Thesis Title

Enhanced Hydrolysis of Polyethylene Terephthalate (PET) plastics by Ozone and Ultrasound Pretreatment

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ABSTRACT

The rapidly accumulating post-consumer polyethylene terephthalate (PET) plastics pose a great threat to our environment as they constitute one of the most used products in our day-to-day life. As a result, degradation of PET and recycling has become the focus of considerable interest in the last decade. Hydrolysis of PET is very challenging as they are extremely resistant to both biotic and abiotic degradation. A technically and economically feasible approach to degrade PET waste from the environment is highly desirable. Physicochemical pre-treatment can play an important role in making PET more degradable by changing their surface properties. Direct recycling of segregated PET has problems of contamination of additives and components used in various PET products. PET hydrolysis however can lead to recovery of the monomers terephthalic acid (TPA) and ethylene glycol (EG) as well as the dimers bis(2-hydroxyethyl) terephthalate (BHET) and mono(2-hydroxyethyl) terephthalate (MHET) which can be reused for making new PETs. This can potentially solve the difficulties associated with PET recycling and lead to a circular economy. The present study reports the effect of ozone and ultrasound pretreatment on both enzymatic and chemical hydrolysis of PET. The results showed that combination of ozone pretreatment followed by ultra-sonication during enzymatic hydrolysis using HiC cutinase enzyme resulted in almost 9-fold increase in TPA and EG recovery compared to enzymatic hydrolysis of untreated PET. However, the long reaction time in enzymatic hydrolysis prompted us to investigate chemical hydrolysis. Although, chemical hydrolysis of pretreated PET films using methanolic sodium hydroxide as solvent resulted in 80% weight loss (at 50C, atmospheric pressure), the recovery of monomers was relatively not as efficient as enzymatic hydrolysis. Size reduction of the PET films followed by chemical hydrolysis gave the highest (90%) breakdown, but it is a very energy intensive process.

Keywords: Enzymatic hydrolysis, chemical hydrolysis, polyethylene terephthalate (PET), pretreatment, ozone, ultrasound.

LAY SUMMARY

Plastic pollution is a major environmental concern in today's world. The enormous use of plastic has led to accumulation of wastes in both terrestrial and marine environments. Polyethylene terephthalate (PET) is one of the most commonly used plastic that are used in food and beverage packaging. Usually, PET products are disposed of immediately after a single use and makes a high contribution of low degradable wastes. The accumulation of PETs in the landfills, waterways and oceans critically affects the ecosystem and food chain. They are broken down in size forming micro plastics but remain unconverted for hundreds of years. These particles can be consumed by animals and aquatic organisms and eventually can enter human food streams. The overall aim of this study was to address this issue and move towards finding a sustainable solution. Use of biological catalysts (enzymes) to break down PET is a potential method for plastic degradation. This will allow of recovery of their building block (monomer) constituents. The novelty of this study was to incorporate physicochemical pretreatments like ozone and ultrasound for enhancing the efficiency of enzymatic hydrolysis. However, the process being slow, chemical breakdown (hydrolysis) was also attempted. This reduced reaction time but needed size reduction, which is energy intensive. Besides the monomers, this process also led to small chain polymer chains. The breakdown of PET to its building block (monomers) can help in their reuse to produce new plastic products again and hence contribute to a sustainable circular economy.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGUREURES

List of Abbreviations

PET	Polyethylene Terephthalate
TPA	Terephthalic Acid
EG	Ethylene Glycol
BHET	Bis(2-hydroxyethyl) Terephthalate
MHET	Mono(2-hydroxyethyl) Terephthalate
HiC	*Humicola insolens* Cutinase
LDPE	Low Density Polyethylene
HDPE	high density polyethylene
PVC	Polyvinyl Chloride
PP	Polypropylene
PS	Polystyrene
PHAs	Polyhydroxyalkanoates
WL	Weight Loss
US	Ultra Sound
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High-performance liquid chromatography
UV	Ultra Violet
OSHA	Occupational Safety and Health Administration
DET	Diethyle Terephthalate
pNP	p-nitrophenol
pNPB	p-nitrophenol butyrate

Chapter 1

INTRODUCTION

The expansion of various industries like construction, automotive and packaging in the twentieth century had led to the revolution of petroleum-based plastic products worldwide (Lintsen *et al.* 2017; Sánchez, 2019). The use of different types of plastic in day to day life has increased 20 folds since 1964 because of its low cost, durability, transparency, chemical inertness, light-weight and versatility (Eriksen *et al.*, 2019; Sánchez, 2019). The global production of plastic is around 335 million tons and is expected to reach 1124 million tons by 2050 which indicates a four-fold increase (EMF, 2016; PlasticsEurope and EPRO, 2017; Young, 2019). The major plastics used in day-to-day life today include low density polyethylene (LDPE), high density polyethylene (HDPE), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyhydroxyalkanoates (PHAs), polylactic acid (PLA) etc. Among various petroleum-based plastic, PET has gained enormous popularity in the packaging industry, especially in beverage and drinking water bottles. Figure 1.1 shows the application of PET in different industrial sectors.

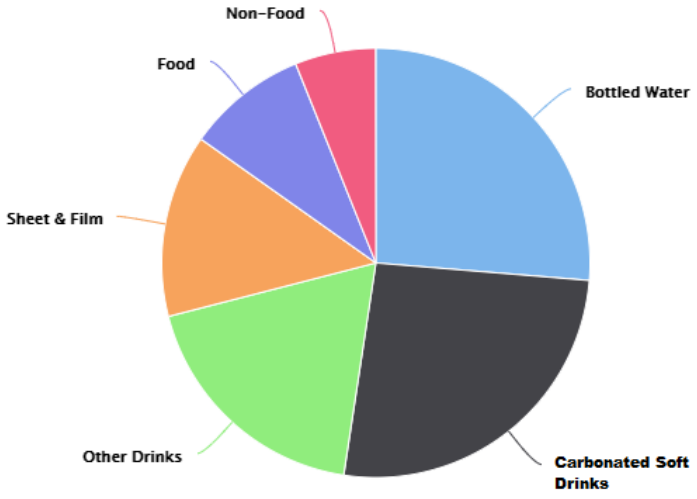

Figure 1.1: Consumption of PET in different sectors globally (source: PlasticInsight.com, 2020)

A report by Global PET-Market, (2017), global PET production was 50.1 million tons and it was expected to reach 87.16 million tons by 2022. PET is a thermostable polyester which is composed of ethylene glycol (EG) and terephthalic acid (TPA) (Wei and Zimmermann, 2020; Liu *et al.*, 2018; Qiu and Chen, 2020; Chen *et al.*, 2020). In 2017, global PET production was 30.3 million tons (PlasticInsight.com, 2020). As regards specifically PET bottles, a recent report by Statista highlighted 485 billion PET bottle production in 2016 and predicted an increase to 583.3 billion by 2021 (Tiseo, 2021). The high volume of production of PET generates a great amount of post-consumer wastes in the environment as most of the packaging is thrown away after a single use. The high molecular weight of PET has made it almost impossible to biodegrade naturally thus can accumulate in the environment for hundreds of years (Chen *et al.*, 2020). Eventually, these post-consumer PETs pose a great threat to the environment by accumulating waste in the ocean and prevailing as micro-plastic in the terrestrial ecosystem (Beaumont *et al.*, 2019; Qiu and Chen, 2020).

