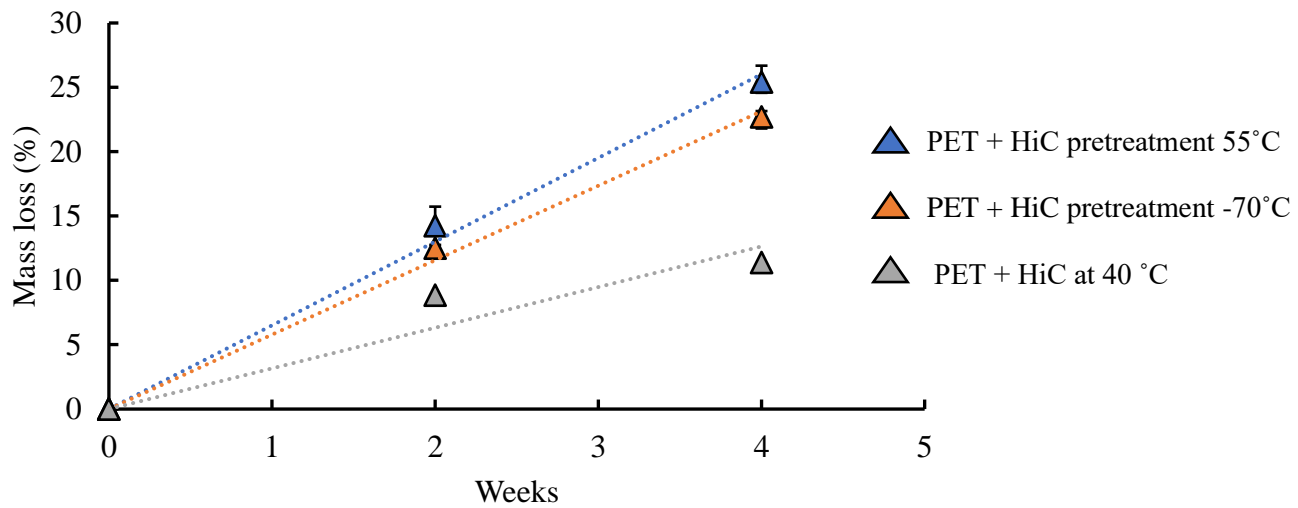
A)



B)



C)



**Figure 2-1.** A) Mass loss in percentage for temperature experiments at 25 °C (green), 40 °C (grey) and 55 °C (blue) PET + HiC condition. B) Mass loss in percent for FTC pretreatments at 6 (orange), 16 (blue) and 26 (green) cycles. Grey data in B and C is from the original baseline 40 °C temperature experiment. C) Mass loss in percent for extreme temperature pretreatments of 55 °C (blue) and -70 °C (orange). For all experiments, PET-only condition samples were used as a control for the experimental condition, PET + HiC.

Given the incubation durations of 16 weeks, 16 weeks and 10 days for the incubation temperatures of 25 °C, 40 °C and 55 °C respectively, this corresponded to total mass losses observed of 2.4%, 50%, and 80%, respectively (Figure 2-1A). PET mass loss rates in the PET + HiC incubations increased with increasing incubation temperature, increasing from 0.08 to 1.6 to 29 year<sup>-1</sup> at the incubation temperatures of 25 °C, 40 °C, and 55 °C, respectively (Table 2-1). In addition to the measurements of PET mass loss rates with time, we also measured the accumulation of dissolved organic carbon (DOC) in solution with time in the incubations for the temperature experiments conducted at 55 and 40 °C. DOC accumulation exhibited upward trends with time in both the 55 °C and 40 °C incubations (Figures AI-1 and AI-2).

**Table 2-1.** Mass loss results reported by different units and rate. Water bottle experiment mass loss rates are not included due to the lack of significant mass loss.

Experiment	Condition	Mass loss (%)	Half-life (weeks)	Surface area-normalized mass loss rate ( $\mu\text{m year}^{-1}$ )	Mass-normalized mass loss rate ( $\text{year}^{-1}$ )
Temperature	25 °C for 16 weeks, PET + HiC	2.4	335	8.6	0.08
Temperature	40 °C for 16 weeks, PET + HiC	50	15	215	1.7
Temperature	55 °C for 10 days, PET + HiC	80	0.9	3196	29
FTC pretreatment	FTC (26 C), incubated at 40 °C for 4 weeks, PET + HiC	23.8	8.4	414	3.1
Extreme temperature pretreatment	Extreme (-70 °C, incubated at 40 °C for 4 weeks, PET + HiC	22.7	8.8	357	3
Extreme temperature pretreatment	Extreme (55 °C), incubated at 40 °C for 4 weeks, PET + HiC	25.4	7.9	407	1.3

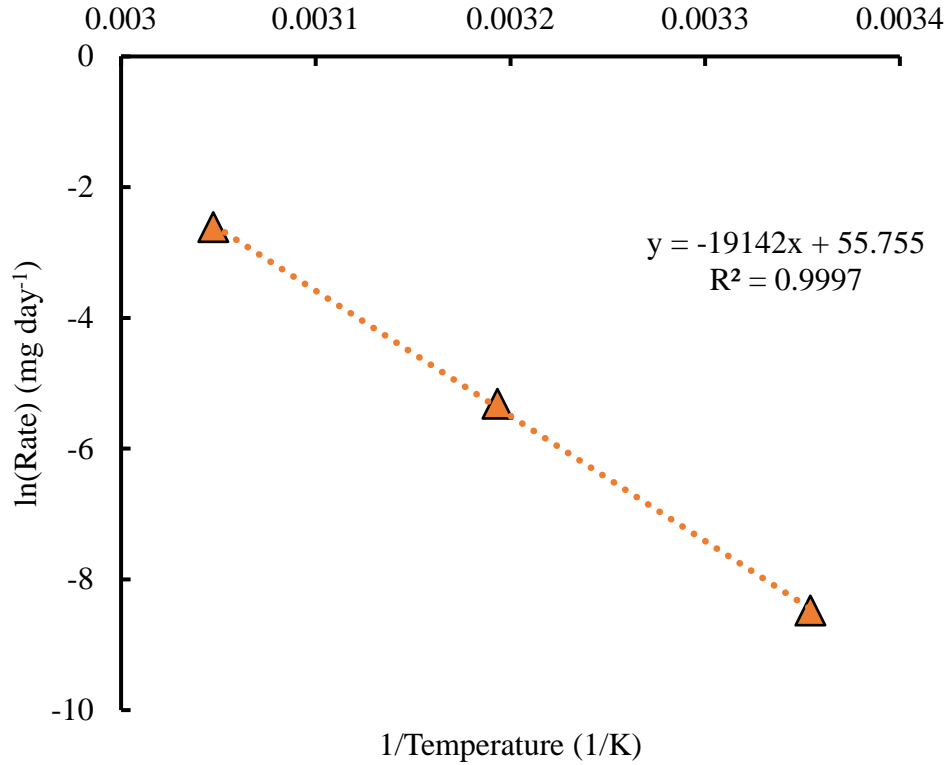
Using Table 2-1 we can estimate the amount of degradation expected per year under ideal laboratory conditions for each temperature and pretreatment. For example, under ideal conditions at 40 °C, we expect to see 0.215 mm of plastic degraded per year. At 25 °C, this number drops to 0.08 mm per year under ideal conditions, with HiC in abundance. While these experiments do not have a direct implication on plastics in the environment, there remains an indirect implication. Even under ideal laboratory conditions, at low temperatures such as 25 °C, a polymer such as PET only degrades 0.008 mm per year. In turn, under environmental conditions without the abundance of enzymes known to degrade PET and low temperatures, a similar plastic will degrade far slower.

To evaluate and compare the temperature sensitivity of the mass loss rates observed with those observed for hydrolysis in other studies, we calculated the activation energy by fitting the Arrhenius equation to the mass loss rates observed at the three incubation temperatures (Figure 2-2):

$$r = Ae^{-\frac{E_a}{RT}} \quad (\text{Eq. 5})$$

Where,  $r$  is the mass loss rate ( $\text{year}^{-1}$ ),  $A$  is the pre-exponential factor,  $E_a$  is the activation energy ( $\text{kJ mol}^{-1}$ ),  $R$  is the universal gas constant, and  $T$  is the incubation temperature ( $^{\circ}\text{C}$ ).

The calculated activation energy was  $153 \text{ kJ mol}^{-1}$ . Reported results in the literature using similar degradation assays resulted in activation energy values under  $100 \text{ kJ mol}^{-1}$  (Sabde et al., 2023; Eugenio et al., 2021). Previous studies have utilized different enzymes, plastic types and crystallinities, and have conducted experiments under different conditions which is likely the cause of the difference between the activation energy calculated and those reported in the literature.



**Figure 2-2.** Temperature and degradation rate using data from all three temperature experiments (at 25 °C, 40 °C and 55 °C) is shown.

Half-life was calculated using the following equation;

$$t_{\frac{1}{2}} = \frac{N_o}{2R} \quad (\text{Eq. 6})$$

At varying temperatures, half-life can be estimated at 1 week, 3.5 months and 6 years for 55 °C, 40 °C and 25 °C, respectively.

#### 2.4.2 Mass loss rates: effect of pre-treatments

Mass loss rates during incubation with HiC at 40 °C for 4 weeks were higher for PET that was exposed to both the FTC or extreme temperature pre-treatments compared to the PET not exposed to the pre-treatments (Figure 2-1). The mass loss rate observed for the PET exposed to 6 FTCs was

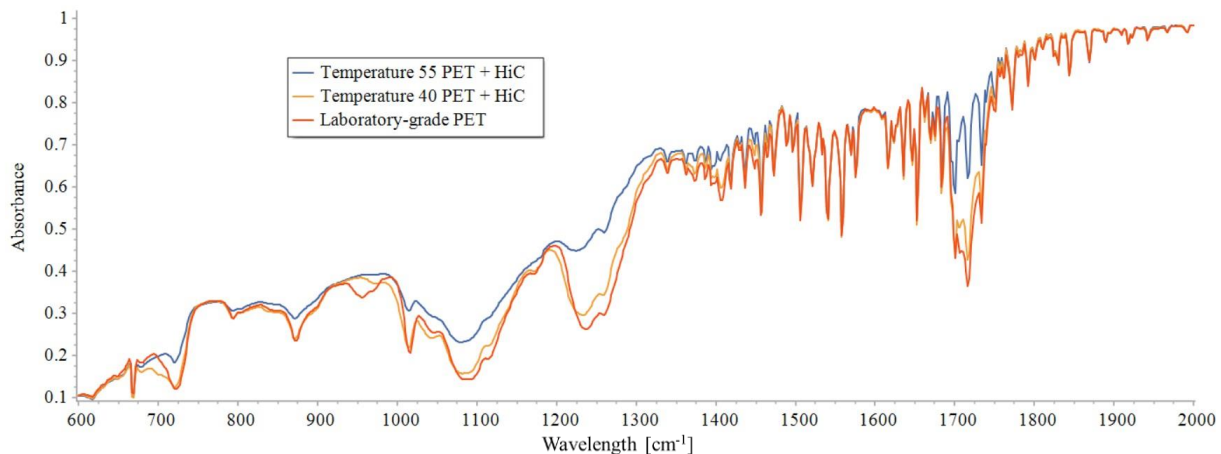
not substantially different relative to the PET not exposed to FTCs. Mass loss rates increased, relative to the PET not exposed to FTCs, with an increasing number of FTCs between 16 and 26 FTCs, increasing from 10% over 4 weeks with no exposure to FTCs to 20% with exposure to 16 FTCs to 23% with exposure to 26 FTCs. There was minimal increase in the mass loss rate with increased exposure to 75 and 100 FTCs compared to that observed for exposure to 26 FTCs.

Mass loss rates during incubation with HiC were also higher for PET that was exposed to extreme temperatures. Exposure to -70 °C for 1 week increased the 4-week mass loss observed from 11% (for the untreated PET) to 23%. Similarly, exposure of the PET to 55 °C for 1 week increased the 4 week mass loss (during exposure to HiC) observed to 25%. These corresponded to mass loss rates of 3.0 and 3.3 year<sup>-1</sup> for the PET exposed (prior to exposure to HiC) to -70 °C and 55 °C, respectively, which are 2.1-times and 2.3-times higher, respectively, than the HiC-induced mass loss rate (1.3 year<sup>-1</sup>) of the untreated PET (Table 2-1).

#### *2.4.3 Mass loss rates: effect of plastic source*

After 10 weeks of enzymatic (HiC) exposure at 55 °C, both consumer-grade plastic water bottle samples showed no mass loss. For plastic type, we observed that laboratory-grade plastics degrade far easier than consumer-grade plastics. This is a reoccurring theme presented throughout these results, as consumer-grade PET proved to be stronger and more durable than laboratory-grade plastic. These results are concerning amidst current efforts to decrease plastic pollution in the environment.

#### *2.4.4 Fourier transform infrared spectroscopy (FTIR)*

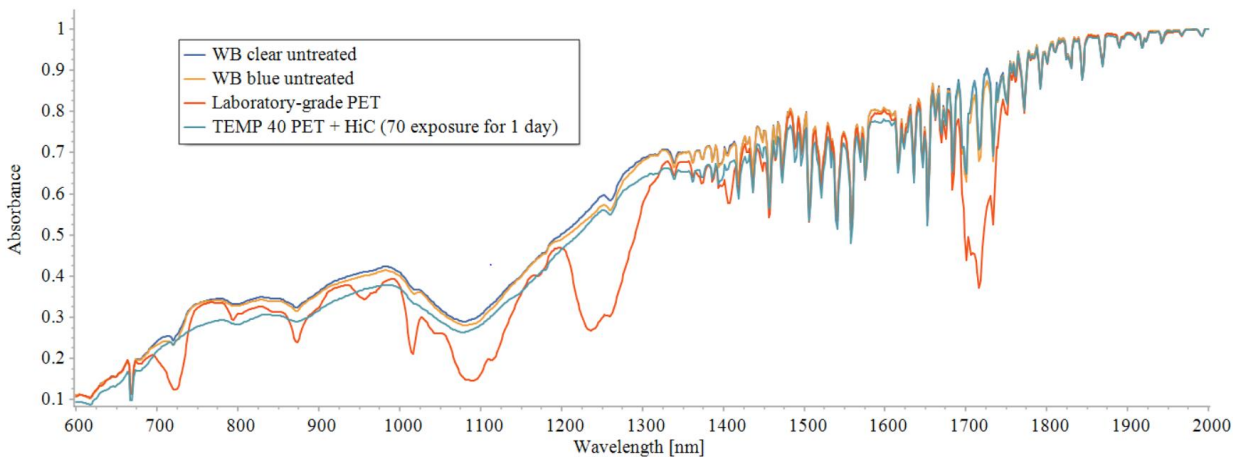


**Figure 2-3.** Spectra of plastic pieces from the 55 °C experiment PET + HiC at 6 days (40% mass loss), PET + HiC incubated at 40 °C for 14 weeks (40% mass loss) and an untreated laboratory-grade PET sample. Spectra have been normalized with the spectrum's highest peak normalized to an absorbance of 1.

Major changes to spectra are seen in two stages, between untreated PET and PET-only condition and between PET-only and PET + HiC (Figure 2-3). Flattening and decreases in peak intensity are seen in PET + HiC and PET-only conditions compared to untreated PET. This trend is repeated between PET-only and PET + HiC, with the latter having the most flattened and shallow peaks.

Minimal changes are seen with 25 °C incubations, as mass loss amount and higher temperatures influence the degree of peak flattening and decreasing. We see decreases in the peaks compared to PET-only condition, suggesting a strong temperature dependence or thermal-driven changes. Several peaks of interest include 1237  $\text{cm}^{-1}$  (Fan et al., 2012), which corresponds to C-OH bending, a double peak at 1016  $\text{cm}^{-1}$  and 1087  $\text{cm}^{-1}$  (C-O-C) (Da Silva et al., 2012; Kannan et al., 2019), 872  $\text{cm}^{-1}$  and finally at 720  $\text{cm}^{-1}$  (presence of a polyethylene chain, aromatic C-H bond)(Portaccio

et al., 2023). Another peak to note that does not fall in the fingerprint region is  $1716\text{ cm}^{-1}$  which is the C double bond to oxygen (Figure 2-3).



**Figure 2-4.** FTIR spectra of WB clear untreated sample, WB blue untreated sample, laboratory-grade PET untreated sample and PET sample from  $40\text{ }^{\circ}\text{C}$  temperature experiment incubated for 16 weeks and exposed to 1 day of  $70\text{ }^{\circ}\text{C}$  vacuum oven exposure. Spectra has been normalized with the spectrum's highest peak normalized to an absorbance of 1.

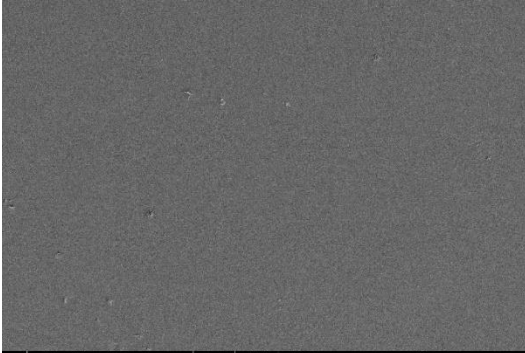
Extreme temperature pretreatment (and later exposure to HiC incubation) does not show any significant changes in spectra. Freeze-thaw cycle (FTC) pretreatment did exhibit the same trends as the temperature experiments but significantly less variation between conditions was seen. For plastic type, it appears both WB clear and WB blue have very similar FTIR spectra (Figure 2-4). Neither show changes between PET + HiC and PET-only conditions after 10 weeks of incubation. Both water bottle plastic types are missing the peak at  $1716\text{ cm}^{-1}$  that the laboratory-grade PET exhibits, and they lack many of the dips and peaks seen in the fingerprint region. Moreover, PET laboratory-grade samples exposed to  $70\text{ }^{\circ}\text{C}$  for 24 hours showed decreases in peak variability in the fingerprint region and resembled water bottle plastics (Figure 2-4).



#### 2.4.5 SEM imaging

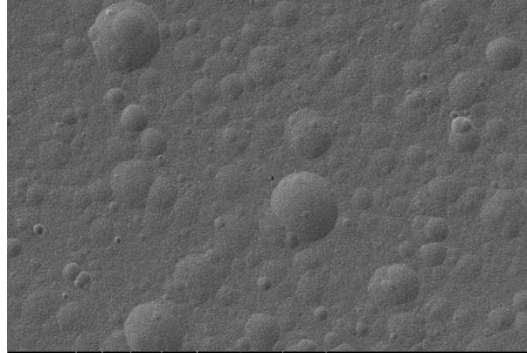
The untreated laboratory-grade PET at 1000-fold magnification shows minimal imperfections or damage to the surface (Figure 2-5). The 40 °C PET+ HiC samples incubated for 16 weeks show significant bubbling and damage to the surface, as seen at 1000-fold and 20 000-fold magnifications (see Figure 2-6 for 20 000-fold magnification). In a similar trend to the mass loss results, the 55 °C incubation at 10 days displays significantly more bubbling and damage to the PET surface. The 25 °C incubation at 16 weeks was more similar to the untreated PET but with an increased number of impurities or pits. The FTC pretreatment of 26-cycles for untreated PET has bigger pits/impurities, but they are less defined than the ones seen in the 25 °C incubation. Untreated water bottle PET looked similar to untreated laboratory-grade PET, and water bottle samples incubated at 55 °C for 10 weeks with HiC showed no significant surface degradation.

A)



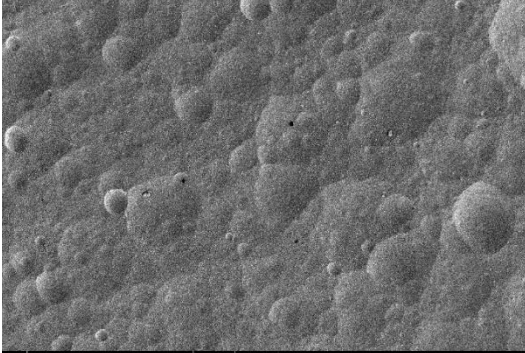
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
1.00 kV	0 V	ETD	SE	13 pA	1 000 x	298 µm	3.9 mm	Canadian Centre for Electron Microscopy

B)



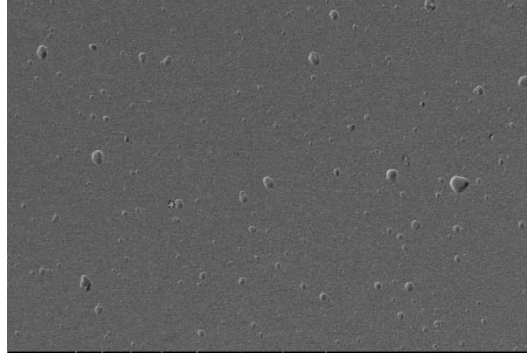
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
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C)



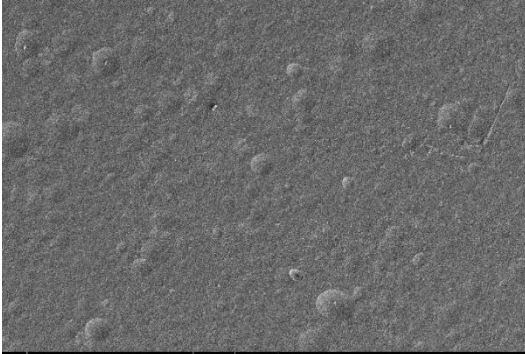
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
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D)



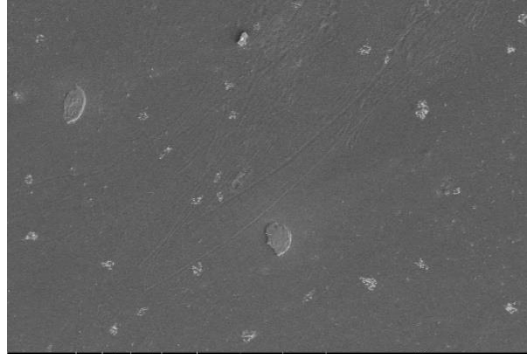
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
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E)



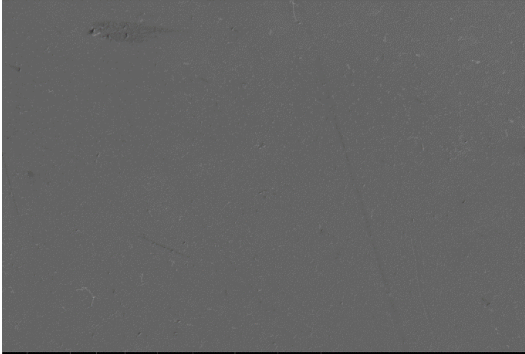
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
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F)



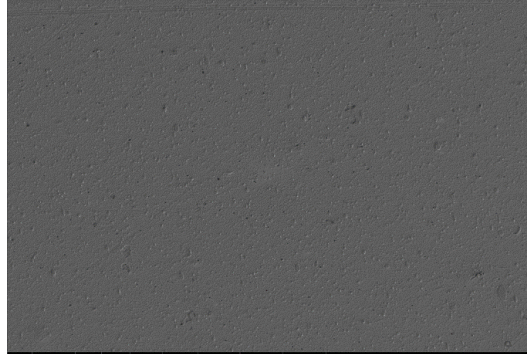
HV	bas	det	mode	curr	mag	HPW	WD	100 µm
1.00 kV	0 V	ETD	SE	13 pA	1 000 x	298 µm	4.0 mm	Canadian Centre for Electron Microscopy

G)



HV	bas	det	mode	curr	mag	HPW	WD	100 µm
1.00 kV	0 V	ETD	SE	13 pA	1 000 x	298 µm	4.0 mm	Canadian Centre for Electron Microscopy

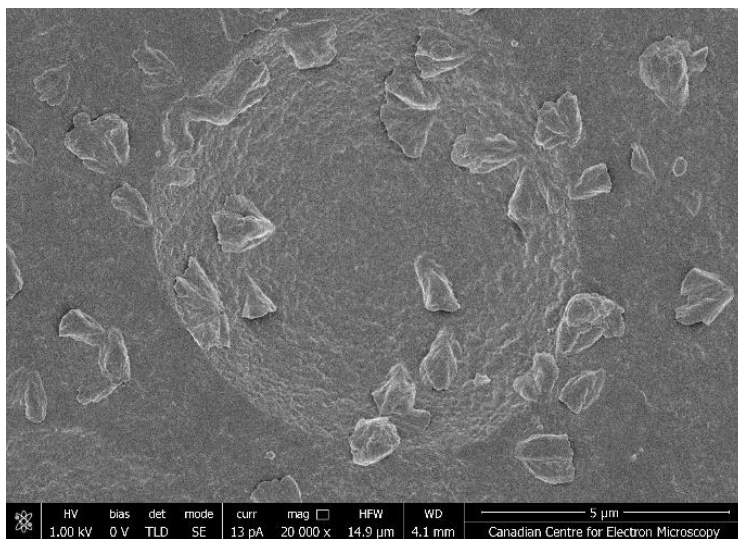
H)



HV	bas	det	mode	curr	mag	HPW	WD	100 µm
1.00 kV	0 V	ETD	SE	13 pA	1 000 x	298 µm	3.8 mm	Canadian Centre for Electron Microscopy

**Figure 2-5.** SEM images of PET, exposed to HiC at different conditions and timepoints, including a blank, untreated sample. A) untreated PET, B) PET from temperature experiment at 16 weeks (40 °C), C) PET from temperature experiment at 10 days (55 °C), D) PET from temperature experiment at 16 weeks (25 °C), E) PET from extreme temperature  $-70$  °C after 4 weeks, F) PET from FTC 26C blank, G) WB clear after 10 weeks at 55 °C, H) WB blue after 10 weeks at 55 °C.

All samples exposed to HiC show small flakes seen in Figure 2-6, however for lesser degraded samples, the flakes are smaller or fewer. Samples at 55 °C (PET + HiC) show more flakes but are smaller in size than those of PET + HiC incubated at 40 °C. These results are unlike previously reported SEM images of hydrolyzed PET. It is more common to see fracturing of the polymer surface or textured surfaces. Other studies often use much higher temperatures than those tested here (Bisceglie et al., 2022; Weinberger et al., 2017).



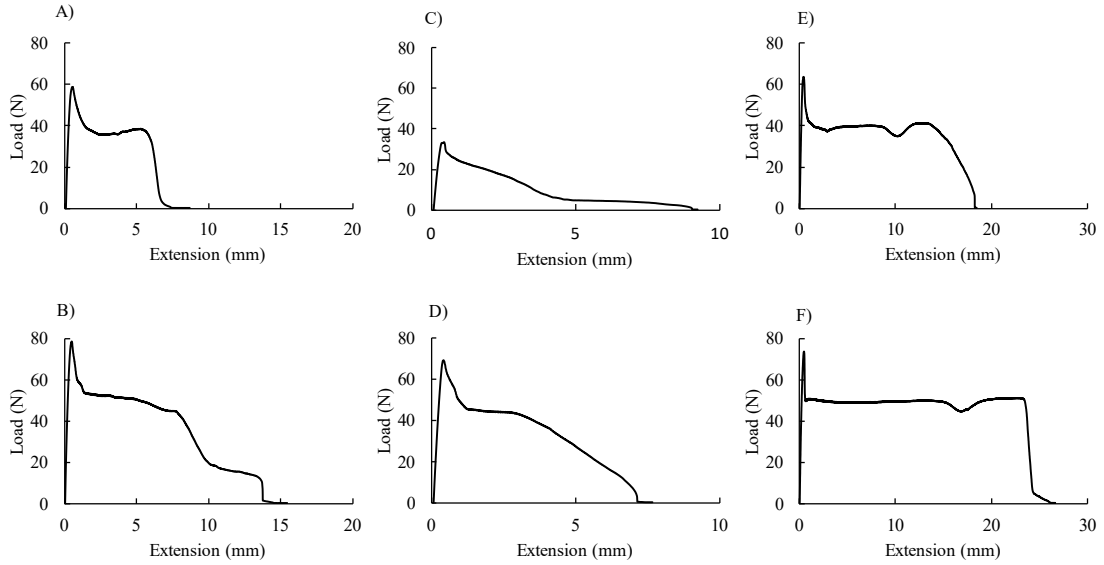
**Figure 2-6.** SEM image at 20 000-fold magnification of a PET + HiC sample incubated at 40 °C for 16 weeks.

#### *2.4.6 Tensile strength test*

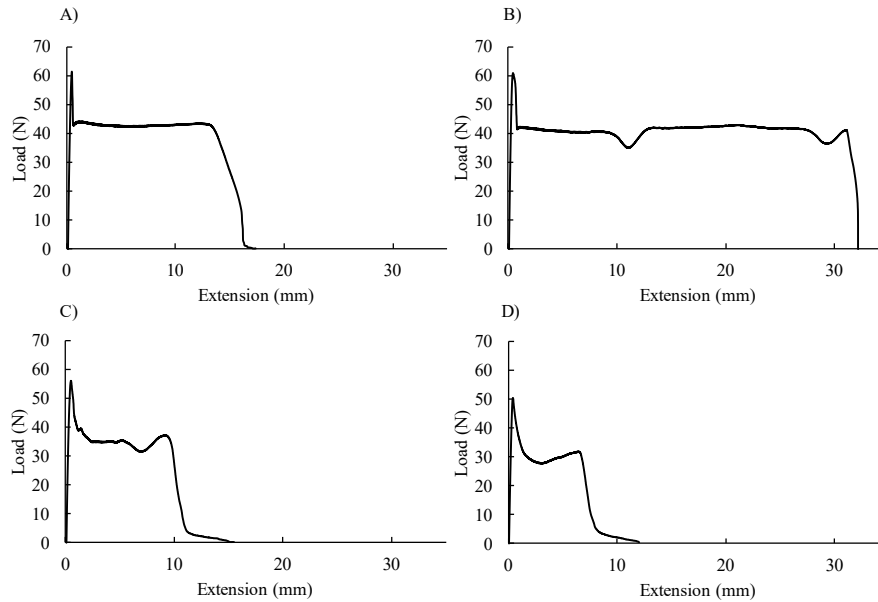
Lower and shorter peaks in the elastic region are seen in temperature experiment samples exposed to HiC. These characteristics translate to laboratory-grade plastic being more ductile and flexible. The peak found in the elastic region was highest at the low incubation temperature (25 °C) and higher incubation temperatures resulted in samples with lower peaks in this region.

Shorter and higher peak behavior indicates more brittle materials while longer and lower peaks indicate more ductile material. The untreated laboratory-grade PET presented as very ductile and flexible. In each temperature incubation's experimental control (PET-only), the peaks are different from the untreated sample, indicating temperature alone is altering plastic morphology (Figure 2-7). Changes between control (PET-only) and experimental (PET + HiC) samples indicate HiC is decreasing the load and extension, suggesting HiC is decreasing the ductility, stability and strength of the plastic. Furthermore, the crystallinity of a plastic affects its strength and durability, as strength/brittleness decreases (y axis values) crystallinity increases (Stearne and Ward, 1969).

Tensile strength graphs for the temperature experiments demonstrate each temperature has a signature appearance (Figure 2-7). Based on comparison with the untreated PET, lower temperature (25 °C) incubation alters the tensile graph shape (not the tensile strength value) more than higher temperatures (55 °C).

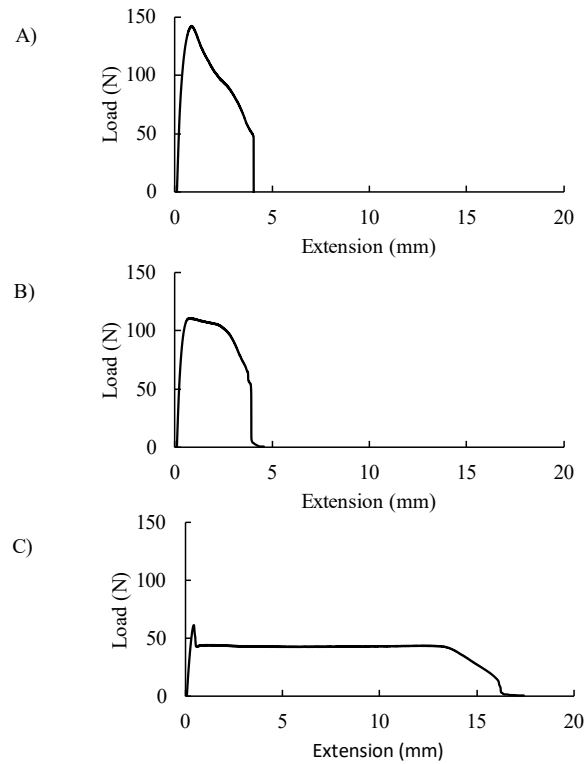


**Figure 2-7.** Tensile strength of temperature experiments at end points of each experiment. A) 55 °C temp PET + HiC (10 days), B) 55 °C temp PET only (10 days), C) 40 °C temp PET + HiC (16 weeks), D) 40 °C temp PET only (16 weeks), E) 25 °C temp PET + HiC (16 weeks), F) 25 °C temp PET only (16 weeks). X axes vary by temperature but remain consistent between the experimental and control condition at each temperature to highlight the changes caused by HiC.