Although PET is considered highly recyclable, the scenario is very poor in actual practice in most countries. The fate of the post-consumer plastics in Canada is shown in Figure 1.2.

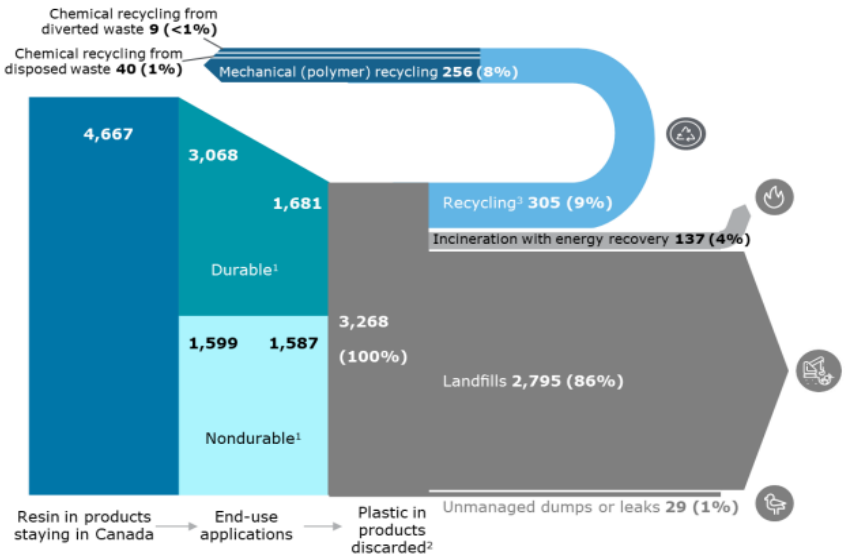

Figure 1.2: Canadian plastic flows in thousands of tons per annum, 2016 (Source: Canadian Plastic Industry, 2019).

Studies reported that approximately 72% of packaging waste is not recovered properly where 32% escapes the collection stream and 40% ends up in landfills and the ocean (Wei and Zimmermann, 2020; Liu *et al.*, 2018). The Recycling Council of Ontario reported that only 9% of plastic waste are recycled in Canada. Difficulties in the segregation of different plastics are one of the major hurdles in PET recycling. Post-consumer PET bottles that are collected from households, industry, commercials, are mixed with other types of plastics. When all these plastics are recycled together, they get contaminated and produce low quality end products (Canadian Plastic Industry, 2019).

Chemical and physical methods including incineration, solvent extraction etc. have been used to get rid of this polymer waste (Gan *et al.* 2009; Phale *et al.* 2019; Kim *et al.*, 2019). However, these physicochemical methods have some disadvantages. They can introduce or convert into other harmful compounds like dioxins and furans leading to more complexity and are costly to implement as well (Phale *et al*. 2019; Kim *et al.*, 2019).

Over the years, research on the biodegradation of plastic is gaining much popularity as it has no adverse effect on the environment (Arutchelvi *et al.*, 2008; Niaounakis, 2013; Tiso *et al.*, 2020; Ghosal *et al.*, 2016; Furukawa *et al.*, 2019). Biodegradation can be done either by the microorganism or by using the hydrolase enzymes produced by the microorganisms. Enzymatic degradation of PET is ecofriendly and hence can play a major role in accomplishing sustainable development (Lorenz and Kandelbauer, 2016; Furukawa *et al.*, 2019). As enzymatic hydrolysis breaks the easter bonds of the polymer chain, there is a high chance of recovering the monomers (Furukawa *et al.*, 2019; Wei and Zimmermann, 2020; Kumar *et al.* 2013).

PET has structural similarities with cutin, a waxy polymer in plant cuticle. Thus cutin degrading hydrolase enzyme cutinase can also degrade polyesters (Furukawa *et al.*, 2019). Cutinase from many bacteria and fungus including *Ideonella sakaiensis, Fusarium oxysporum, Fusarium solani, Thermomyces insolens, Thermobifida alba, Thermobifida fusca, Humicola isolens, Penicillium citrinum, and Bacillus subtilis* showed PET degradation capability (Nimchua *et al.* 2008; Liebminger *et al.*, 2009; Ronkvist *et al.*, 2009; Ribitsch *et al.*, 2011, 2012; Yoshida *et al.*, 2016).

However, the enzyme sometimes lacks the ability to cleave the polymer bonds efficiently. In order to make the polymer more prone to hydrolytic cleavage, different physical and chemical pretreatment methods have been studied. These include mechanical size reduction by grinding, UV irradiation and weathering effect, chemical treatment etc. (Esmaeili *et al.*, 2013; Tian *et al.*, 2017; Tribedi and Dey, 2017; Farzi *et al.* , 2019). These physicochemical pre-treatments help to loosen the polymer bonds, increase functional groups on the polymer surface, change the surface topography resulting in efficient hydrolysis. For instance, oxidation of polymer surface by ozone causes formation of active functional group and ultrasound causes formation of microbubbles on the surface, hence, the polymer become more prone to hydrolytic cleavage (Pellis *et al.*, 2016; Tian *et al.*, 2017). To the best of our knowledge, ultrasound and ozone for the hydrolysis of PET have not been studied extensively yet. Therefore, the rationale of this study is to understand the effects of ozone pre-treatment and ultrasound treatment on PET followed by enzymatic and chemical hydrolysis. These hydrolysis processes cloud led to accumulation and recovery of the monomers which overcomes the difficulty of mixing different types of plastics. The overall aim of this study was to incorporate pretreatment such as ozone and ultrasound in order to improve the hydrolysis process efficiency without using harsh chemicals.

<u>The specific objectives of this study were to:</u>

1) Evaluate the effect of ozone and ultra-sonic pretreatment on chemical and enzymatic hydrolysis of PET.

2) Evaluate the effect of a combination of ozone and ultrasonic pretreatment on chemical and enzymatic hydrolysis of PET.

3) Estimate the recovery of the monomers (TPA and EG) and dimers (BHET and MHET) after hydrolysis of PET.

Chapter 2

LITERATURE REVIEW

2.1. The problem of Plastic Pollution

Plastics are synthetic polymers with excellent physicochemical properties which result in prolonged life even in harsh environmental conditions(Shimao, 2001; Kawai, 2010). Plastics can be classified in two broad groups, petroleum-based plastic and bio-based plastic. Generally, petroleum based plastics are made of polymer derivatives such as polyethylene, polystyrene and polyvinyl. Under natural condition these plastics can persist in the environment for hundreds of years. With their wide range of application, plastic pollution has become a major (Cózar *et al.*, 2014; Klein *et al.* 2015). As a consequence, bio-based plastics like polyhydroxy alkanoate (PHA), polyhydroxy butyrate (PHB), polylactic acid (PLA) etc. have emerged. These products are made from renewable biomass source like corn starch, vegetable fats and oils, wood hemicellulose etc. which can be biodegraded easily (Varsha *et al.* 2011). Unfortunately, all bio-based plastics are not degradable (Ribitsch *et al.*, 2012). Nonetheless, it has been reported (Muller *et al.*, 2005; Esmaeili *et al.*, 2013; Tournier *et al.*, 2020) that different types and combinations of physicochemical treatments have shortened the degradation period from hundreds of years to months. This thesis is an attempt to contribute to this effort.