**Figure 2-8.** Tensile strength of FTC experiments, A) untreated laboratory-grade PET, B) FTC untreated after 26 cycles, C) FTC (26 cycles) exposed to PET + HiC (4 weeks), D) FTC (100 cycles) exposed to PET + HiC (4 weeks).

Compared to the laboratory-grade PET untreated sample, the FTC untreated PET (which underwent 26 cycles, no HiC degradation) showed the same elastic peak but had a much longer extension in the plastic region, almost double that of the laboratory-grade untreated PET. The elastic peak remains similar across different numbers of cycles; however, the elongation shortens with increasing FTC and exposure to HiC. Necking is seen in the FTC samples that underwent HiC exposure (PET + HiC condition). The peak shape resembles those of the temperature experiment at 40 °C, suggesting different temperatures will affect plastic performance differently. The extreme temperature experiments follow the original temperature experiment trends.



**Figure 2-9.** Tensile strength graphs of different plastic types tested, A) consumer-grade WB blue (untreated), B) consumer-grade WB clear (untreated), C) laboratory-grade PET (untreated).

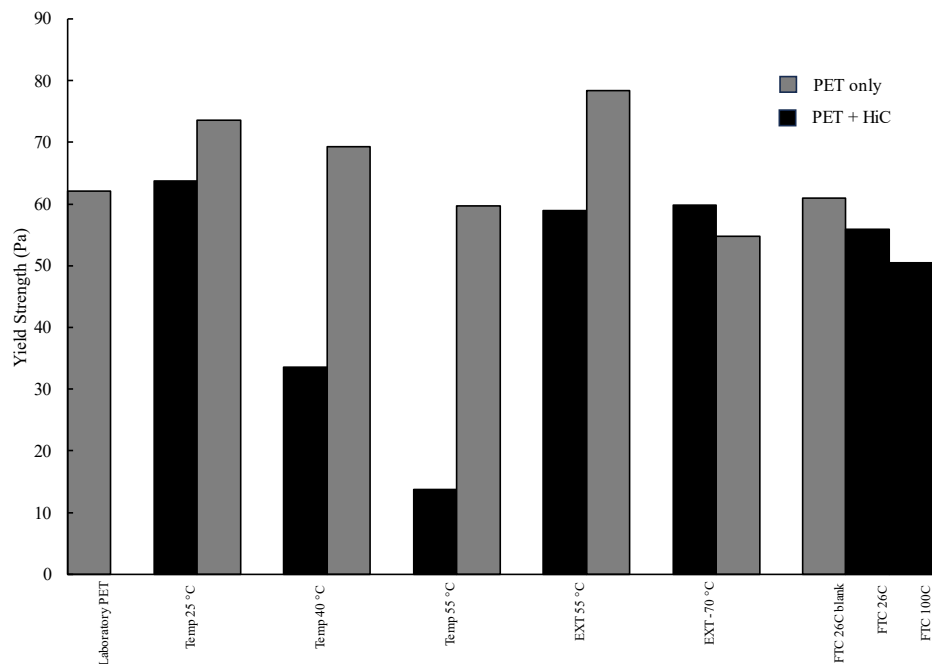
Varying plastic type results in major differences in tensile strength. Laboratory-grade untreated plastic has an elastic peak at 60 Pa, while consumer-grade untreated water bottles were 110 and 140 Pa. Differences in extension in the plastic region were apparent, with laboratory-grade plastic having approximately 16 mm of extension while both consumer-grade plastics had 4 mm of extension.

**Table 2-2.** Ultimate tensile strength and yield strength of PET samples exposed to various treatments. Yield strength was unable to be determined for the water bottle plastics due to the differences in chemical properties.

<b>Experiment series</b>	<b>Treatment</b>	<b>Yield strength (MPa)</b>	<b>Ultimate tensile strength (MPa)</b>
	Laboratory-grade PET, untreated	62.1	41.3
Incubation temperature	Incubation at 25 °C for 16 weeks with HiC (PET + HiC)	63.8	41.3
Incubation temperature	Incubation at 25 °C for 16 weeks with HiC (PET only)	73.5	51.1
Incubation temperature	Incubation at 40 °C for 16 weeks with HiC (PET + HiC)	33.6	5.3
Incubation temperature	Incubation at 40 °C for 16 weeks with no HiC (PET only)	69.3	44.0
Incubation temperature	Incubation at 55 °C for 10 days with no HiC (PET only)	13.8	9.2
Incubation temperature	Incubation at 55 °C for 10 days with no HiC (PET only)	59.7	43.6
Pretreatment: exposure to FTCs	Exposure to 26 FTCs, untreated	61.0	41.3
Pretreatment: exposure to FTCs	FTC 26 cycles, incubated for 4 weeks, PET + HiC	56.0	37.1
Pretreatment: exposure to FTCs	FTC 100 cycles, incubated for 4 weeks, PET + HiC	50.5	31.9
Pretreatment: exposure to extreme temperature	Exposure to 55 °C, followed by incubation for 4 weeks with HiC (PET + HiC)	58.9	38.6



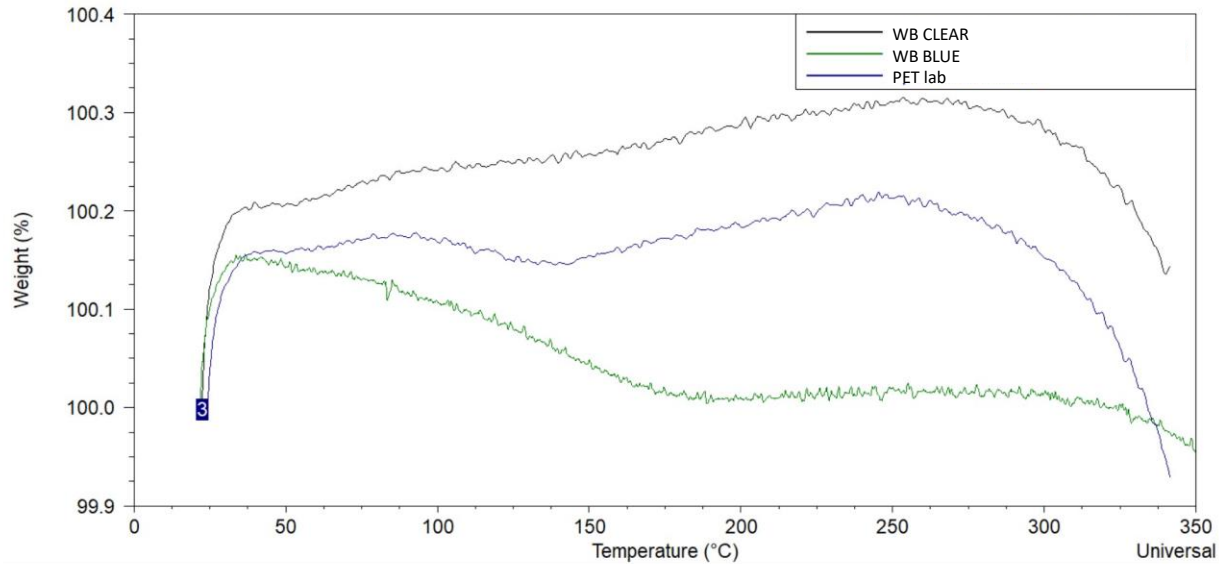
Pretreatment: exposure to extreme temperature	Exposure to 55 °C, followed by incubation for 4 weeks with no HiC (PET only)	78.4	19.6
Pretreatment: exposure to extreme temperature	Exposure to -70 °C, followed by incubation for 4 weeks with no HiC (PET only)	59.9	38.4
Pretreatment: exposure to extreme temperature	Exposure to -70 °C, followed by incubation for 4 weeks with no HiC (PET only)	54.8	50.6
Consumer-grade PET	Clear coloured water bottle PET (WB clear), untreated	N/A	100.5
Consumer-grade PET	Clear coloured water bottle PET (WB clear), incubated with HiC for 10 weeks	N/A	102.0
Consumer-grade PET	Blue coloured water bottle PET (WB blue), untreated	N/A	142.6
Consumer-grade PET	Blue coloured water bottle PET (WB blue), incubated with HiC for 10 weeks	N/A	79.5



**Figure 2-10.** Yield strength of temperature and pretreatment experiments. Untreated laboratory-grade PET is included as a reference. All samples were taken from end points; temp 25 °C (16 weeks), temp 40 °C (16 weeks), temp 55 °C (10 days), EXT 55 °C (4 weeks), EXT -70 °C (4 weeks), FTC 26C and FTC 100C (4 weeks).

#### 2.4.7 Thermogravimetric analysis (TGA)

Before DSC could be performed, TGA was performed on untreated samples of both the laboratory and consumer-grade PET to measure the thermal stability of the polymers. Based on the TGA curves produced, the laboratory-grade PET and the clear consumer-grade water bottle PET had similar TGA curves whereas the blue consumer-grade water bottle PET does not have similarities.



**Figure 2-11.** TGA graph of all untreated PET polymer types, laboratory and consumer-grade.

#### 2.4.8 Differential scanning calorimetry (DSC)

The general trend seen is increases in crystallinity percentage with increasing degradation. This remains true for all samples. This matches mass loss and FTIR data for conditions that produced more degradation. Between PET + HiC, and PET only conditions, PET + HiC shows high peaks, and range of heat flow is altered. See AI-3 for differences in the DSC graph between PET-only and PET + HiC plastic samples.

**Table 2-3.** Parameters calculated from DSC spectra including: crystallinity, glass transition, cold crystallization temperature, melting point, and crystallization point. DSC was run for only the endpoint plastics of PET + HiC condition.

<b>Experiment series</b>	<b>Treatment</b>	<b>Crystallinity (%)</b>	<b>Glass transition (°C)</b>	<b>Cold crystallization (°C)</b>	<b>Melting point (°C)</b>	<b>Crystallization point (°C)</b>
	Untreated laboratory-grade PET	9.2	80.0	133.5	247.4	185.6
Temperature	25 °C for 16 weeks, PET + HiC	10.1	80.3	132.5	247.5	185.8
Temperature	40 °C for 16 weeks, PET + HiC	12.3	81.6	133.0	247.0	186.6
Temperature	55 °C for 10 days, PET + HiC	16.0	80.8	129.2	244.8	186.1
Pretreatment	FTC (26C), incubated at 40 °C for 4 weeks, PET + HiC	10.3	84.6	133.5	247.2	185.3
Pretreatment	FTC (100C), incubated at 40 °C for 4 weeks, PET + HiC	11.2	84.4	133.0	245.1	186.1
Pretreatment	Extreme (-70 °C, incubated at 40 °C for 4 weeks, PET +HiC	9.3	84.4	133.2	248.2	185.1
Pretreatment	Extreme (55 °C), incubated at 40 °C for 4 weeks, PET +HiC	10.5	82.6	132.7	247.2	185.1
	Untreated WB clear	6.4	89.6	N/A	247	186.0
WB	Incubated at 40 °C, 10 weeks	2.5	100.4	N/A	252.2	185.5

	Untreated WB blue	7.0	74.6	N/A	248.4	192.1
WB	Incubated at 40 °C, 10 weeks	3.7	99.8	N/A	249.0	193.1

A disclaimer is present for DSC analysis for two reasons; the first DSC analysis of polymers always has some type of error associated which is not the instrument error and the second, as noted before, the consumer-grade plastic did not have a cold crystallization peak and as such crystallinity percentages were calculated differently for laboratory and consumer-grade PET. This leads to consumer and laboratory-grade crystallinity percentages being incomparable due to different methods of calculating crystallinity. As for the associated error of DSC for polymers, some literature suggests that during the re-cooling phase of DSC, the polymer can crystallize some amorphous regions resulting in higher levels of crystallinity than before the plastic underwent DSC analysis.

## 2.5 Discussion

### 2.5.1 Polymer Characterization

Characterization of the polymers used during this series of experiments was deemed essential for results to be comparable to literature. As previously noted, different polymer formulas and manufacturing methods alter the rate of degradation. Many studies on polymer degradation are lacking information on the type of plastic used within the experiment, making results incomparable. The laboratory-grade PET used for this series of experiments was found to have a crystallinity of 9.2%, meaning it would be classified as a low crystalline PET. Most PET is between 0-50% crystalline (Kaushal et al., 2021) so the laboratory-grade plastic used was lower than average. Both the FTIR spectra and the tensile graph for untreated laboratory-grade PET are

unique data sets which provide additional information on the polymer. DSC analysis allows the crystallinity percentage of a polymer to be calculated, which is a large factor determining degradation. FTIR spectra yields important information on the functional groups that make up the polymer, which is important in determining which methods of degradation will be most effective. Finally, tensile strength determines the load and elongation properties of the polymer, which helps to determine if additives such as plasticizers or alternative manufacturing methods have been used to alter the chemical composition. Overall, the combination of DSC, FTIR and tensile strength analysis for characterizing a polymer provides a basic overview of the polymers chemical qualities with instruments that are fairly common and accessible. We suggest these three analyses should be implemented as baseline analyses in all plastic research.

The consumer-grade PET utilized in this study was also characterized through the three analyses recommended above. Crystallinity was calculated using a different formula due to the differences in DSC graphs, however it was found to be 6.4% and 7% for clear and blue PET, respectively. FTIR and tensile strength graphs differed, with consumer-grade plastics identified as more brittle than the laboratory-grade PET. See section 2.5.4 for more information about differences between laboratory and consumer-grade PET.

### *2.5.2 Effect of Temperature*

Laboratory-grade PET exhibits increasing degradation with increasing temperature, resulting in a strong temperature dependence on degradation rates. Through tensile strength graphs, temperature alone is confirmed to affect plastic composition, not the polymers surface. SEM images of PET-only samples incubated at 25, 40 and 55 °C show minimal changes to the plastic surface, thus surface degradation is not occurring, rather re-configuration of the polymer molecules is more likely. This could be as temperature increases, the motion of polymer chains increases, leaving the

polymer more open and vulnerable. The concept of polymers opening and taking up material solution at elevated temperatures has been proven with red dye (Maes et al., 2017; Cole, 2016; Erni-Cassola et al., 2017) so it is possible the surface molecules are up-taking HiC better at higher temperatures. SEM images of PET + HiC samples do show increasing bubbling and surface changes with increasing temperatures and degradation. However, PET-only SEM images do not show any changes to the polymer, as no enzyme was present to take advantage of the open polymer. Additionally, HiC functions better at higher temperatures, so its possible temperature has a two-fold effect on the plastic structure and HiC function which has resulted in higher rates of degradation. At lower temperatures the polymer could be more closed and possibly entrap solution. Regardless of the specific temperature, tensile strength and crystallinity differences suggest temperature is allowing for enough energy to pack the polymer differently resulting in intermolecular chain changes which in turn affects degradation rates (Guseva et al., 2015; Panowicz et al., 2021).

FTIR analysis shows significant changes from untreated PET to PET-only samples incubated at higher temperatures which again suggests some changes to the polymers structure without any degradation occurring. This is more drastically seen with the 55 °C incubations. PET + HiC condition samples show flatter peaks than those of the PET-only samples, and these peaks continue to decrease in intensity as degradation of the PET continues. The flattening of these peaks indicates degradation or weathering of the plastic and the loss of functional groups (Ioakeimidis et al., 2016).

The activation energy calculated from the temperature experiments matches well with the literature values for laboratory-grade PET that were also hydrolyzed with HiC (Sabde et al., 2023). However, it does suggest that PET with higher crystallinity will take significantly longer to degrade. Degradation will be further inhibited by lower temperatures that are more realistic to

environmental conditions as enzymes (specifically HiC) are not at optimal temperatures nor is the polymer open.

Future work needs to be conducted investigating the inhibition of degradation caused by materials (specifically organic compounds) absorbing on PET in the environment. This study employed clean PET sheets and PET was cleaned at every step to ensure no contaminants however the majority of PET retrieved from the environment is contaminated with surrounding materials, often organic, such as algae or dirt.

### *2.5.3 Effect of Pretreatments*

As mentioned in the previous section (2.5.2), changes in temperature alter the polymers molecules and arrangement. The pretreatments are likely having a similar effect, however more drastic given the extreme temperatures and temperature changes of the pretreatments. These pretreatments cause changes that lead to quicker degradation but also compromise the integrity of the PET.