2.2. Plastic Degradation

As mentioned earlier, plastic degradation has become a major concern as it tends to remain unchanged for hundreds of years after being disposed of. Plastic pollution poses a major threat to our environment, marine ecology, wildlife as well as human life itself. Generally, mechanical, thermal, photo-oxidative or microbial methods are used for plastic degradation. However, there are some drawbacks and difficulties with these methods and need improvement. Some of these processes are described below.

2.2.1. Mechanical Degradation

Mechanical strength plays an important role in the durability and reliability of plastics. Plastics can undergo several mechanical degradations at the time of processing, storage, and use. Mechanical degradation can take place because of mechanical stress such as shear forces, tension and/or compression (Briassoulis, 2006, 2007). Mechanical degradation comprises of four major routes including regrinding, adhesive pressing, compression molding and injection molding (Bhuvaneswari, 2018).

2.2.1.1. Regrinding: The process is also known as powdering because plastic waste is ground into a powder and reused for different applications. The two methods used here include ball milling for grinding rigid polymer and roll milling for flexible polymer. One of the common applications of these powdered polymers is as fillers for the production of elastomers or polymer foams (Bhuvaneswari, 2018). The energy costs involved for size reduction of plastics are high which do not make them economically feasible.

2.2.1.2. Adhesive pressing: It is a simple and rapid recycling process. Adhesive pressing is one of the oldest processes for the physical recycling of plastic foam. In this method, the surface of plastic particles such as polyurethane is coated using an adhesive binder and bonded in a heated press. The recycled products can be used as carpet underlay, production of mats, sports hall floor parts etc. (Bhuvaneswari, 2018). This method does extend the use of the plastics used, but is limited by the amount that can be reused in this way and the quality of new product.

2.2.1.3. Compression molding: This is a process where the molding of plastic particles occurs at high temperature and pressure that helps the flow of the neat particles without the need for any additional binders. The end product of this procedure increases the stiffness which is used in automotive parts (Bhuvaneswari, 2018). Contaminants present in the recycled plastics obtained need to be segregated or separated, which is not an easy task.

2.2.1.4. Injection molding: This is also a type of molding that helps the recycling of cross-linked polymer products. Usually, this method is used for mixing different thermoplastics (Singh and Sharma, 2008; Yousif and Haddad, 2013; Bhuvaneswari, 2018).

In mechanical degradation, the segregation of plastics is not done precisely. As a result, there is a high chance of contamination or the mixing of different types of plastics in the waste which eventually decreases the quality of the recycled products (Ragaert *et al.* 2017).

2.2.2. Thermal Degradation

Molecular deterioration of plastics as a result of overheating is known as thermal degradation. The three reactions occur during nonoxidative thermal degradation are depolymerization, random chain scission and side-group elimination (Król-morkisz and Pielichowska, 2019). A recombination process that leads to cross-linking or cyclization may occur during the process (Kaczmarek *et al*., 2012) . In most cases, more than one degradation mechanism takes place simultaneously.

In depolymerization, which is a free radical process, polymer chains are degraded to monomers, dimers and/or oligomers at high temperatures. It is a continuous process that lasts until the polymer is completely depolymerized (Król-morkisz and Pielichowska, 2019). Thermal depolymerization is usually suitable for those polymers that are generated by chain polymerization such as polymethyl methacrylate (PMMA), polystyrene, and polyoxymethylene (POM). It is the opposite of polycondensation of polymers (Król-morkisz and Pielichowska, 2019).

The second mechanism in polymer thermal decomposition is random chain scission. This process generally follows multiple free-radical generating routes including initiation, propagation and termination steps (Ray and Cooney, 2012). These steps cause fragmentation of the polymer. The fragmented molecules differ in chain length as well as some volatile molecules. In the case of end-chain scission, process monomers are removed from the chain ends which is another possible pathway for the polymer thermal degradation (Król-morkisz and Pielichowska, 2019). The molecular weight and mechanical strength of polymer decrease drastically in chain scission processes. Random chain scission is a classic degradation mechanism for polyolefins (Ueno *et al*., 2010; Wilkie and Morgan, 2010).

Side group elimination is the third degradation mechanism in which the bonds between the side groups and the main chains are broken. The free side groups can react with each other parts of the same molecule resulting in cyclic structures. The molecules

formed are usually volatile products of degradation (Ueno *et al.*, 2010). In case of PET, although the glass transition temperature, T_g is 67 C for amorphous PET and 81 C for crystalline PET, thermal degradation can occur at extremely high temperature, approximately 250-400 C, by random chain scission of ester bonds. This is because the melting point (T_m) of PET is very high which is 260 C. The end-groups in PET are mostly hydroxyl-ester, hence, they form vinyl-ester end groups and carboxyl end groups after thermal degradation (Sarker and Rashid, 2013). However, thermal degradation is not environmentally friendly. This process produces many harmful compounds that are eventually released into the atmosphere. Greenhouse gases like carbon dioxide, methane, toxic oxygen-based free radicles, heavy metals are released in this process (Webb *et al.*, 2013).

2.2.3. Photo-Oxidative Degradation

Photon energy in light causes photodegradation. Most polymers are susceptible to this degradation initiated by UV and visible light (Bhuvaneswari, 2018). Generally, the lifespan of polymers for outdoor applications is determined by the extent of exposure to near-UV radiation in the sun light (290-400 nm) (Singh and Sharma, 2008). Photodegradation changes the physical and optical properties of the plastic. Different polymers require different wavelengths for degradation based on the bonds present in the polymers. For instance, polypropylene needs around 370 nm wavelength for degrading whereas polyethylene requires around 300 nm (Bhuvaneswari, 2018).

The mechanisms involved in photodegradation are expressed by the Norrish reactions that transform the polymers by photoionization (Norrish I) and chain scission (Norrish II) (Singh and Sharma, 2008). Photodegradation can conduce Norrish reactions, cross-linking reactions or oxidative processes (Niaounakis, 2013). Photo-oxidative degradation involves both oxygen and ultraviolet radiation which results in breaking of chemical bonds, polymer chains, produces radicals and reduces the molecular weight (Lucas *et al.*, 2008).

Photodegradation and thermal degradation can be accelerated by the use of oxidants. In an atmosphere containing oxygen, the onset of decomposition temperature decreases. The degradation rate and its mechanism may drastically change in the presence

of oxidants (Król-morkisz and Pielichowska, 2019). Although photo-oxidative degradation is environmentally friendly compared to thermal degradation, the process is very slow. It might take 50 years or even more to degrade polymers via this process. However, the slow changes of surface topography of polymer make them more susceptible to microbial degradation as they provide better binding site for microbial attachment.

2.2.4. Microbial and Enzymatic Biodegradation

When micro-organisms bring about the breakdown of polymers, they are called microbial biodegradation. The steps involved are the attachment of microbes on the plastic surface, growth of microbes using the plastic as the carbon source (Yousif and Haddad, 2013). When microbes attach to the surface of the polymer, they secrete enzymes that break down the polymer into monomers or oligomers. For aliphatic polyesters, the breakdown products are consumed by the micro-organisms as carbon sources for growth. The biopolymers are degraded into CO_2 and water in aerobic conditions and CO_2 and CH_4 in the anaerobic environment (Arutchelvi *et al.*, 2008a; Singh and Sharma, 2008).