Increased mass loss was found for PET + HiC samples that were exposed to pretreatment compared to samples with no pretreatment exposure. This supports the claim that pretreatments alter chemical interactions within the PET leading to increased degradation when exposed to HiC. Not all pretreatments have the same rate of increasing degradation, as 55 °C pretreatment induced more degradation than -70 °C. No degradation was seen with the PET-only samples which confirms these pretreatments did not cause degradation, rather they altered the polymer molecules leaving it more susceptible to HiC.

When freeze-thaw pretreatments are used, mass loss increases compared to PET + HiC samples with no pretreatment. With mass loss alone, increasing the number of FTCs results in increasing mass loss however there is a threshold. When exposed to 26, 75, or 100 cycles, all produced a mass



loss around 28% after 4 weeks of HiC exposure at 40°C. It remains unclear if this is solely for mass loss, as small tensile strength, DSC, and FTIR changes were seen between samples which underwent 26, 75 and 100 cycles. Combined, there is some evidence which suggests the threshold is only for mass loss, but other analyses continue to see changes.

The results predict that some plastics will not be able to withstand manufacturers claimed parameters if exposed to these kinds of temperatures or temperature changes. Previous work of this kind has focused on PVC pipes however PET is found in many important materials such as concrete, asphalt and car parts. These materials are used because of their durability and are considered strong. PET is often used in these materials because it is resistant to deformation, fatigue and is long lasting. These pretreatments, which are more extreme conditions than environmentally realistic, suggest that the reliability of these materials can be compromised which can have grave consequences (Tournier et al., 2023). Many materials containing PET are used in areas where temperatures fluctuate, to build infrastructure such as bridges, roads, building and more. For PET found as plastic waste in the environment this provides some optimism that PET which has been exposed to environmental conditions, including temperature fluctuations, is more degradable than untreated PET, provided that there are the microbes and enzymes present to carry out degradation. However, as mentioned before, environmental temperatures do not reach the optimal temperature of these enzymes, slowing down the process, so it is unknown if the effect of temperature changes will have any significant effect on increasing the degradation of PET in the environment.

#### *2.5.4 Consumer versus laboratory-grade PET*

The most concerning result of this study regards the complete lack of degradation seen in consumer-grade PET samples. A similar study conducted a 3-week enzyme incubation at 55 °C

with amorphous PET bottle film (10% crystallinity) which resulted in 50% mass loss (Müller et al., 2005). The lack of any measurable degradation in this experiment suggests one of two things; either HiC is not able to act upon this type of consumer-grade plastic and bind effectively or the plastic is more resistant to degradation. Initially, additives found in consumer plastic were thought to be inhibiting degradation but a difference in manufacturing process may be more likely. Regardless, consumer-grade PET does not degrade even under ideal laboratory conditions.

FTIR spectra confirms that no changes to the consumer-grade plastic had occurred after incubation with HiC. Both water bottle spectra (consumer-grade PET) are identical and differ widely from that of the laboratory-grade PET. The consumer-grade PET lacks many of the peaks and dips in the fingerprint region and is missing the C=O bond at  $1716\text{ cm}^{-1}$  that the laboratory-grade PET presents. Previous research regarding the hydrolytic degradation of PET states the rate of degradation is affected by the carboxyl end group (Negoro et al., 2016). The lack of C=O suggests that its absence inhibits degradation.

PET bottles used for commercial purposes are manufactured differently than laboratory-grade PET, where bottles are made through stretch blow molding (SBM) and secondary heating, resulting in a barrier that is more difficult to penetrate. In this case, it could affect the binding of HiC to the polymer substrate (Tournier et al., 2023). This manufacturing process also results in a lower crystallinity level compared to PET sheets, which is supported by DSC analysis conducted.

To determine if secondary heating could be a factor inhibiting degradation and if the consumer-grade PET used had undergone secondary heating, a piece of laboratory-grade PET was incubated at  $70\text{ }^{\circ}\text{C}$  for 1 day to mimic the secondary heating process. FTIR results showed the plastic sample had identical peaks to that of the consumer-grade PET and it differed largely from the original untreated laboratory-grade plastic (Figure 2-4). Furthermore, the sample also lost its C=O peak.

Manufacturing procedures for the water bottle PET are unknown however based on these results we can estimate the plastic underwent a secondary heating below the melting point that made it significantly more resistant to degradation. In addition, this provides evidence that reheating PET under the glass transition state will inhibit polymer degradation by forming an outside layer of plastic that is more crystalline (Negoro et al., 2016).

The DSC analysis conducted on the consumer-grade PET resulted in two unexpected results. Firstly, it revealed both water bottle-derived PET do not have a cold crystallization peak that PET typically has. Secondly, crystallinity decreased after incubation with HiC. This is a strange occurrence but could be explained by moisture and water absorption. Water absorption increases over time and increases in moisture within the polymer lead to decreased crystallinity (Negoro et al., 2016). Regardless, it remains unknown as to why laboratory-grade PET did not decrease in crystallinity if that is the case. Water absorption observed with laboratory-grade PET decreased over time while water absorption for consumer-grade plastic seemed to remain constant over the experiment.

The consumer-grade WB clear plastic showed similar trends to that of laboratory-grade PET for TGA analysis, indicating a similar thermal stability. Based on FTIR results they may originate from similar plastic composition but have undergone different manufacturing processes. Additives and the manufacturing process are major contributors increasing the degradation resistance of consumer-grade plastics, which will lead to increasing amounts in the environment with few proven methods of recycling and degrading (Wiesinger et al., 2021).

### *2.5.5 Implications*

Through this study multiple analyses were conducted to present a well-defined and explored rate of PET degradation. Existing studies which have investigated degradation, in general, utilize FTIR

and mass loss due to ease of access and the size of plastics. We demonstrate why this is an incomplete approach to measuring degradation and characterizing the plastic used. Alternative tests, such as tensile strength and DSC, can be useful techniques to determine chemical property changes of degraded polymers. Through this study, a trend emerged with the following analyses: mass loss, tensile strength and DSC (crystallinity percent). With increase mass loss, decreases in tensile strength and an increase in crystallinity was seen. As a result, there are several analyses recommended for polymer research concerning degradation. SEM imaging is best for visualizing degradation, however minimal degradation is not likely to be seen, so mass loss must be significant. Tensile strength and FTIR are best for determining chemical property changes to the polymer while TGA simply gives the polymers stability. DSC is a useful starting point to determine the crystallinity and thus degradability of the polymer but crystallinity does not increase significantly after degradation, making the analysis more useful to characterize the polymer. Finally, mass loss is by far the most important measure of degradation when using macroplastics due to ease of access to equipment and comparability in literature.

Based on the extensive list of analyses conducted and the characterization of the polymer, we recommend a classification system for plastics used in research. Industry often produces plastics with higher crystallinity, which have high tensile strength and are less affected by solvents, making them ideal for consumer safety but these high crystallinity plastics are more difficult to degrade. We suggest classifying PET used in research by crystallinity percent and manufacturing source, adding a layer of complexity for researchers but resulting in more comparable results in the field. This addition will require additional testing of materials before use to classify the PET crystallinity and source, with identifiers such as hcPET (high crystallinity) and lcPET (low crystallinity)

(Maurya et al., 2020), and source, such as laboratory-grade and consumer-grade PET. This will aid in making research more comparable and creating standards for the field of plastic research.

A larger implication regarding the changes in degradation rates between the consumer and laboratory-grade plastic relates the FTIR spectra presented. While secondary heating is known to be a method polymer manufacturers utilize to increase the durability of plastics, very little literature can be found on its effects to plastic lifespan or degradation. In this paper, we observe consumer-grade plastics lack the same FTIR peaks laboratory-grade plastic, which have not undergone a secondary heating exhibit. This is supported by the spectra from a laboratory-grade sample heat treated to induce a secondary heating, which resulted in a loss of FTIR peaks. To date, no polymer degradation literature has shown explicit evidence that secondary heating is responsible for reduced degradability of a polymer until now. Furthermore, the lack of degradation to consumer-grade plastic under ideal conditions, with HiC and high temperatures is especially concerning for degradation at environmentally realistic conditions without the abundance of HiC.

## **2.6 Conclusions**

In this study, PET plastic is proven to degrade at accelerated rates when exposed to higher temperatures or exposed to temperature pretreatments. FTIR shows changes to the fingerprint region when PET is exposed to high temperatures and/or HiC. The activation energy of laboratory-grade PET was calculated to be  $153 \text{ kJ mol}^{-1}$  and was highly dependent on temperature. Through exposing consumer-grade PET water bottles to HiC, a disturbing lack of degradation occurred signaling many previously thought methods of plastic recycling and breakdown may not be effective on consumer-grade plastics. This demonstrates a large concern regarding plastic research and efforts to clean up plastic pollution, as many methods and clean-up techniques are tested on

laboratory-grade PET (lacking impurities and additives, while utilizing different manufacturing processes) but are likely to not translate to success on consumer plastics. Visualized by multiple analytical techniques, consumer-grade plastic does not degrade implying even longer lifespans than previously estimated and an alarming realization that PET laboratory-grade plastic and consumer-grade PET are not interchangeable, and thus must be classified as different polymers types when conducting research.

### **3. Investigation of polyethylene terephthalate (PET) and polyamide (PA) microplastic degradation *in situ* in stormwater pond sediments**

#### **3.1 Introduction**

The prevalence of plastic pollution and consequently microplastics in the environment is an increasing environmental disaster and health concern (Chamas et al., 2020; Berry et al. 2023). In 2022 alone, it is estimated there was over 400 million metric tonnes of plastic produced globally (Lampitt et al., 2023). By 2035, annual plastic production is expected to increase to over 515 million tonnes (Production of key thermoplastics, 1980-2050 – Charts – Data & Statistics). Plastic presence has polluted every system of human life, from tea bags (Hernandez et al. 2019), water (Mason et al., 2018; Gambino et al., 2022) and clothing (Henry et al., 2019) to human blood (Leslie et al., 2022). Not only does this pose serious health and environmental concerns but since 2019 many of the recycling and waste management systems have collapsed resulting in lower rates of recycling for many plastics leading to increased presence in landfills (Franklin-Wallis, 2023). While recycling can be a good step to mitigate polymers from entering the environment, unfortunately even if plastics are eligible for recycling (Berry et al. 2023), the process results in decreased quality of the plastic. This results in plastic being recycled several times before being deemed unfit for use and discarded (Franklin-Wallis, 2023).

Polyethylene terephthalate (PET) is one of the most commonly produced plastics, used to make clothing fibres, food packaging such as plastic bottles and used in many industrial materials including automotive parts, concrete and asphalt. It is heat resistant, lightweight, strong and has

good chemical stability that makes it a versatile material with many functions however these mechanical properties are what make PET so difficult to degrade (Sui et al., 2023). Its crystalline structure also aids to reduce any potential degradation. Polyamide (PA) is another polymer widely used commercially, often found in textiles, furniture, and car parts. Like PET, it has good chemical and thermal resistant properties that make it difficult to degrade. Materials which contain PET or PA produce high levels of secondary microplastics which results in increasing levels found in the environment with limited methods to degrade the polymers (Thomson et al., 2022).

Stormwater ponds are often constructed in urban environments to collect stormwater runoff from rain or snowmelt events coming from impermeable surfaces. They are used to improve water quality by collecting pollutants, which includes microplastics and expelling less polluted water. As a result, they are often a pathway for residential and industrial microplastic pollution to enter larger waterways (Lui et al., 2019). These ponds are very effective at reducing microplastic loads from urban areas by acting as a microplastic sink (Olesen et al., 2019). Studies have shown pond sediment is highly concentrated with microplastics, reaching levels of  $0.4 \text{ g kg}^{-1}$ . Increased plastic presence within ponds has resulted in higher microplastic accumulation in vertebrates ponds (Olesen et al. 2019) and negative impacts to microbial community abundance and diversity (Seeley et al., 2020). Based on this knowledge, determining the rate of degradation of the microplastics found within the pond sediment would be largely beneficial to these stormwater pond systems.

Several long-term field studies have been conducted to determine the degradation of polymers in the environment, in marine ecosystems, landfills, lakes and soil (Chamas et al., 2020). However, there are very few that have investigated the degradation of plastics in stormwater pond sediment, sediment that is reported to have the highest rates of microplastics and who's presence is likely affecting surrounding organisms (Lui et al., 2019). A large degradation influence is sunlight



exposure (Delre et al., 2023) and mechanical weathering from water movement, both of which are absent in sediment. This leaves most of the degradation potential to microorganisms held within the sediment. Microbial degradation relies on microbes using plastic as a carbon source and past literature has identified many organisms which can degrade plastic.

**Table 3-1.** Degradation experiments previously conducted in sediment with various polymers and varying conditions. Mass loss was a required analysis which greatly limited the number of studies listed below. Studies which focused on single enzymes or bacteria (isolation) that degrade polymers were not included.

Plastic Type	Conditions	Duration	Mass loss	Mass loss rate (%/year <sup>-1</sup> )	Analysis conducted	Reference
HDPE	3m deep in Bay of Bengal, free in water	6 months	0.5-0.8%	0.01-0.016	FTIR, DSC, SEM, biofilm analysis	Sudhakar et al., 2007
LDPE	3m deep in Bay of Bengal, free in water	6 months	1.5-2.5%	0.03-0.05	FTIR, DSC, SEM, biofilm analysis	
PP	3m deep in Bay of Bengal, free in water	6 months	0.5-0.6%	0.01-0.012	FTIR, DSC, SEM, biofilm analysis	
PET	1m deep in Bay of Bengal, tied to floating raft frame with biofouling	1 year	7.49%	0.07	FTIR, Tensile strength, TGA,	Muthukumar et al., 2011
LDPE,	(1) Direct sunlight, (2) buried in soil, (3) in marine water (Bay of Bengal)	(1) and (2) for 30 days, (3) 150 days	Lowest mass loss in soil (~1%), highest in sunlight (5.4%), marine (5.7%)	(1) 0.65, (2) 0.12, (3) 0.14	SEM, TGA, FTIR	Muthukumar et al., 2010
HDPE	(1) Direct sunlight, (2) buried in soil, (3) in marine	(1) and (2) for 30 days, (3) 150 days	Lowest mass loss in soil (~1%), highest in sunlight	(1) 2.64, (2) 0.12, (3) 0.18	SEM, TGA, FTIR	

	water (Bay of Bengal)		(22.7%), marine (7.8%)			
LDPE	(1) Pellets from SS Hamada wreck, (2) pellets from beach	~29 years	No significant mass difference	N/A	FTIR, DSC, GPC	Brümmer et al., 2022
PLA blend 1 and 2	Buried in soil	1 year	0.23 and 0.57%	0.002 and 0.006	TGA, tensile strength, SEM	Slezak et al., 2023
PLA blend	Buried in sediment	1 year	Under 1%	0.01	FTIR, DSC	Beltrán-Sanahuja et al., 2020
PP	Incubated in marine sediment	1 year	0.39%	0.004	Tensile strength, SEM, GLM	Welden and Cowie, 2017
PA	Incubated in marine sediment	1 year	1%,	0.01	Tensile strength, SEM, GLM	
PE	Incubated in marine sediment	1 year	0.45%	0.005	Tensile strength, SEM, GLM	

In this study, PA and PET plastic were inserted into a local stormwater pond sediment for 16 months. Our objective was to determine the degradation rate of both PET and PA using strips of plastic and if there were any changes in degradation by sediment depth. Plastic strips were used instead of microplastics, as larger pieces of plastic were easier to weigh and collect. Geochemical analysis of the pore water was also taken by depth to provide a chemical profile of the pond sediment. This project used pore water samplers (peepers) to house plastic inserted into the pond sediment. Additional peepers inserted at the same depth were used to collect pore water chemistry.

## 3.2 Materials and Methods

### 3.2.1 Field site

A stormwater pond in the City of Kitchener, Ontario, Canada was selected for this experiment, named Bingemans Pond. The pond is maintained by the City of Kitchener and is located beside the Grand River. It has not been dredged for 9 years, resulting in a sufficient accumulation of sediment for installing the passive porewater samplers. The pond receives runoff from the surrounding industrial and commercial buildings, parking lots and roads. Discharge runs into the Grand River, which feeds into Lake Erie. Serval criteria were used to evaluate the most appropriate pond; minimum of 80 cm sediment depth to accommodate peeper installation, low water level under 1.5 m for feasible retrieval of peepers, easy road access for sampling every, and a secluded area with little human traffic to avoid disturbances to the experiment.