Although biodegradation of plastic has been reported in many research studies for the last few decades, under normal condition the process is very time consuming (Albertsson, 2004; Koutny *et al.*, 2006; Arutchelvi *et al.*, 2008a). The slow rate of degradation by micro-organisms is attributed to physical limitations, lack of functional group, hydrophobicity and high molecular weight of polymer(Koutny *et al.*, 2006). The degradation can occur by single microbial colonies or complex microbial communities. The type of polymer used as a substrate can influence the structure of a microbial community isolated on a polymer surface during biodegradation experiments. It has been proven by several studies that the ability of microbes to form a biofilm structure depends on the physicochemical nature of the surface (Donlan, 2002; Restrepo-flórez *et al*. 2014).

The organisms that are generally involved in polymer biodegradation are fungi, bacteria and algae (Leja and Lewandowicz, 2010). In simple words, biodegradable polymers are the food for these micro-organisms. The enzymes, either intracellular or extracellular, produced by the microbes chemically react with polymer and cause the polymer chain to break (Nair *et al.*, 2017).

Considering all the degradation methods, microbial biodegradation seems to be a promising way to reduce the environmental effects of plastic wastes. The limitations of this method can be overcome by using genetic engineering technology that will lead to the generation of novel microbes and species with high efficiency of polymer degradation. Further details on recent research on microbial and enzymatic biodegradation of PET is given in a following section. The use of select microorganisms and enzymes capable of breaking down PET is presently the focus of research in our research group.

2.3. Factors Affecting Plastic Biodegradation

There are certain attributes that determines how well and rapidly a plastic will biodegrade. The general factors that affect plastic biodegradation are discussed below.

2.3.1. Hydrophobicity

Hydrophobicity is the physical property of the polymer that makes them repelled by water because of the contact angle of the polymer surface with water. The higher the contact angle with water the more hydrophobic the polymer surface (Arutchelvi *et al.*, 2008b; Roy *et al.*, 2008; Restrepo-flórez *et al.* 2014). The hydrophobicity of a polymer surface relies on the exposure, concentration, and nature of the functional groups available in the polymer. Hence, hydrophobicity is an important attribute in biodegradation which, in turn, determines the colonization of microbes on polymer surface (Restrepo-flórez *et al.* 2014). It further supports the reports that hydrophilic surfaces are more susceptible to microbial attack (Donlan, 2002; Wang *et al.*, 2012).

2.3.2. Degree of crystallinity

Crystallinity is another parameter that affects biodegradation which defines the degree of structural order of the polymer material. The rigidity and density of plastic increase with the increase in the degree of crystallinity. The polymer structure consists of the amorphous region and the crystalline region. The crystalline microstructures are surrounded by amorphous regions (Restrepo-flórez *et al.* 2014)). Several studies report the breakdown of the amorphous region before the crystalline structures due to their accessibility. This increases the percentage of crystallinity (Raghavan, 1992; Manzur,

Cuamatzi and Favela, 1997; Santo, Weitsman and Sivan, 2012). The fate of the crystalline region is not yet clear. However, it has been suggested that after consuming the amorphous region microbes attack and consume small crystal parts of polymers, increasing the percentage of larger crystal parts(Manzur, Cuamatzi and Favela, 1997; Restrepo-flórez, Bassi and Thompson, 2014). Therefore, after a certain period, the biodegradation rate decreases. In our study, we used an amorphous PET plastic sheet that have been used as a standard in many researches.

2.3.3. Surface Topography

The surface of plastic is usually even when molded for their respective applications. A change in the surface topography indicates physical degradation of the plastic. It has been proven that the colonization of micro-organisms on the polymer surface results in a change of surface topography. When micro-organisms attack the polymer, they tend to colonize on the surface depending on the availability of some functional groups (Bonhomme *et al.*, 2003; Hadad, Geresh and Sivan, 2005; Koutny *et al.*, 2006; Fontanella *et al.*, 2010; Pramila and Ramesh, 2011; 'Low-density polyethylene degradation by Pseudomonas', 2013). The chances of microbial attack increase with the increase of the number of functional groups available on the polymer surface. The functional groups on the polymer surface can be studied by FTIR spectroscopy. The most common functional groups responsible for microbial attachment on polymer surfaces are esters (1740 cm^{-1}), double bonds (908 cm^{-1}), carbonyls (1715 cm^{-1}) and vinyls (1650 cm^{-1}) (Restrepo-flórez, Bassi and Thompson, 2014).

2.3.4. Molecular Size

The high molecular size of plastic makes them resistant to microbial attack. The decrease in molecular weight is a common effect after a microbial attack due to the ingestion of smaller molecular size chains by the microbes(Lee and Johnson, 1992; Yamada-onodera, Mukumoto and Katsuyaya, 2001; Hadad, Geresh and Sivan, 2005; Santo, Weitsman and Sivan, 2012) although some authors conclude that abiotic factors like UV radiation affect molecular size rather than a direct microbial attack (Fontanella *et al.*, 2010). For the determination of molecular weight distribution two approaches have been discussed in many studies which are size exclusion chromatography and rheological

measurements (Yamada-onodera, Mukumoto and Katsuyaya, 2001; Bonhomme *et al.*, 2003; Hadad, Geresh and Sivan, 2005; Koutny *et al.*, 2006; Fontanella *et al.*, 2010).

2.4. Polyethylene Terephthalate (PET)

Polyethylene terephthalate (PET) has resin code number 1 and is composed of ethylene glycol (EG) and terephthalic acid (TPA) (Boyle and Örmeci, 2020). It has high molecular weight ranging from 192.2 – 228.19 g/mol of repeat units depending on the crystallinity. Degree of polymerization of PET depends on the purpose of the product used and can be ranged from 300-3000 repeat units. Figure 2.1 shows a general structure of PET.

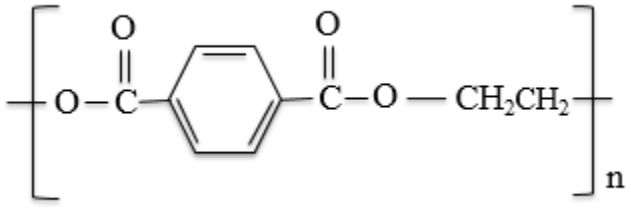

Figure 2.1: General structure of PET

In the food packaging system, PET is one of the most commonly used plastics that includes packaging of soft drinks, single-served water, sports drinks, oils, ketchup and salad dressing to name but a few. The main reason behind this increasing use of PET is its chemical and physical stability. They are lightweight, transparent and have high recyclability. Moreover, PET bottles provide an adequate gas barrier against carbon dioxide, oxygen, moisture which make them suitable liquid food packaging especially carbonated beverages (Ghoshal, 2019). Some of the widest use of PET bottles in liquid food packaging are discussed below.

2.4.1. Packaging of Water

Water is an essential everyday need of human life. Since the beginning of human culture, water has been stored and transported by vessels. Nowadays, the demand for filtered water leads to the popularity of the commercialization of bottled water (Ghoshal, 2019). Light weight, handy, sleek and contoured PET bottles gained popularity among consumers.

2.4.2. Packaging of Fruit Juice

Packaging organic juice requires sophisticated material to enhance shelf life in terms of the time frame of realistic usability. PET bottles are increasingly used for fruit juice packaging, because of having suitable properties like UV resistance, clearness, good oxygen barrier (Ghoshal, 2019).

2.4.3. Packaging of Carbonated Beverages

Carbonated beverages like soda, soft drinks contain carbonated water which causes pressure inside the package. Glass, metals and PET bottles can withstand such pressure. Unlike glass and metal, PET bottles are durable, light weight, low cost and easy to transport which make PETs perfect packaging material (Ghoshal, 2019).

Due to these vast use PET bottles are produced in huge numbers across the world which results in an increasing amount of waste generation. Since PET is non-biodegradable, it can remain unchanged for hundreds of years. According to a report by the "Environment and Climate Change Canada, 2019" annual waste generation by the packaging sector is around 43% of total plastic waste. The majority of waste generation is occupied by the post-consumer PET bottles. To manage this huge amount of PET waste, Canadian Plastics Industry Association (CPIA) introduced the 5Rs hierarchy that includes reduce, reuse, recycle, recover, and retain.

2.5. Recycling of PET Bottles

As mentioned earlier, the rate of recycling PET is considerably low. The Recycling Council of Ontario reported (2016) that only 9% plastics are recycled in Canada. As a result, the majority of the post-consumer PET ends up in landfills and oceans. Eventually, this building up of plastic waste in the environment poses a great risk towards all living beings. Difficulties in the segregation of different plastics are one of the major hurdles in PET recycling.

2.5.1. Source of Contamination in Recycled PET

Post-consumer PET bottles that are collected from households, industry, commercials, are mixed with other types of plastics. When all these plastics are recycled

together, they get contaminated and produce low quality end products (Post-consumer Plastic Recycling, 2019). The contaminant sources are being divided into three broad categories. They include the chemicals used specifically for the recycling process, input contaminants and the degradation products. Besides, the different color blends also contribute to contamination during recycling. Studies showed that in the USA approximately 43% of recycled PET bottles are turned into fiber whereas 25% of recycled PET bottles are again used as bottles (Gopalakrishna and Reddy, 2019). Because of contamination, PET bottles lose their quality thus recycled PET bottles cannot give the ubiquitous properties of the primary PET bottles. The additives present in PET bottles also makes the recycling process difficult (Gopalakrishna and Reddy, 2019).

2.5.2. Regulation of PET Recycling

In order to recycle and recover PET bottles, different technologies and methods are used which follow the rules and regulations set by different countries. According to US regulations, the facilities that are responsible for PET recycling should follow some regulations to reduce contamination (Hurd, Seattle, WA, 98121). The dust exhaust and filtration system of grinders of PET plastics should follow the OSHA requirements. The Department of Transportation regulation is responsible for the transport of PET for recycling (Gopalakrishna and Reddy, 2019). At the time of transportation, PET bottles are restricted to different bales according to their type, size and density. Usually, three classes of PET bales are available. So called ‘soda bales’ containing only the carbonated beverage bottle. ‘Curbside bale’ contains custom PET containers along with soda bottles. ‘Custom bale’ contains only the custom PET bottles (Gopalakrishna and Reddy, 2019).

These strict regulations and the issues related to contamination have reduced the recycle of PETs. If recycle streams can be broken down to monomeric or dimeric level, it might solve the problems associated with contamination as well as serve as a potential for reproducing good quality PETs from the recovered monomers and dimers. Eventually, this will become a sustainable option by evolving into a circular bioeconomy.

2.6. Current Knowledge on Microbial and Enzymatic Degradation of PET

As mentioned earlier, biodegradation of PET has been gaining popularity over the past few decades because of the process being ecofriendly. Microorganisms from PET accumulation environment were investigated in many research and their ability to degrade amorphous and low crystallinity was discovered (Chen *et al.*, 2020; Sim *et al.*, 2021). Several bacterial and fungal microorganisms including *Psudomonas species, Streptomyces species, Enterobacter species, actinomycetes- Thermobifida fusca, Thermobifida alba, thermobifida cellulosilytica, Pichia pastoris, Humicola insolens, Fusarium solani* showed biodegradtion capability by convirting PET into its' oligomer and monomer, Bis-2-hydroxyethyle terephthalate (BHET) and mono-2-hydrosyethyl terephthalate (MHET) (Kawai, 2019). However, microbial degradation being very slow, approaches were taken to modify the genes of the microorganisms for improving their ability to degrade PET. The mutant microbes showed enhanced biodegradation in many studies.

Yoshida *et al.*, (2016) discovered a new bacterial strain, *Ideonella sakaiensis* 201-F6 that can use PET as its major carbon source by using a cutinase like enzyme PETase. This enzyme can act on MHET to release TPA and EG. They have reported conversion of 75% PET carbon using consortia of bacteria and yeast like cells (Yoshida *et al.* 2016). Chen *et al.*, (2020) engineered a similar PETase enzyme in yeast cell that increased enzyme activity by 36-fold compared to pure PETase. Furukawa *et al.*, (2019) did mutation of TfCut2 with similar sequence from PETase and constructed TfCut2 G62A/F209A. The mutant version of enzyme could biodegrade PET ~90% within 30 hours which is the highest biodegradation of PET so far.

Janczak et al. (2018) studied PET biodegradation by different rhizosphere microorganism in soil compost. After 6 months in compost, they recorded 20% loss of strength of the polymer. Because of the recalcitrant nature of PET, many researchers worked with the dimer oligomer such as Diethyle terephthalate (DET) and BHET for PET biodegradation studies. Liu *et al.*, (2018) studied mineralization of DET by *Delftia* spp. WL-3. They found ~94% degradation of DET in 7 days. Table 2.1 summarizes recent studies on Microbial biodegradation of PET.

Table 2.1: Recent Studies on Biodegradation of PET

Sample	Pre-treatment	Microorganism	Enzyme	Result	Reference
PET strip 25x5 mm	UV 30 mins, 365nm	*Pseudomonas* spp	Lipase	Increase in aliphatic index	Vague, 2019
PET powder	Mechanical	*Streptomyces* spp	-	68% biodegradation	Farzi *et al*. 2019
1.9% LC PET film	-	*Ideonella sakaiensis* 201-F6	PETase	75% PET carbon converted into CO_2	Yoshida *et al.*, 2016
lc PET film (250um goodfellow)	N/A	*Thermobifida fusca* KW3	TfCut2 & TfCut2s mutant	90± 4.5% biodegradation	Furukawa *et al.*, 2019
hc PET 6mm	N/A	*Pichia pastoris*	Engineered PETase	Enzymatic activity increased 36-fold	Chen *et al.*, 2020
DET PET	N/A	*Delftia* spp. WL-3	N/A	94% degradation of DET in 7 days. Pits and cavities on PET surface by SEM	aLiu *et al.*, 2018
PET Film	Extruded and then film formation	*Arthrobacter sulfonivorans, Serratia plymuthica, Laccaria laccata, Clitocybe* spp.	Fungi-cellulose Bacteria-Lipase	20% strength loss	Janczak *et al.*, 2018
BHET	N/A	*Enterobacter* spp. HY1	Esterase-recombinant EstB	84.4% degradation rate of BHET	Qiu and Chen, 2020

Table 2.1: Recent Studies on Biodegradation of PET (continued).