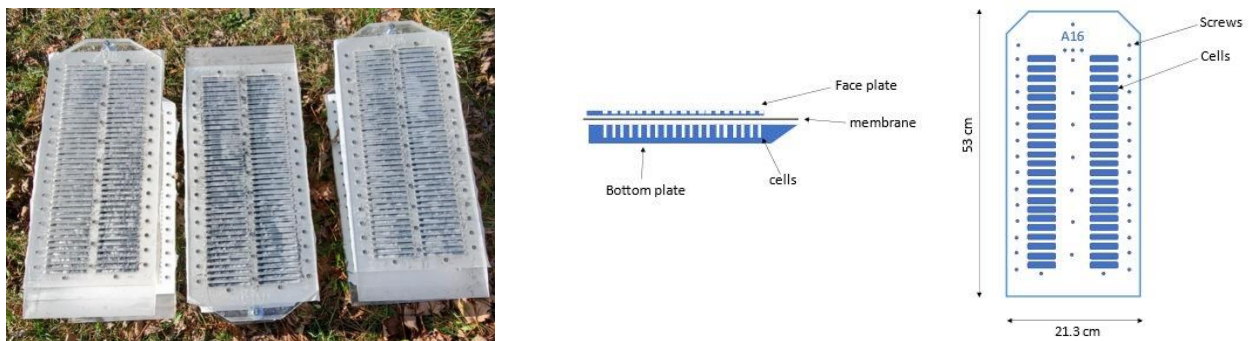
**Figure 3-1.** Areal view image of the stormwater pond.

### 3.2.2 Materials and Analysis

Both PA and PET clear plastic sheets having thicknesses of 0.2 mm and 0.25 mm, respectively, were obtained from Delta Scientific Laboratory Products Ltd. A grid of 6 cm x 0.6 cm pieces was drawn to guide the cutting of the sheets into 6 cm x 0.6 cm pieces using an exacto knife. A grid was not drawn directly onto the plastics to avoid contamination on the plastic surface. The plastic pieces were cleaned with 70% filtered ethanol and allowed to air dry. Each piece of PET and PA plastic was weighed and given a corresponding number for future mass loss analysis.

### 3.2.3 Peeper design and preparation

Peeper are equilibrium dialysis samplers that allow for the passive diffusion of pore water with the environment. This is achieved using a porous membrane which is situated between the inner cells of the peeper and the outside environment. The peepers used in this experiment are an altered design from the ones first created, from Hesslein et al. (1976). The peeper body is created from Plexiglass™ with dimensions 53 cm x 21.3 cm with a Plexiglass™ face plate (48 cm x 21.3 cm) held together with stainless steel screws. Each peeper contains 80 sample cells, with 40 cells on each side, with cells spaced 0.4 cm apart vertically. The volume of water in each cell is 3.5 cm<sup>3</sup> (72 mm x 6 mm x 8 mm) due to rounded cells.



**Figure 3-2.** Pore water samplers (peepers) design and measurements.

All peeper units used in the experiment were rinsed with 70% filtered ethanol and allowed to air dry to clean and sterilize them before deployment to the field. One set of peepers prepared (8 in total) were the peepers to be used for equilibrating and sampling porewater (“pore water peepers”). These pore water peepers were prepared by pipetting 4 mL of MQ water into each cell, adding the membrane and face plate. The membrane used was a 0.2  $\mu\text{m}$  polysulfone membrane (Gelman HT-200 polysulfone membrane, Pall Corporation) and was secured between the peeper body and faceplate with screws. Once filled, the pore water peepers were stored in an anoxic box filled with MQ water with a steady flow of nitrogen gas for approximately 24 hours.

Another set of peepers prepared (8 in total) were used for installing and incubating the plastic (PA and PET) in the sediment (“sediment peepers”). These peepers were prepared by packing sediment collected from Bingemans stormwater pond into each cell and placing a single piece of plastic (PA or PET) with clean tweezers into the sediment inside the cell.

Surface sediment from the stormwater pond was collected the same day that the peepers were packed with sediment and plastic, and installation occurred the next day. The sediment was homogenized, sieved through a 4.76 mm sieve and packed into each cell of the sediment peepers. Excess sediment was carefully removed from the top of cells and surrounding areas before placing the membrane and face plate on the peepers. A 5  $\mu\text{m}$  membrane was used for the sediment peepers to allow for diffusion of microorganisms (which have an average cellular diameter of 2  $\mu\text{m}$  or less) through the membrane and to then interact with the plastic and sediment in the peeper cells. All of the plastic peepers were secured into an anoxic box and left for approximately 18 hours with a steady flow of nitrogen gas to remove oxygen. Boxes used to create anoxic conditions were air-tight Plexiglass™ boxes.

### *3.2.4 Installation, retrieval and in situ analysis*

The day of installation, the nitrogen gas flush was stopped and the boxes were transported to the field site. Upon arrival, the boxes were unsealed and one pore water peeper and one sediment peeper were removed at a time. Peepers were laid horizontally, with the face plate up to eliminate water or sediment loss before installation into the pond sediment. One pore water and one sediment peeper were attached with a metal bar to create a peeper pair. Once all peeper pairs were attached, each pair was laid horizontally in the pond water for 30 seconds before being turned vertically and inserted into the pond sediment. Peepers were inserted with the top 5 cm (5 cells) of the peeper in the bottom of the water column and the remaining 35 cm (35 cells) of the peeper found in the sediment column. Temperature loggers (HOBO Onset) were installed on either end of the peepers in the field to monitor the pond water column temperature. On the day of peeper installation, the pond water column temperature, pH, redox potential, and electrical conductivity were also measured on site using a handheld YSI Quattro unit.

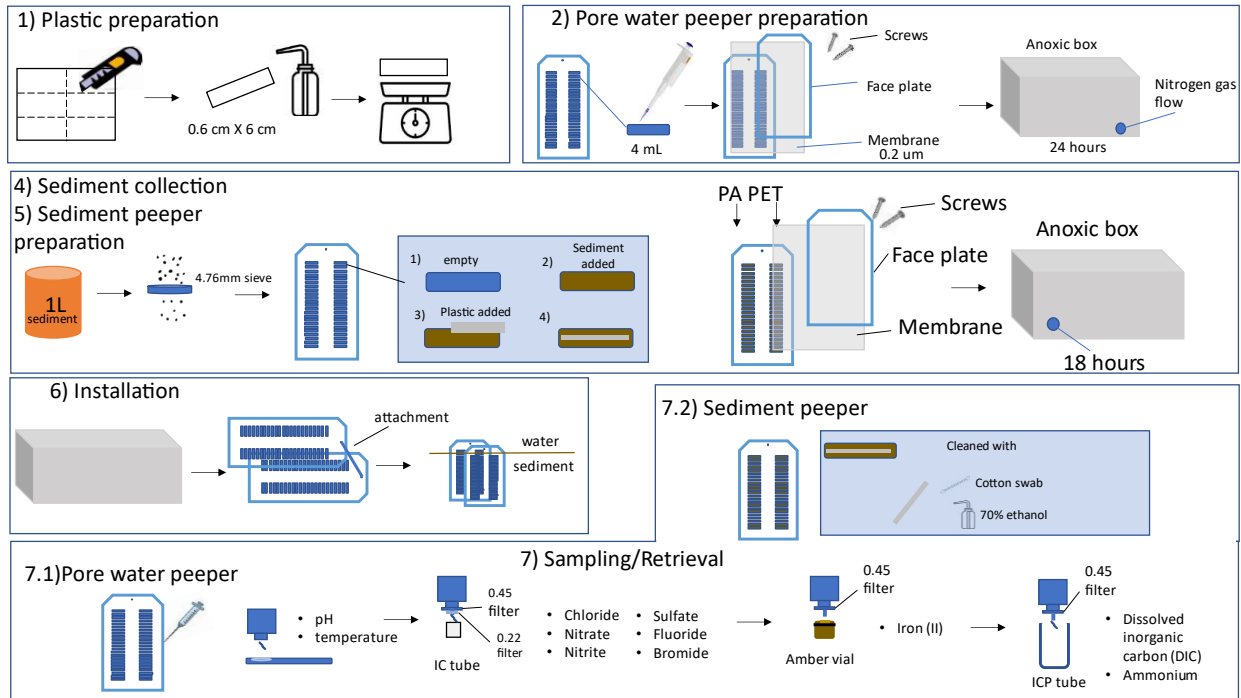
Retrieval occurred approximately every 2-4 months, with one pore water and one sediment peeper pair being removed at each timepoint. Peeper pairs were removed vertically from the sediment and left in the water while removing the connector bar holding the peeper pair together. Once separated, each peeper sampler was flipped horizontally in the water, face plate up to avoid water loss from the peeper cells and removed from the water.

The “porewater peeper” was sampled in the field. Starting from the bottom of the pore water peeper, a 22G needle and syringe was used to puncture the 0.2-micron membrane and remove ~4mL of pore water. Several drops were allocated directly to a handheld pH meter (LAQUA Twin meters, model Horiba B-213) to determine pH and temperature of each cm depth in field. A 0.45-micron filter was added to the syringe and 2mL of pore water was allocated to the acidified tubes.

This was done by piecing the septa caps with the 22G needle. The remaining pore water was added to the tubes for analysis of concentrations of dissolved inorganic carbon (DIC) and ammonium ( $\text{NH}_4$ ) using a Total Organic Carbon (TOC) analyzer (Shimadzu TOC-LCPH/CPN, method detection limit = 0.03 mM) and Gallery Discrete Analyzer (Thermo Scientific™ Gallery™ discrete analyzer), respectively. The other cell extraction included the same step of piercing the membrane with a 22G needle and adding both 0.22 and 0.45  $\mu\text{m}$  pore size membrane filters (Thermo Scientific Polysulfone filter) before adding porewater into the tube for major ions, including, chloride ( $\text{Cl}$ ), nitrate ( $\text{NO}_3^-$ ) and sulfate ( $\text{SO}_4^{2-}$ ) using ion chromatography (IC, Dionex ICS-5000 with a capillary IonPac® AS18 column;  $\pm 3.0\%$  error and  $\pm 1.6\%$  precision; MDL 2.14, 0.81, and 1.32  $\mu\text{mol L}^{-1}$ , respectively). The 0.22  $\mu\text{m}$  filter was removed and the remaining pore water was filtered with a 0.45  $\mu\text{m}$  membrane filter for ammonium and dissolved inorganic carbon. Iron (II) was measured using the Gallery Discrete Analyzer. All the porewater sampling tubes were prepared beforehand for acidification in the field to measure iron (II) ( $\text{Fe}^{2+}$ ) concentrations. 200  $\mu\text{L}$  of 1M HCl was added to each vial and vials were moved to an anaerobic chamber overnight with the caps removed to allow the vial headspaces to equilibrate with the anaerobic chamber atmosphere. The following day, the vials were sealed with unpierced septa caps in the chamber and removed.

The “sediment peeper” was placed into the anoxic box carrier for transport to the lab in the Ecohydrology Research Group. The face plate and membrane were removed in lab and ethanol-cleaned tweezers were used to remove the plastic strips from each cell. Ethanol-cleaned spatulas were used to remove samples of sediment into cryotubes and flash frozen with liquid nitrogen for later analysis of DNA extraction and gene sequencing. Plastic strips were stored at room temperature until the plastic pieces were cleaned. Based on the cleaning methods tested in Chapter 2, plastic was cleaned using MilliQ water and cotton swabs. Plastic was removed from the peeper

cell, sprayed with MilliQ water two to three times to remove the sediment, a wet cotton swab was used to removed any remaining sediment and finally the plastic strip was sprayed with MilliQ water a final time before being left to air dry before weighing. Very little visible microbial growth or damage was observed, plastic pieces were cleaned until not visible debris was seen under a microscope.



**Figure 3-3.** Overall schematic of peeper preparation, installation and retrieval methods.

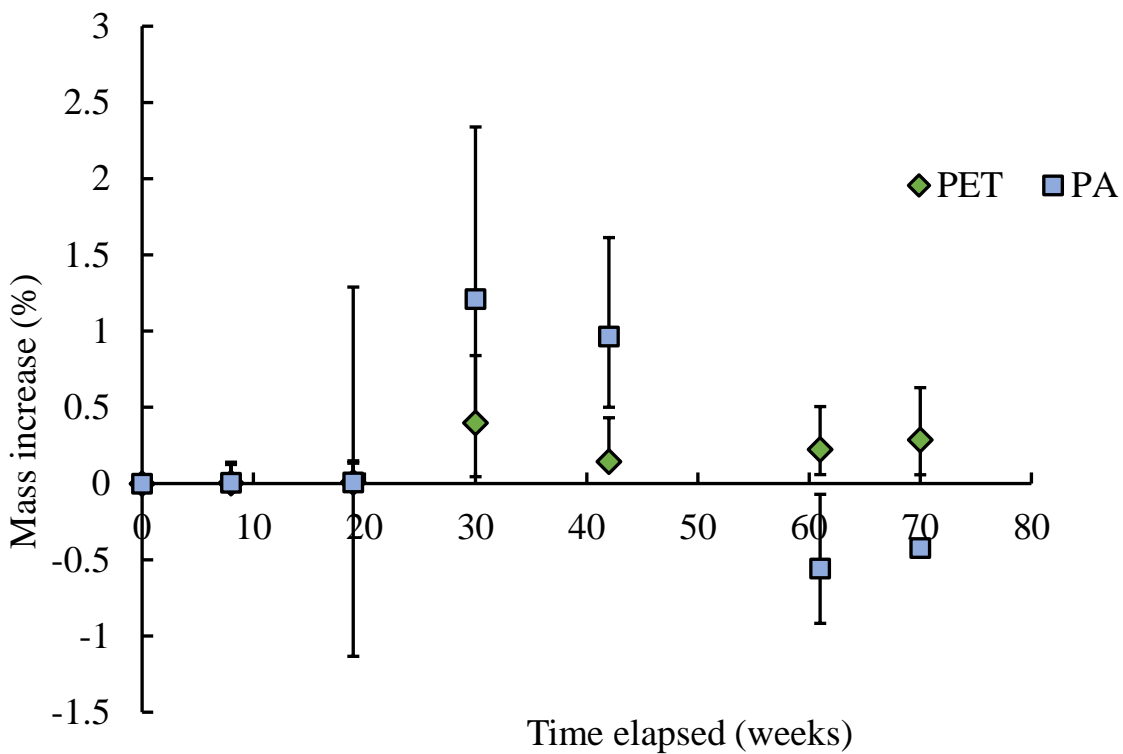
Peeper were installed November of 2022 and the first timepoint was January 2023 (2 months later). The second timepoint was in March (4 months), the next in June (7 months), August (9 months), January 2024 (14 months) and the last included in this paper was March 2024 (16 months).



### 3.3 Results

#### 3.3.1 Mass loss

The main analysis performed for this study was mass loss of the plastic strips incubated in the pond sediment. Plastic strips were weighed before installation in the pond and after incubation in the pond. After 16 months of incubation within the pond sediment it was observed that PA strips degraded an average of 0.42%. At 16 months, PET plastic had an overall net positive weight, meaning the final weight was greater than the weight of the plastic before installation in the pond. No changes to mass loss by depth over the 16 months were seen.

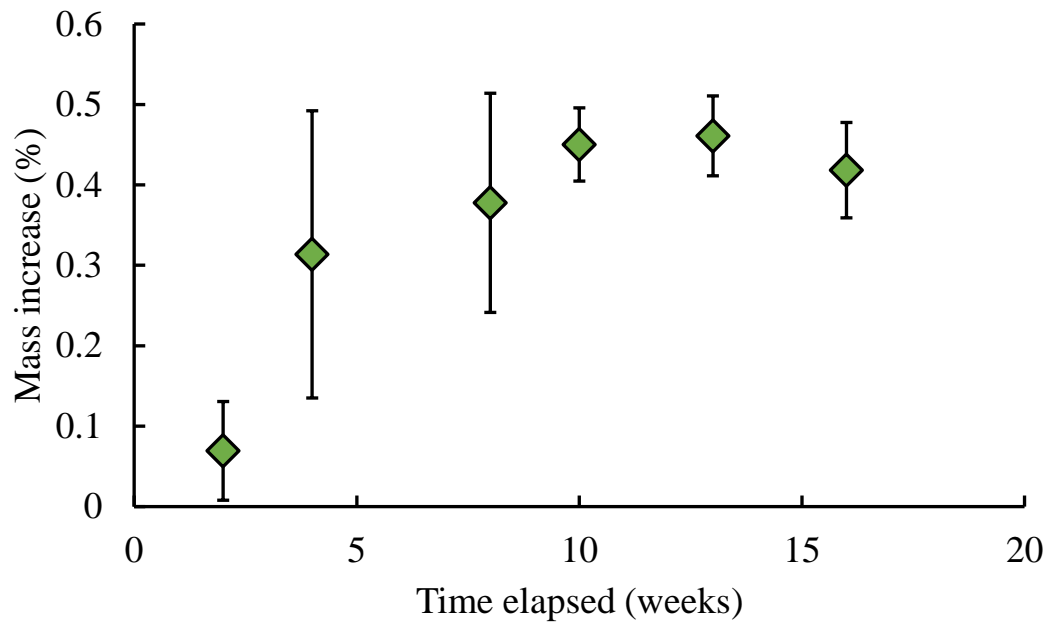


**Figure 3-4.** Mass loss of in situ plastic over a period of 71 weeks (16 months). PET is seen in green and PA is seen in blue. 95% confidence intervals are built in as error bars.