Sample	Pre-treatment	Microorganism	Enzyme	Result	Reference
Lc PET	N/A	Consortia with *Ideonella sakaiensis* 201-F6	Mutants of PETase	Degradation rate 0.13 mg/(cm^2 day)	Taniguchi *et al.*, 2019
Lc PET film 15x15 mm	N/A	Humicola insolens Pseudomonas mendocina Fusarium solani	HiC PmC FsC	97 % weight lossby HiC 5% weight loss by PmC and FsC	Ronkvist *et al.*, 2009
PET film 10mm x 5mm	N/A	Fusarium. solani	FsC	7-fold increase in hydrolyzed product	Eberl *et al.*, 2009
PET film 0.5 cm	N/A	Humicola insolens *Candida antarctica* lipase B	HiC CLAB	7.7 fold increase in TPA yield	Carniel *et al.*, 2016
PET film PET powder	Ultrasound -assisted	*Thermobifida cellulosilytica*	Thc_Cut 1	Film = 1.2-fold increase in TPA release Powder = 5.2-fold increase in TPA release	Pellis *et al.*, 2016
50 mg polyester powders	N/A	*Thermobifida cellulosilytica*	Thc_Cut 1	10 times increase in hydrolyzed product	Gamerith *et al.*, 2017

Similarly, Qiu and Chen, (2020) found ~84% degradation of BHET by *Enterobacter* species. Figure 2.1 shows the hydrolysis pathway of PET.

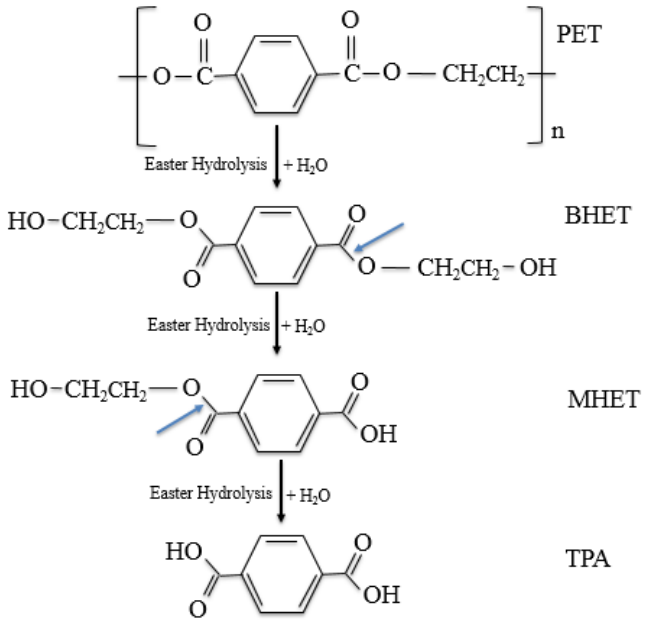

Figure 2.2: Hydrolysis Pathway of PET to TPA (Modified from Qiu and Chen, 2020)

Some microorganism secrete the hydrolase enzyme responsible for plastic biodegradation. The chain of PET polymer has easter bonds hence they are susceptible to enzymatic hydrolysis. To date, several studies reported PET hydrolyzing activity by lipase, cutinase, esterase and carboxylic esterase (Ronkvist *et al.*, 2009; Janczak *et al.*, 2018; Furukawa *et al.*, 2019; Qiu and Chen, 2020). All these enzymes basically belong to the serine esterase group of α/β superfamily which catalyzes the ester groups of polymer (b Liu *et al.*, 2018). They have similar catalytic centre called catalytic triad of Ser160-Asp206-His237 (Lenfant *et al.*, 2013). These enzymes break the covalent bond resulting in decreasing molecular weight.

Enzymatic breakdown of PET got highlighted when Muller *et al.*, (2005) discovered PET hydrolyzing enzyme TfH from *Thermoibifida fusca* that resulted in ~14% weight loss in 1 week and ~50% of weight loss in 3 weeks. Ronkvist *et al.*, (2009) reported weight loss of lc PET film of 97% by HiC and 5% by PmC and FsC after 96 hours. From HPLC analysis they found the presence of TPA only. In another study done by Carniel et al., (2016) showed combination of HiC and CLAB enzymes from *Humicola insolens* and *Candida antarctica* lipase B increased TPA release by7.7-fold. An interesting study by Pellis *et al.*, (2016) reported 1.2-fold increase of TPA release when enzymatic hydrolysis

was carried out in presence of ultra-sonication. Table 2.2 highlights PET hydrolyzing enzymes and their source organism.

Table 2.2: PET hydrolyzing enzymes and their source organism (adapted from Kawai *et al.,* 2020).

Name	Source	GenBank Accession	Sequence Identity (%)
lipase	Streptomyces exfoliatus		62.8
BTA-1 (TfH)	*Thermobifida fusca* DSM43793	AJ810119.1	100
Tfu_0882	*Thermobifida fusca* YX	AAZ54920.1	93.1
Tfu_0883	(*T. fusca* WSH03-11)	AAZ54921.1	100
TfCut1	*Thermobifida fusca* KW3	CBY05529.1	94.3
Est1	*Thermobifida alba*	BAI99230.2	83.1
Est119	AHK119	BAK48590.1	82.4
Thc_Cut1	*Thermobifida*	ADV92526.1	100
Thc_Cut2	*cellulosilytica* DSM44535	ADV92527.1	93.1
Thf42_Cut1	*Thermobifida fusca* DSM44342	ADV92528.1	97.7
Tha_Cut1	*Thermobifida alba* DSM43185	ADV92525.1	98.5
Thh_Est	*T. halotolerans* DSM44931	AFA45122.1	75.1
TfaXE	*Thermobifida fusca* NTU22	ADM47605.1	98.9
LC-cutinase	leaf-branch compost	AEV21261.1	56.6
Cut1	*Thermobifida fusca* NRRL	JN129499.1	93.1
Cut2	B-8184	JN129500.1	100
Tcur1278	*Thermonospora curvata*	ACY96861.1	61.9
Tcur0390	DSM43183	ACY95991.1	61.9
Cut190	*S. viridis* AHK190	AB728484	65.6
PETase	*Ideonella Sakaiensis* 201-F6	GAP38373.1	51.7
EstB	*Enterobacter sp.* HY1	MK681894	99.5
BsEstB	*Bacillus subtilis* 4P3-11	ADH43200.1	N/A
HiC	*Humicula insulens*	4OYY	N/A

Usually, cutinase activity is measured by the release of pNP from model substrate p-nitrophenol butyrate (pNPB) in order to compare polymer hydrolysis reaction. However, Heumann *et al.*, (2006) showed there is no correlation of cutinase hydrolyzing activity of PET with its enzymatic activity on pNPB.

While increasing studies about enzymatic hydrolysis of PET exist, knowledge gaps and lack of standard analytical method cause trouble in understanding the full extent of the degradation.

2.7. Chemical Hydrolysis of PET

Chemical hydrolysis of PET has been investigated by several research as there is potential for recovering monomers like enzymatic hydrolysis. Studies by Oku *et al.,* (1996); Lopez-Fonseca *et al.,* (2009); Rahman and East (2009) reported very high temperature and longer reaction time of chemical hydrolysis that makes the process costly. However, Bhogle and Pandit, (2018) investigated alkaline hydrolysis in presence of ultra-sonication in aqueous medium and non-aqueous medium. In non-aqueous medium the weight loss oh PET was 42% weight loss whereas in aqueous medium it was 20% because non-aqueous NaOH gives better affinity to the PET surface. In this process, firstly, sodium hydroxide (NaOH) cleaves ester bond of PET making disodium terephthalate (Na_2TA) and EG. Secondly, by using concentrated sulphuric acid (H_2SO_4), Na_2TA is neutralized and precipitated TPA as white solid. Figure 2.2 shows the mechanism of action of PET hydrolysis by NaOH.

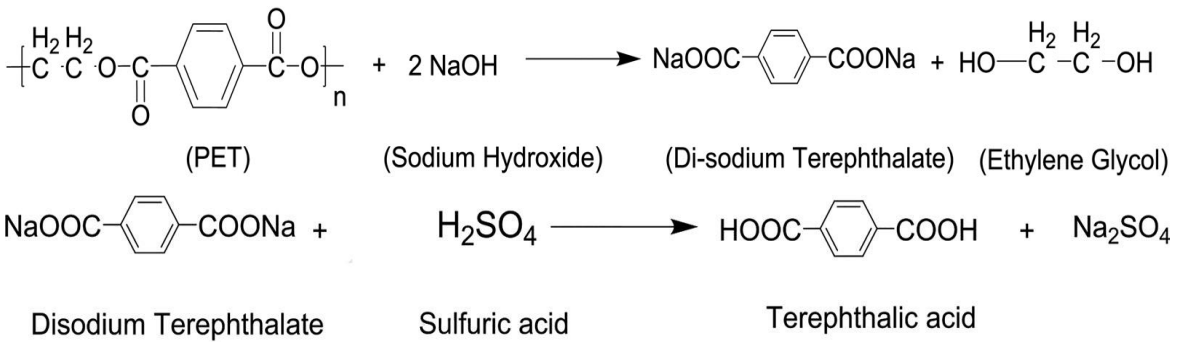

Figure 2.3: Formation of TPA by Chemical Hydrolysis of PET (Bhogle and Pandit, 2018).

Similar study was performed by Paliwal and Mungray, (2013) in presence of phase transfer catalyst and yielded 99% PET conversion to TPA. Ultrasound causes microbubbles on PET surface. This mechanical phenomenon increases hydrolysis.

Kamaruzamal (2014) studied solubility of PET in different solvent. Table 2.3 summarizes the resistance of PET from different chemicals.

Table 2.3: Chemical resistance by PET (Kamaruzamal, 2014).

Chemicals	**Resistance by PET**
Concentrated acids	Good
Diluted acids	Good
Alkali	Poor
Alcohols	Good
Aromatic hydrocarbons	Fair
Halogens	Good
Ketons	Good
Greases and Oils	Good

2.8. Pretreatment of PET for Hydrolysis

Physicochemical pretreatments showed an increase in biodegradability in other plastics. In the case of PET plastic, several micro-organisms show enzymatic hydrolysis effects but the rate of degradation is not up to the mark. So, pre-treatment of PET plastic can enhance its biodegradability Bhuvaneswari, (2018) Pre-treatment with ozone followed by ultrasound assistance during hydrolysis will cause change in hydrophobicity, crystallinity, surface topography of the PET by introducing oxygen to the surface. As a result, carbonyl (CO) and hydroxyl (HO) group will form on PET surface (Boyle and Örmeci, 2020). Eventually, these will lead to the increase of functional groups on the PET surface and will make a favorable environment for hydrolysis (Bhuvaneswari, 2018) Table 2.4 summarizes the effects of pre-treatment on plastic biodegradation.

Table 2.4: Biodegradation of Pretreated Plastics.

Plastic	Pre-treatment	Microorganism	Biodegradation without pretreatment	Biodegradation with pretreatment	Reference
LDPE films	UV irradiated	*Lysinibacillus xylanilyticus* and *Aspergillus niger*	15.8% (weight loss)	29.5%	Esmaeili *et al.*, 2013
LDPE films	UV treated	*Soil microcosms*	3.5% (gravimetric loss)	6%	Tribedi and Dey, 2017
PS films	Ozonation	*Penicillium variabile*	0.010 ± 0.003% mineralisation	0.15 ± 0.03%	Tian *et al.*, 2017
PET powder	Mechanical	*Streptomyces spp*	N/A	~70% weight loss	Farzi et al., 2019
PET powder	Mechanical	*Ideonella sakaiensis* strain 201-F6	N/A	90% de-polymerization	Tournier *et al.*, 2020
PET film	Thermal	*Thermobifida fusca*	N/A	49.7% weight loss	Muller *et al.*, 2005

2.8.1. Ozone Pretreatment

Ozonation had been used in wastewater treatment and polycyclic aromatic hydrocarbon (PAH) remediation in soil (Nam and Kukor, 2000; Wu *et al.*, 2021). Naphthalene, fluorine, phenanthrene and anthracene were efficiently removed from PAH contaminated soil using ozonation treatment followed by biodegradation (Nam and Kukor, 2000). Ozone can successfully oxidize organic contaminant in wastewater as it is a very strong oxidant (Nam and Kukor, 2000; Mohan *et al.*, 2019). Because the presence of a very unstable “nascent” oxygen atom in ozone molecule makes them highly reactive. Ozone transforms organic compounds into oxygenated intermediates either by direct oxidation or by generating hydroxyl radical. Hence, these intermediates become more soluble and

degradable (Nam and Kukor, 2000; Wu *et al.*, 2021). Figure 2.4 shows general reaction of ozone with aromatic structure.

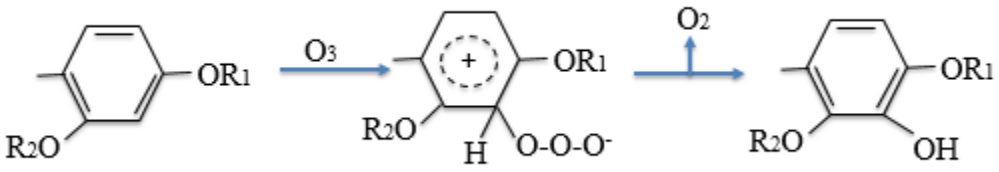

Figure 2.4: Reaction of Ozone with Aromatic Structure

Tian *et al.*, (2017) used ozone pretreatment for polystyrene mineralization by *Penicillium variabile*. They reported that ozonation formed active groups such as carbonyl groups on the polymer chain. However, in plastic degradation little research have been done incorporating ozonation process. To the best of our knowledge, our study is the first to analyze effects of ozone pretreatment for PET hydrolysis.

2.8.2. Ultrasound Treatment

Like sound wave, ultrasound is an elastic wave. But ultrasonic wave has higher frequency and low wave length, thus differ from sound wave propagation (Vikulina and Vikulin, 2018). Ultrasonication has been used in wastewater treatment and degradation of organic chemicals (Okitsu *et al.*, 2009). Usually, ultrasound causes formation of micro-cavities in compounds. These cavities generate shock waves by increased temperature and pressure inside the bubbles. As a results, breakage of weak bonds like hydrogen bonds and van der Walls bonds occurs (Pellis *et al.*, 2016; Vikulina and Vikulin, 2018). Figure 2.5 shows general mechanism of ultrasonic cavitation on polymer.