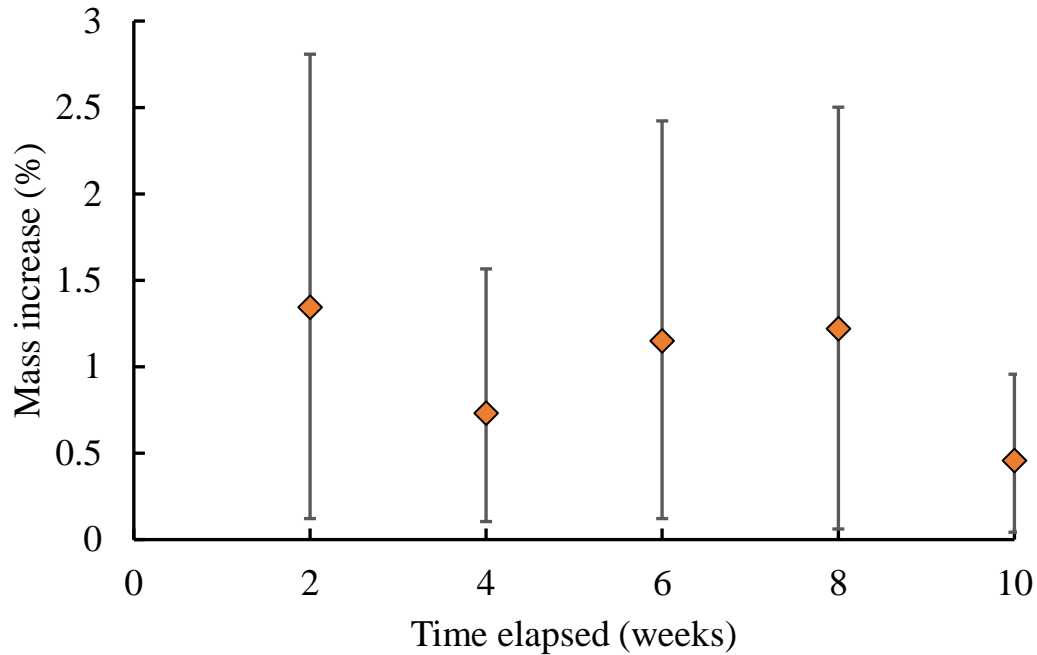
We only observe mass loss occurring after 60 weeks of incubation, and mass changes do not occur in a linear fashion (see Figure 3-4). As a result of degradation not following a linear pattern, linear estimates of further degradation cannot be accurately estimated nor compared to current literature rates of polymer degradation.

In earlier months, very minimal changes are seen however at sampling points June 2023 (7 months/30 weeks) and August 2023 (9 months/42 weeks) we observe mass increase for both polymer types. This mass increase peaks at 30 weeks before decreasing in later sampling points. Mass increase was seen to be the highest at 30 weeks, with a mass increase of 1.2% for PA and 0.4% for PET.

Mass loss was greater at 14 months for both polymer types compared to 16 months. For PET, an average mass increase of 0.22% was observed at 14 months and rose to 0.28% mass increase at 16 months. PA was found to have an average mass loss of 0.56% at 14 months and decreased to a mass loss of 0.42% at 16 months. Based on these rates, it does suggest that PA degrades faster than PET which is highly supported by literature based on the differences in crystallinity percent. Higher crystallinity plastics, such as PET, which was characterized to have a crystallinity percentage of 9.2% in chapter 2 degrade far slower in this study compared to PA, a plastic which characteristics suggest is lower in crystallinity. Crystallinity and water absorption have an inverse relationship, with high crystallinity levels resulting in low water absorption and low crystallinity levels causing high water absorption.



**Figure 3-5.** Water absorption of PET under laboratory conditions for 16 weeks, incubated at 25°C with MQ. Error bars indicate standard deviation.



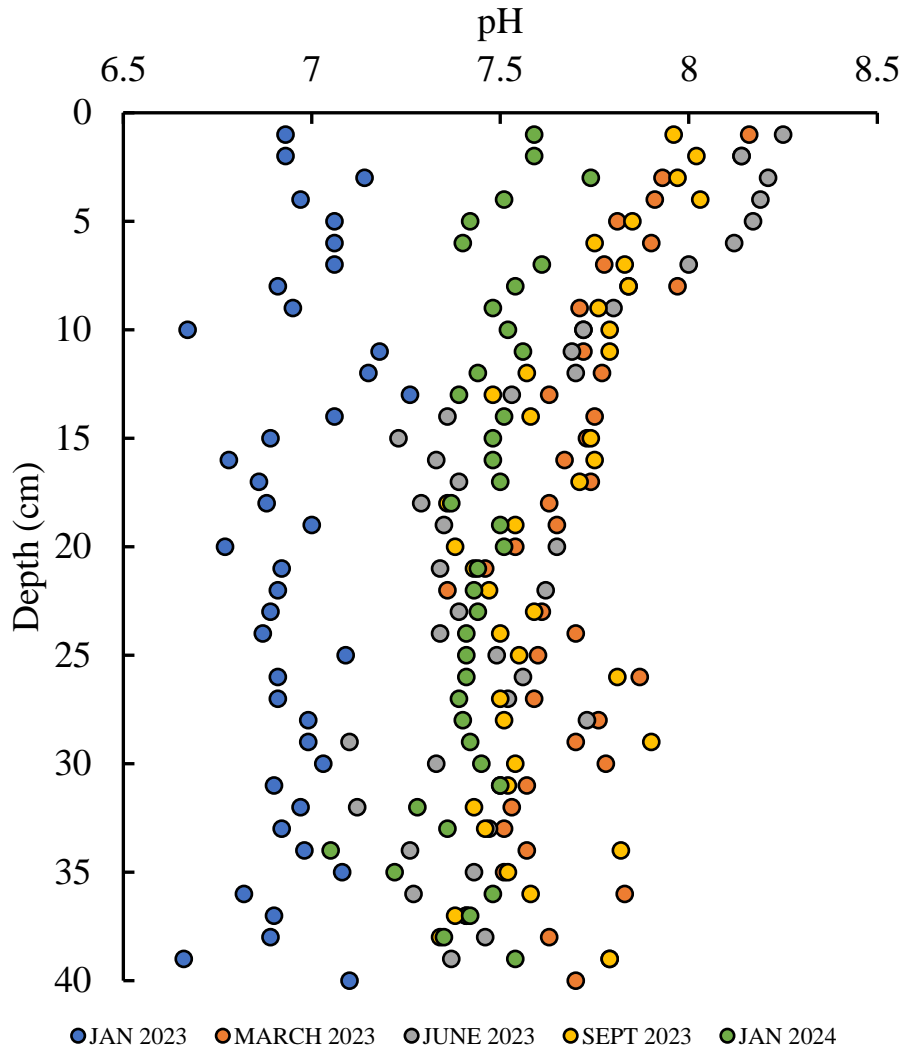
**Figure 3-6.** Water absorption of PA under laboratory conditions for 10 weeks, incubated at 25°C with MQ. Error bars indicate standard deviation.

To approximate the crystallinity of PA and confirm the cause of the mass increase during weeks 30 and 42 of the field study was due to water absorption (Negoro et al., 20016), a laboratory experiment was conducted on both plastic types. Plastic strips were cleaned, left to air dry and then submerged in tubes containing MilliQ water in an incubator set to 25 °C. PET was held in water for 16 weeks, with sacrificial triplicate sampling every two weeks (Figure 3-5). It was found that PET increases 0.5% due to water absorption. Later a water absorption test for PA found a maximum of 1.5% mass increase over 10 weeks in the same 25°C incubator (Figure 3-6). The results from this test proved that PET and PA intake water, resulting in mass increases. The duration of mass increase was significantly shorter in the laboratory experiment, lasting approximately 2 months compared to the mass increases seen for 8 months in the in-situ peepers

however the percentage of mass increase was similar for both lab and in situ. The difference in duration could be due to temperature variation, as the laboratory experiment held a consistent 25°C temperature while in situ plastic was held at a lower temperature in the sediment. Previous research has shown that water absorption is dependent on temperature and moisture content (Jabarin et al., 1986). Finally, in regard to estimating crystallinity percentage of PA we know PET has a crystallinity percentage of 9.2% and water absorption of 0.5%. PA has a water absorption rate three times that of PET, so crystallinity is expected to be one third of 9.2%, approximately 3% crystallinity.

### *3.3.2 Temperature and pH*

Temperature ranged from 3°C in colder months to 25°C in hotter months. Temperature by depth remains consistent during cold months but in warmer months temperature and pH decrease with depth. Average pH ranged from an average of 6.9 to 7.7, with an overall annual average of 7.5, and over the course of 16 months the pH ranged from 7 to 8, depending on month. Notably, January 2023, the first sampling point (2 months after initial incubation) had the lowest pH of all sampling points (see Figure 3-7). Degradation of polymers in sediment and the water column are dependent on water quality conditions such as pH and temperature which as noted above, change seasonally.

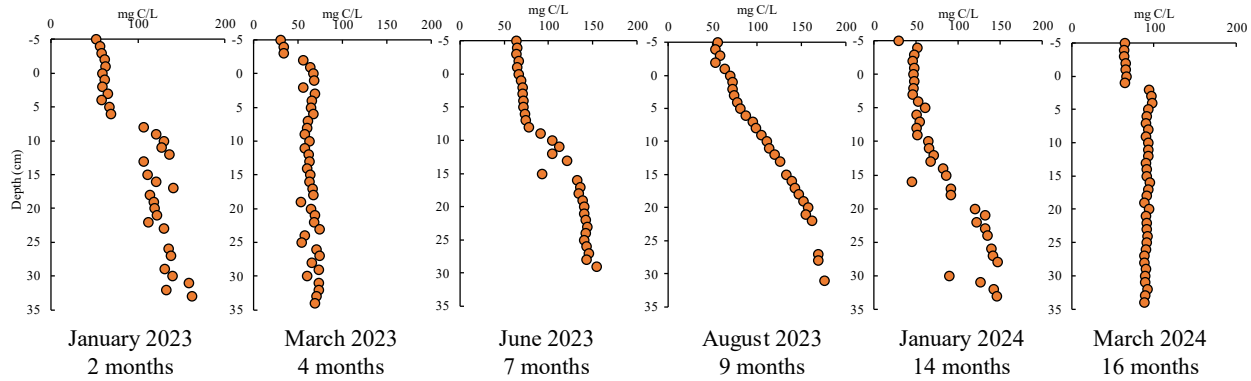


**Figure 3-7.** pH by depth from January 2023 (2 months) to March 2024 (16 months).

### 3.3.3 Sediment profile

The sediment profile of the stormwater pond was characterized using pore water retrieved from the pore water peepers at every timepoint tested. This included 40cm of depth, with the first 5 cells (5 cm above the sediment line, in the water) with the remaining 35 cm below the sediment line.

Dissolved inorganic carbon was measured throughout the year, with an annual average of 88.4 mg L<sup>-1</sup> of carbon. Values increased with increasing sediment depth except for the second timepoint (MARCH 2023, 4 months), where values were almost the same, regardless of depth.

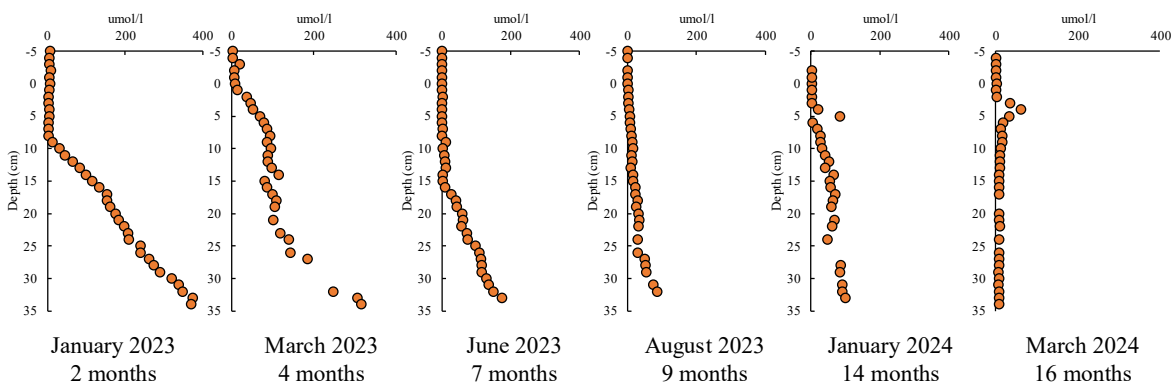


**Figure 3-8.** DIC depth profile through 35 cm of sediment and 5 cm of the water column over the course of 16 months. JAN 2023 (2 months) is the farthest left and increases to MARCH 2024 (16 months) on the far right.

The iron (II) profile followed expected results, with little to no iron (II) present in the first 10 cm of depth, before steadily increasing with increasing sediment depth. Throughout the year, iron levels in general decreased with the highest rates in January 2023 before decreasing consistently through MARCH 2023 (4 months) and JUNE 2023 timepoints, reaching the lowest at SEPT 2023 (10 months). Rain and snow events are large contributors to iron level changes, as snow melts or rain falls, the water seeps into soil and rock containing iron and the iron is dissolved into the water. We expect seasons which experience high rain and snow melt to produce higher rates of iron concentration and minimal changes to iron concentration in dry seasons. Changes to concentration

by sediment depth are a result of ferric iron ( $\text{Fe}^{3+}$ ) changed to ferrous iron ( $\text{Fe}^{2+}$ ) with the absence of oxygen farther down the sediment column (Iron in Well Water - MN Dept. of Health).

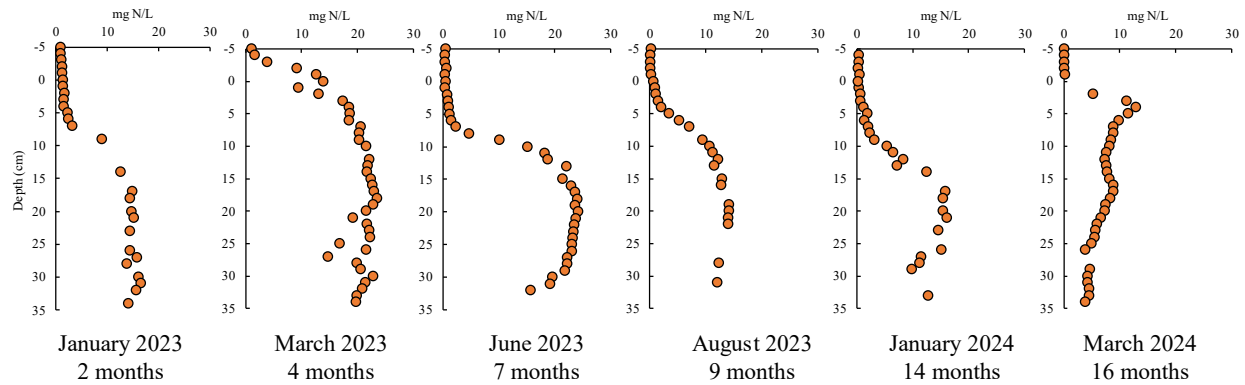
Interestingly, January 2024 saw a much lower amount of iron compared to January 2023. Iron levels only rose by  $20 \text{ umol L}^{-1}$  from the summer season, compared to January's levels of  $128 \text{ umol L}^{-1}$ . The annual average iron (II) level was  $56.9 \text{ umol L}^{-1}$ . This could be a result of less rain and snow melt experienced in January 2024 than January 2023.



**Figure 3-9.** Iron depth profile through 35 cm of sediment and 5 cm of the water column over the course of 16 months. JAN 2023 (2 months) is the farthest left and increases to MARCH 2024 (16 months) on the far right.

The annual average ammonium concentration was  $9.9 \text{ mg L}^{-1}$  of nitrogen, with averages ranging from  $18 \text{ mg L}^{-1}$  in June to lows of  $7.0 \text{ mg L}^{-1}$  in September. Concentration increases by depth, peaking at 20 cm below the sediment and decreasing slowly after.





**Figure 3-10.** Ammonium depth profile through 35 cm of sediment and 5 cm of the water column over the course of 16 months. JAN 2023 (2 months) is the farthest left and increases to MARCH 2024 (16 months) on the far right.

Other anions tested included bromide concentration, which changed seasonally, with an average concentration of  $0.22 \text{ mg L}^{-1}$ . Sulfate varied in concentration in the top 15 cm of the sediment before decreasing to under  $5 \text{ mg L}^{-1}$  for most time points for the remaining sediment depth tested. Warmer months saw higher concentration in the water column and the top 15 cm of sediment. The average concentration was  $13.5 \text{ mg L}^{-1}$ . Nitrate concentrations varied in the water column but remained consistent in the sediment by depth, with all values under  $10 \text{ mg L}^{-1}$  in the sediment column. The average concentration was just over  $1 \text{ mg L}^{-1}$ . Phosphate concentration varied in the sediment column with an average of  $1.4 \text{ mg L}^{-1}$ . Presence of phosphate was more commonly detected in later months of experiment, with most phosphate data obtained at September 2023, January 2024 and March 2024 timepoints. Chloride concentration varied in the top 15 cm of the sediment column and water but remained consistent farther down the sediment column, with all values under  $6000 \text{ mg L}^{-1}$ . The 16-month average was  $1061 \text{ mg L}^{-1}$ . Variation in the water and sediment was seen in colder months, while hotter months saw no variation by depth. Fluoride was

detected at several time points, with most values falling under  $2 \text{ mg L}^{-1}$  and an overall average of  $0.56 \text{ mg L}^{-1}$ . See supplementary figures for more information on pore water chemistry.

### **3.4 Discussion**

In this experiment, mass loss was not observed in PET plastic, a mass gain was seen, unlike previous studies. PA plastic degraded slightly however significantly less than other polymer studies reported in Table 3-1. Not only does this suggest that microbes in the sediment were better able to degrade PA than PET, but it also indicates the reduced activity or ability of these microbes to degrade the polymer compared to other sediments.

In broader degradation studies, degradation occurring on land or under biological processes, are higher than degradation in sediment. On land, little degradation is seen while biological degradation ranges from 0 to  $1.1 \times 10^1 \mu\text{m year}^{-1}$  (Chamas et al., 2020). Comparing mass loss of degraded polymers suggests sediment is a location which results in the least amount of degradation to polymers in the environment. Using studies listed in Table 3-1, we find all rates of polymer degradation in sediment are under 1% after 1 year of incubation. The study by Beltrán-Sanahuja et al (2020) found mass loss under 1% with PLA which is considered a bioplastic and is more degradable than PA. A study by Welden and Cowie (2017) found PA degraded by 1% after 1 year under marine sediment. PE, a plastic similar to PET, was buried in the same study and degraded 0.45% after 1 year. This strongly suggests stormwater sediment is less capable of degrading polymers compared to other sediment. There are several factors which may be affecting the degradation within sediment: microorganism presence, pH and mechanical degradation.

Microorganism presence, diversity and abundance can influence the rate of polymer degradation in sediment. Oceans and large lakes contain more biodiversity and organisms known to degrade

polymers. Additionally, stormwater ponds act as pollutant catchment sites before water enters larger rivers and lakes. Most city water is treated with high levels of fluoride, nitrate and chloride which negatively affect biodiversity and presence of species in the pond. Based on the pore water chemistry collected, elevated concentrations of some elements were found. High levels of fluoride, chloride or sulfate can affect microbial community, biomass, composition, and inhibit the activity of some enzymes (Zhang et al., 2019; Zhang et al., 2022; Lackner et al., 2020). For heavy metals, such as iron (II), a high concentration results in a decline of microbial biomass, activity and diversity (Chen et al., 2014). A previous study investigating microplastic degradation in marine environments in polluted and non polluted sediment found variations in degradation rates and abundance of microbial community (Beltrán-Sanahuja et al., 2020). The elevated levels of elements found within the sediment are likely a factor inhibiting polymer degradation.

Secondly, the pH of the pond could be a characteristic influencing the rate of degradation. For studies conducted in the ocean, salt water is a better buffer for controlling pH which plays a large role in determining which species and organisms thrive (Luan et al., 2023; Bartram et al., 2014). Contrarily, fresh water does not have the same buffering capacity. More neutral pH values result in increased degradation of polymers (Lyu et al., 2007). The pH changed seasonally in this study and should be investigated further in the future to determine the impact of pH changes. Lastly, the usage of pore water samplers (peepers) to house polymer samples for this study removed the possibility of mechanical degradation caused by continuous water movement or friction between sediment and polymer. Many previous studies were done with larger plastic samples, which formed biofilms and did not account for the possibility of mechanical degradation.

The timeline shown in this study demonstrates mass loss is not linear under these conditions. To date we have very little information of how plastics degrade over time, be that linear or non-linear

degradation. Many previous studies have estimated lifespans on short term mass loss results and assuming said mass loss is linear however it remains unclear if that is accurate for all environments. This represents a gap in literature that has not been filled, as most studies measure weight of plastics before and after degradation, but rarely during.

Previous work has demonstrated stormwater ponds to be sinks for microplastics, however it's possible microplastics degrade at decelerated rates in stormwater ponds compared to seawater due to a lack of biodiversity caused by water pollution or altered pH levels. Mechanical breakdown caused by water current and mixing may also be a factor affecting the low degradation of polymers seen during this study.

### **3.5 Conclusion**

Stormwater ponds are known to be entrapment sites for microplastics and heavy metals however microplastics within the sediment are degrading at decreased rates despite having higher abundances. Both polymers saw a mass increase from water absorption over the first 6 months, which later began decreasing. After 16 months, PA was degraded by 0.42% and PET still had a net positive mass. This suggests while stormwater ponds are effective at trapping microplastics, they are not a viable solution to degrade plastics and may cause pond sediment microbial changes.

## **4. Conclusion and future research recommendations**

### **4.1 Summary of key findings**

The degradability of polymers in the environment directly affects plastic pollution clean up efforts through the amount of plastic present in oceans, rivers and land. Since the mass production of plastics began, scientists and communities have been raising the alarm regarding plastic waste despite research claims funded by fossil fuel companies, similar to claims made about gas, oil and coal use. The degradation potential of plastics dictates how long these polymers will persist in the environment and how many generations will have to deal with the lingering toxic chemicals released as plastic degrades gradually. By determining the rates of plastic degradation, realistic estimates of lifespan can be determined and extrapolated, to develop technologies and methods that can degrade plastic waste. Current technologies use outdated research, not by fault of their own, but as a result of plastic producers changing commercial plastic composition in efforts to make the polymer more resistant to degradation. There are valid reasons for these changes in polymer composition, as new plastics and manufacturing processes were discovered.

In Chapter 2, a series of laboratory experiments were conducted with a PET degrading enzyme to determine several factors that affect degradation rates of laboratory grade polyethylene terephthalate (PET). Temperature was tested as the first factor, followed by two types of pretreatments; freeze thaw cycles (FTCs) and exposure to extreme temperatures (+55 °C or -70 °C). Finally, rates of degradation between laboratory PET and commercial/consumer grade PET water bottles were explored. Experiments ranged from 10 days to 16 weeks, with samples scarified in even dispersion. Activation energy was determined using the temperature experiment data. Exposure to higher temperatures or any exposure to pretreatment increased degradation of

laboratory grade PET compared to plastic at lower temperatures or plastic without pretreatment. When consumer grade PET water bottles were exposed to the hydrolysing enzyme at various temperatures for an extended period of time, no significant degradation was observed. This is especially concerning as it demonstrates laboratory grade PET does not degrade at similar rates as commercially available PET, with the latter more abundant in the environment. A mass of analyses conducted demonstrate this lack of degradation through DSC (crystallinity percentages and a lack of Tg), FTIR analysis, tensile strength, mass loss, and TGA. The list of analysis conducted also aided to determine which were the most successful at detecting degradation.

In Chapter 3, we examined the degradation of PA and PET plastic in stormwater pond sediment over 16 months. Pore water peepers were installed along with pore water peepers filled with the pond sediment and plastic pieces. They were sacrificed periodically over 16 months and data was collected by depth. Both plastic types were found to intake water for the first 8 months before mass began to decrease. At 14 and 16 months, PA was found to have mass loss however PET still had a mass increase compared to the original weight of the plastics in the sampler. Pore water chemistry revealed seasonal changes and aided to establish sediment zones of the pond. While previous research has proven microplastics are great sinks for microplastics, were these plastics are stored in the sediment, we see significantly lower rates of degradation in the sediment compared to other studies investigating degradation in the water column and for floating plastics.

The combination of Chapter 2 and 3 provide a contrast between laboratory “ideal” degradation rates possible under ideal condition compared to more realistic conditions in the environment. In chapter 2, conditions such as temperature were high to increase degradation and a hydrolytic enzyme was present to mimic enzymatic degradation, the enzyme simply degrades the polymer at an accelerated rate so mass loss can be measured in a shorter time frame. The pH level of the

laboratory experiments conducted in Chapter 2 were also controlled to maximize the efficiency of the enzyme used. Under environmental conditions in Chapter 3, pH, temperature and microorganisms were not controlled, leaving the polymer to degrade under changing conditions. Using the degradation rates of laboratory plastic in Chapter 2 and 3 which found laboratory plastics degrade quickly with the use of an enzyme at high temperatures but minimally in the environment, specifically sediment, we can infer consumer plastic which did not degrade under ideal conditions in Chapter 2 would take considerably longer to degrade under the environmental conditions seen in Chapter 3. These results demonstrate the importance of environmental conditions on degradation rates and prove even laboratory grade plastics take considerable lengths of time to degrade in the environment. We find even under ideal conditions, consumer plastic which makes up most of the plastic produced worldwide, does not degrade. Under environmental conditions the lack of degradation is even more likely and without large efforts to minimize plastic from entering the environment, plastics will settle and accumulate in sediment with very little chance of degrading.

## **4.2 Recommendations for future research**

In Chapter 2, a series of experiment were conducted utilizing differences in temperature and plastic type, along with the presence of pretreatment, either extreme temperatures or FTCs. Additional experiments could be conducted to identify more environmental conditions that have an effect on degradation. Specifically, Chapter 3 saw seasonal changes to the pond's pH level, which could have an impact on degradation rates. By conducting an experiment similar to those performed in Chapter 2 under laboratory settings, the impact of pH on PET degradation could be observed.

During the series of experiments outlined in chapter 2, we did not test the solution plastic samples were held in for microplastic presence. These experiments were conducted with macroplastics because mass loss would be significantly easier to detect however its possible the enzyme was cleaving areas of the plastic off into microplastics, instead of degrading the plastic piece. It would be beneficial to determine if degradation of polymers using enzymes results in microplastics and if so, the amount.

A significant research recommendation regards the FTIR analysis conducted in Chapter 2 with differences between consumer and laboratory-grade plastics. A diagnostic library could be created to identify environmental polymer samples which were heat treated (secondary heating) as we have proven a secondary heating likely reduces the degradability of a polymer greatly. Using a library such as this could be used to identify abundances of plastics that have undergone a secondary heating and any trends in polymer type or by location.

Mass loss results for plastic in the stormwater sediment from Chapter 3 are strikingly lower than rates reported in literature, even under similar conditions. Conducting similar long term experiments in multiple ponds would aid in strengthening evidence put forth in Chapter 3. One large aspect of this project which was not explored was the microbial communities present in the stormwater pond. Oxygen availability was another line of evidence that was not collected which could provide useful information for microbial community health. Sediment was collected, incubated in the porewater samplers with the plastic pieces and sampled, stored using liquid nitrogen however a microbial community assay to characterize microbes was never conducted. This line of research could contribute to explaining the low rate of degradation seen if few microbial communities were present, there was an alternative food source, or microbes were under stress from environmental factors.



The majority of this work saw most difficulties with producing degradation under realistic conditions or using “real world” consumer plastics. More aggressive methods of degradation may be needed to degrade consumer plastics however these methods cannot come at the expense of producing more toxic chemicals or waste that will only continue to negatively impact the environment and species in the surrounding area. If research into waste cleanup, reduction and alternatives to plastic is not continued, not only will plastic pollution continue to contribute to climate change but landfills and their negative effects will continue to affect communities across the globe. We have viable alternatives that would heavily reduce our consumption of plastic and this work aids in displaying why a switch from plastic is gravely needed given what is known about polymer degradation.

## **Data availability**

The experimental data presented in Chapters 2 and 3 are openly available on Federated Research Data Repository (FRDR) at <https://doi.org/10.20383/103.0897> (Chapter 2) while data for Chapter 3 is available on FRDR at <https://doi.org/10.20383/103.0926>.

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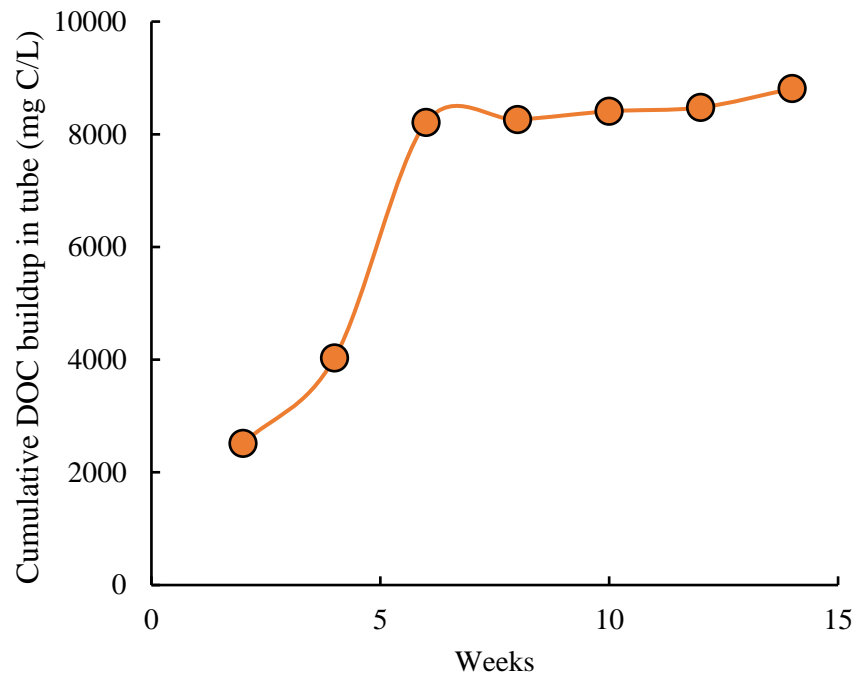
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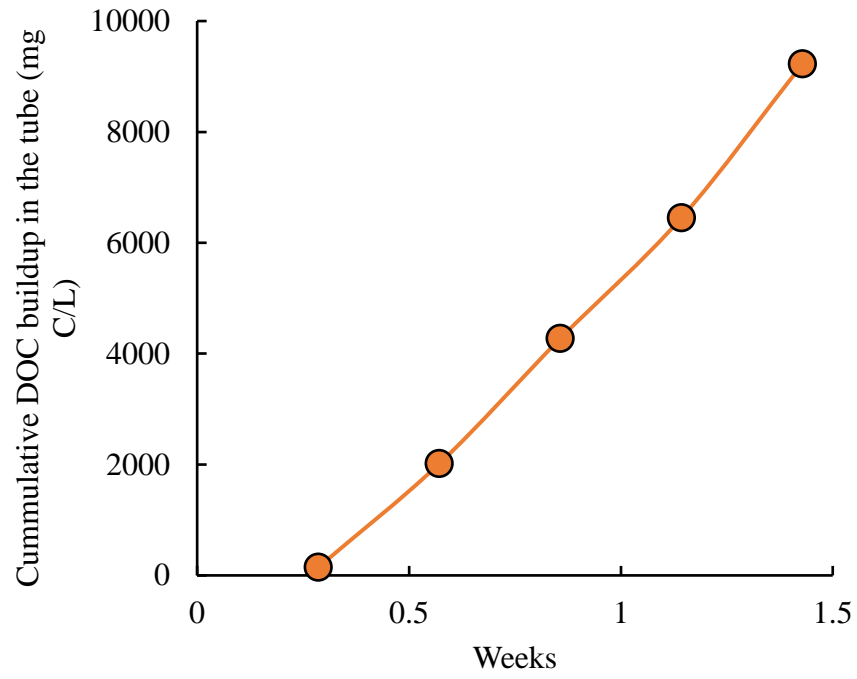


## Appendix

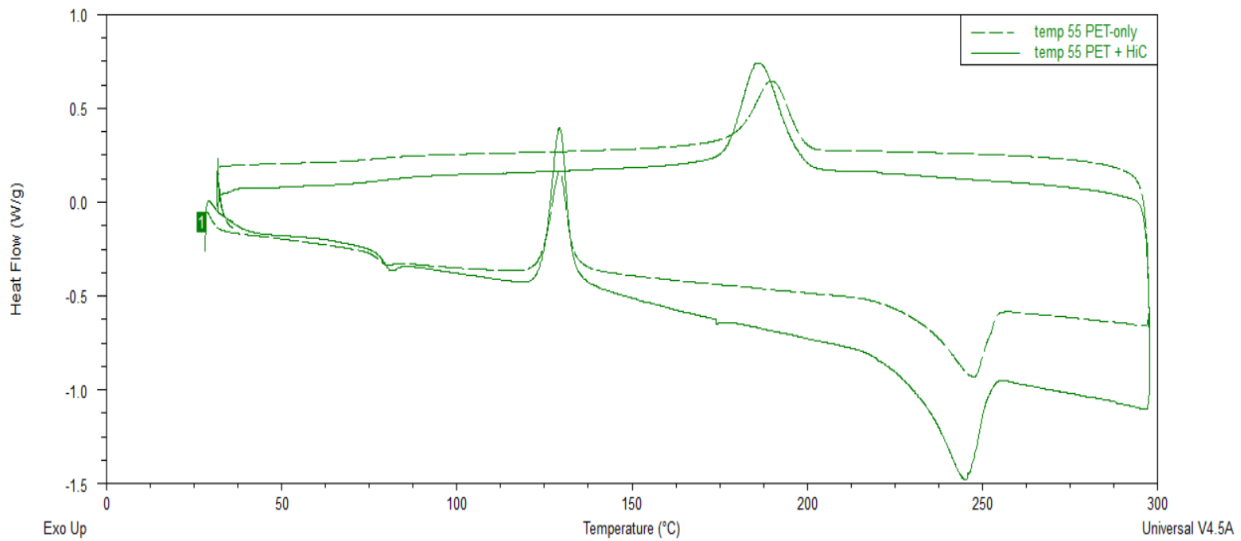
### Appendix I-Additional experimental results for Chapter 2



**Figure AI-1.** Cumulative DOC buildup in the tube for the temperature experiment at 40 °C, PET + HiC for 16 weeks.

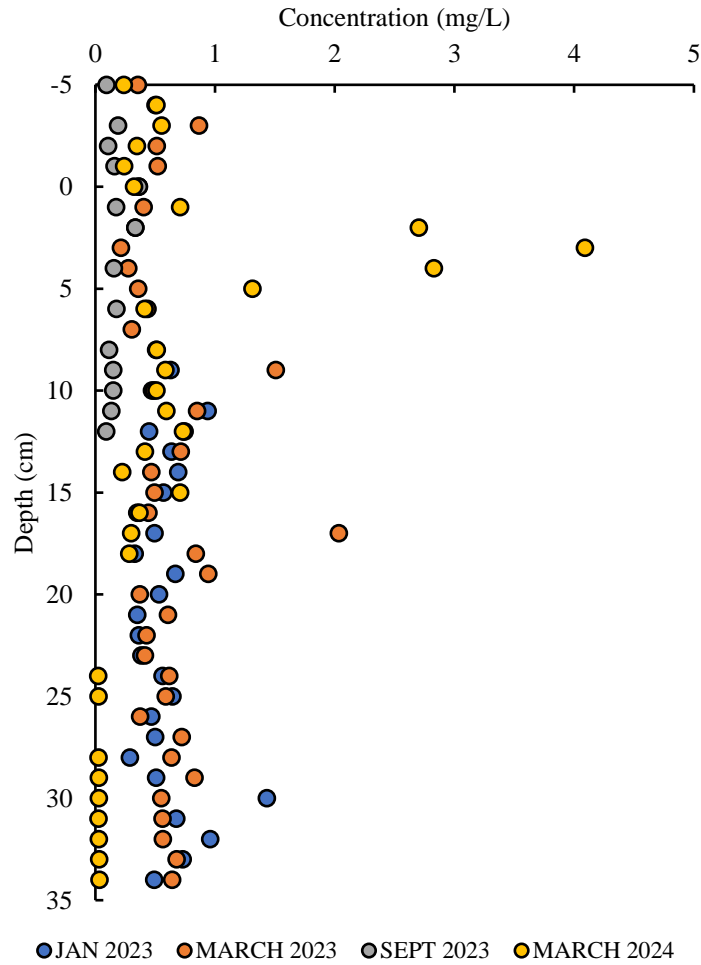


**Figure AI-2.** Cumulative DOC buildup in the tube for the temperature experiment at 55 °C, PET + HiC for 10 days.

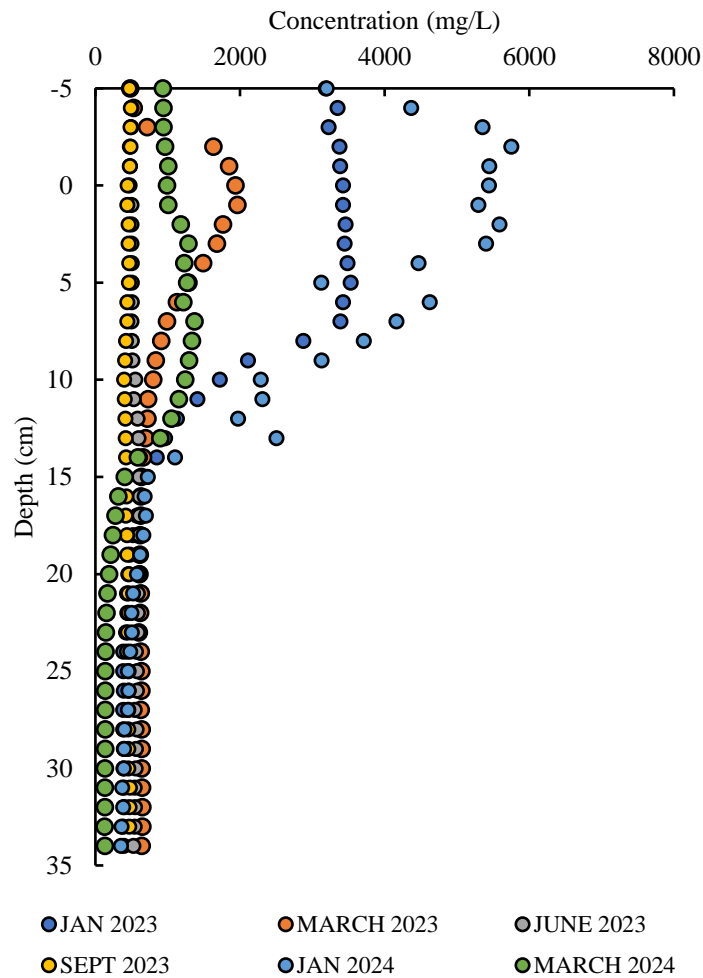


**Figure AI-3.** DSC scan of PET + HiC and PET-only samples incubated at 55 °C, no pretreatment.

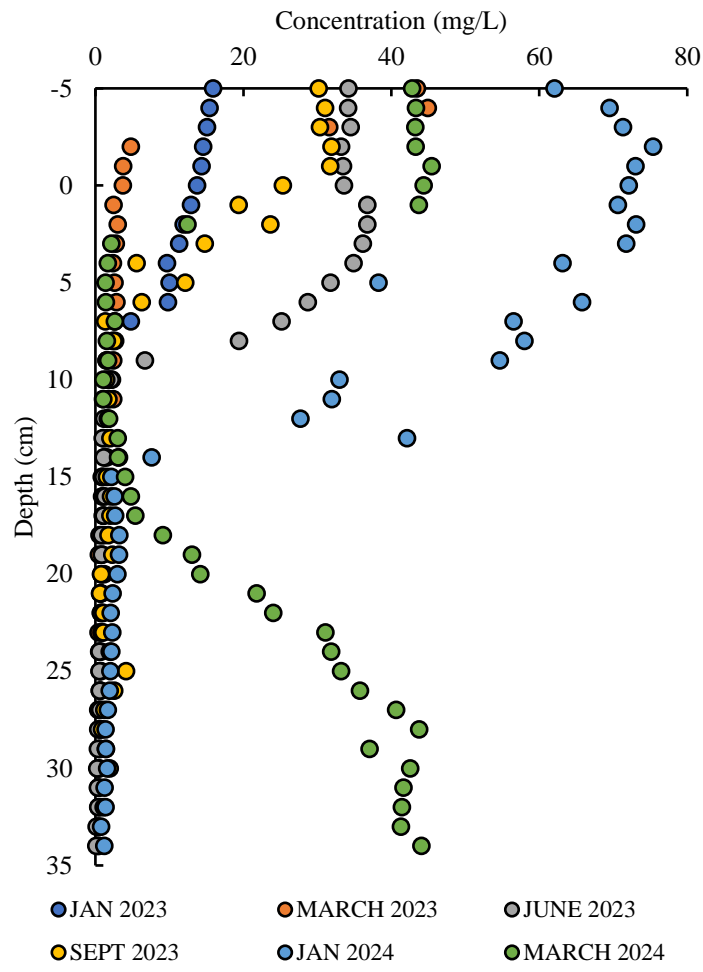
## Appendix II-Additional experimental results for Chapter 3



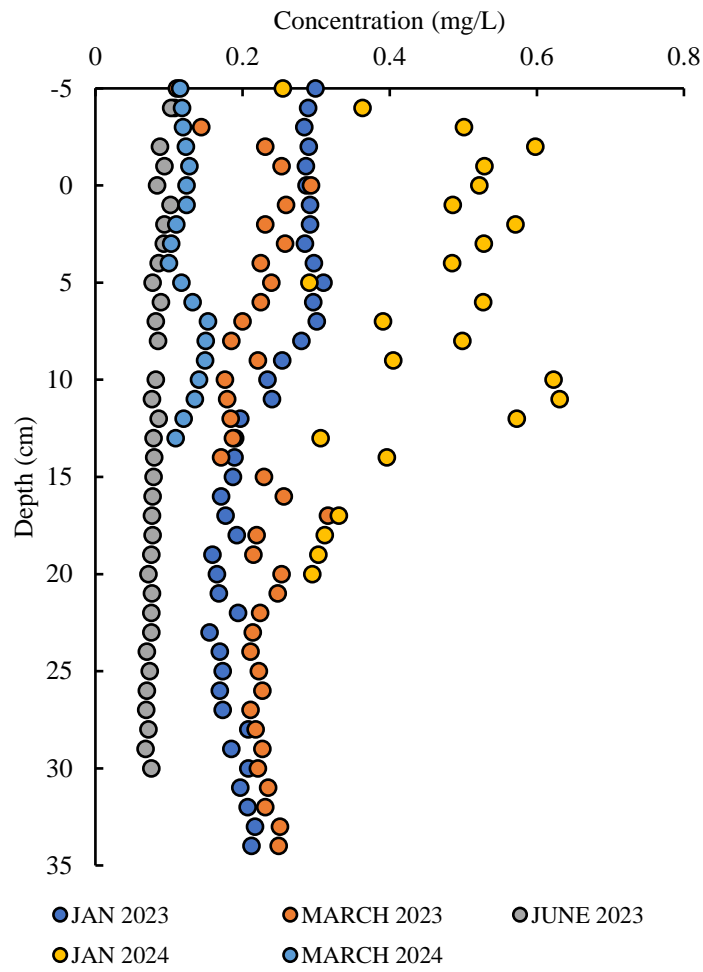
**Figure AII-1.** Fluoride concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of fluoride.



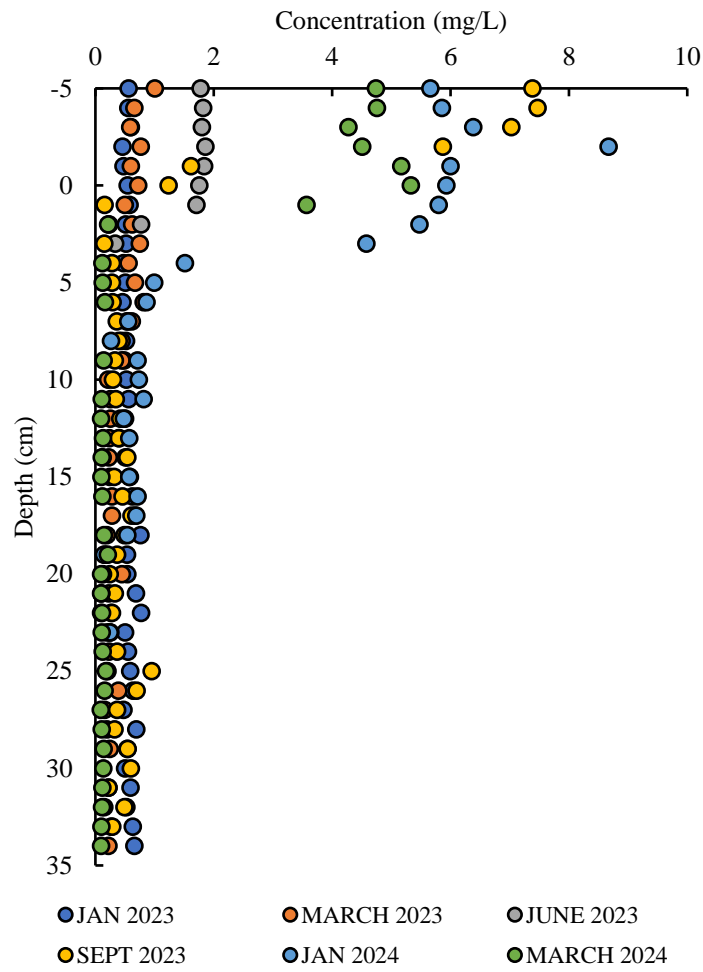
**Figure AII-2.** Chloride concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of chloride. Seasonal variability is seen higher in the sediment and water column.



**Figure AII-3.** Sulfate concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of sulfate. Seasonal variability is seen higher in the sediment and water column.

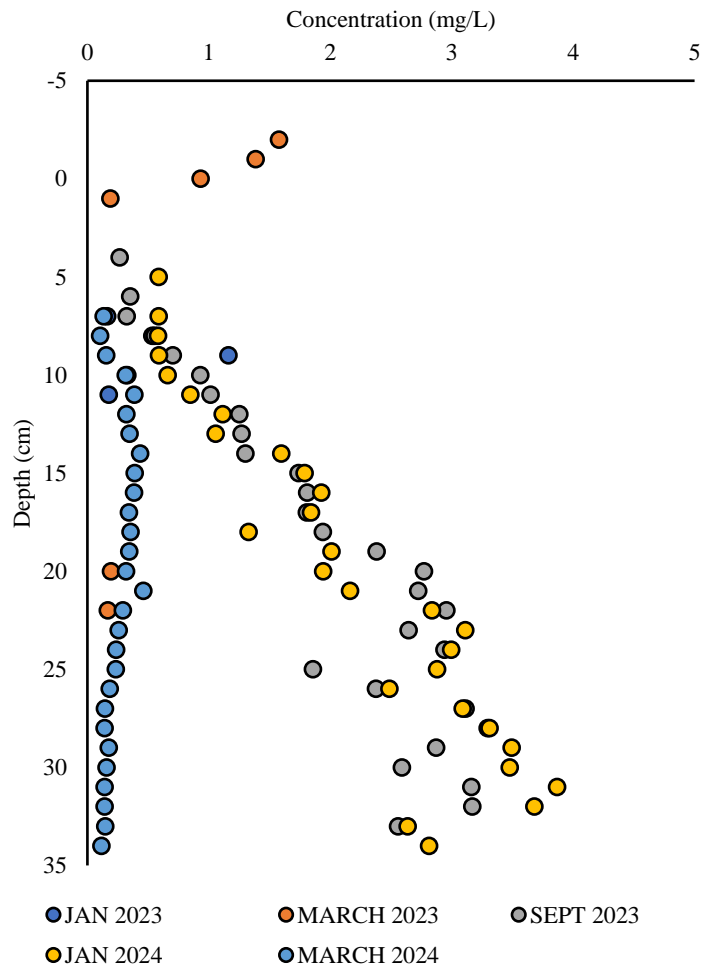


**Figure AII-4.** Bromide concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of bromide. Seasonal variability is seen higher in the sediment and water column.



**Figure AII-5.** Nitrate concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of nitrate or above the method detection limit (MDL).





**Figure AII-6.** Phosphate concentration ranging from January 2023 to March 2024 by depth. All timepoints were tested and missing datapoints indicate no detection of phosphate.