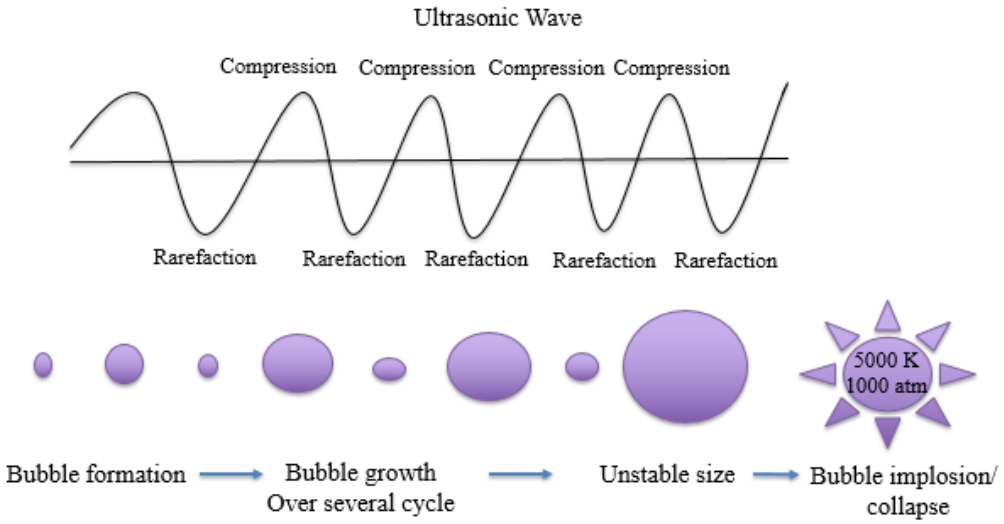

Figure 2.5: Ultrasonic Cavitation in Polymer

Chakraborty *et al.*, (2004) studied ultrasonic degradation of polybutadiene. Degradation of polypropylene and low-density polyethylene under acoustic induced cavitation had been reported by Desai *et al.*, (2008). Ultrasonic degradation of carboxymethyl cellulose and polyvinyl alcohol had been studied by Mohod and Gogate (2011). Daraboina and Madras, (2009) reported ultrasonic degradation of poly alkyl methacrylate. All these studies positively support application of ultrasound in depolymerization. As mentioned earlier, Ultrasound also helped in both chemical hydrolysis and enzymatic hydrolysis of PET (Paliwal and Mungray, 2013; Pellis *et al.*, 2016; Bhogle and Pandit, 2018).

From these studies we realized that there is considerable potential for improving effects of ozone and ultrasonic pretreatment for PET hydrolysis.

Chapter 3

MATERIALS & METHODS

3.1. Materials

The materials used throughout this study and the sources from which they were obtained are given in the following section.

3.1.1. Enzyme Catalyst

Immobilized HiC (cutinase, NZ51032, Novozymes, listed as Lipase by the supplier) was purchased from Cedarlane, Canada. The source organism for the cutinase enzyme was *Humicola insolens.* The enzyme was absorbed into polymethacrylate divinylbenzene copolymer beads and the particle size ranges from 250 to 700 μm. This was the only enzyme available commercially in the market at this time.

3.1.2. Plastic

Transparent, amorphous polyethylene terephthalate (PET) film (thickness 0.25 mm) was purchased from Goodfellow (Goodfellow Cambridge Limited, England).

3.1.3. Chemicals

p-Nitrophenyl Butyrate (CAS: 2635-84-9) was purchased from Cederlane. Analytical grade Bis-2-hydroxyethyle terephthalate (BHET), terephthalic acid (TPA) and ethylene glycol (EG) were purchased from Sigma-Aldrich.

3.1.4. Ozone Generator

For ozone pretreatment, Titan 1 Ozone Generator (WD8) was purchased form Longevity Ozone Resources Inc. Compressed Oxygen (UN 1072, class 2) obtained from Praxair was used as feed gas for the ozone generator.

3.1.5. Ultrasonic Bath

CPX Series Digital Ultrasonic Bath (40 Hz, CAT: 15-337-419, Fisherbrand™) from Fisher Scientific available in our lab was used for ultrasound treatment.

3.2. Methods

The enzyme assays and analytical assay methods used in this study are as follows.

3.2.1. Enzyme Activity Assay

Enzymatic activity assay for cutinase biocatalyst used in this study was adapted from (Carniel *et al*., 2016). Esterase activity of HiC was measured using spectrophotometric analysis of production of p-Nitro phenol (pNP) from the substrate p-Nitrophenyl butyrate (pNPB). A standard curve of optical density vs. pNP concentration in tris-HCl was prepared for concentration ranging from 1 to 25 mM (See Appendix B). The equation derived from the standard curve using excel graph was used to calculate esterase activity later. The equation with the best fit is-

$y = 5.7569x + 0.0894$……(1)

Where, y is absorbance and x is concentration of pNP in solution.

For the assay, 10 µL of 100 mM pNPB substrate were added to different centrifuge tubes and 1 mL of 0.2 M Tris-HCl buffer (pH8) was added to each tubes. Then 5 mg of immobilized cutinase enzyme was added to each tube and the reaction was allowed to occur for 8 minutes at 350 rpm. The temperature was 60C ± 2C. After 8 minutes, the solution was taken out using a micropipette into a microplate and absorbance of the solution was measured. The solutions were diluted few times to get absorbance in the range 0.2-0.8. Absorbance was measured by BioRad xMark microplate spectrophotometer at 405nm and the concentration of the released pNP was calculated using the equation relating absorption to concentration using the standard curve. Finally, enzymatic activity and specific activity of HiC on pNPB substrate was calculated using equation 2 and 3. The experiment was done in triplicate.

Enzyme activity, U (m mol/min) = $\frac{C*df*v}{t}$ …….. (2)

Where, C is concentration, df is dilution factor, v is total volume and t is time.

Specific activity (U/g) = $\frac{U}{mass\ of\ enzyme}$ ……. (3)

3.2.2. Ozone Production Analysis

Ozone density test was done to evaluate the production of ozone by the ozone generator. A 125 mL Erlenmeyer flask was flushed with pure oxygen gas for 5 minutes. A

50 mL syringe was 1st weighed empty and then with oxygen filled in it. Then the flask was flushed with ozone gas for 5 minutes at a flow rate of 6 L/min and ozone dosage of 3 g/hr, 6 g/hr and 12 g/hr. For each dosage, the syringe was weighed with ozone. Then density, mass fraction and ozone production per hour was calculated (See Appendix C) and compared with the data sheet provided by the company.

3.2.3. Pretreatment of PET film

The following pretreatments were done to PET films prior to the hydrolysis assays.

3.2.3.1. Ozone

This method was adapted from Tian *et al.*, (2017). For pre-treatment, 1x5 inch polyethylene terephthalate (PET) films were washed with diluted soap water and rinsed with mili Q water 3 times and then air dried over-night. The films were then hung with clips and placed inside the ozone chamber that was placed inside the fume hood and connected to the ozone generator. Oxygen from a cylinder was fed to the ozone generator at a flow rate of 6 L/min and 9 L/min in separate experiment. Ozone flow was interrupted after 0 hour, 2 hours and 4 hours for each flow rate. After reaction, the samples were removed, washed with methanol and air dried over-night before being used for the hydrolysis experiments.

3.2.3.2. Ultrasound

For ultrasound pretreatment, 1x1 inch PET films were weighed in electric balance and was put into 50 mL beaker. 15 mL methanol was added to the beaker and an ultrasound horn (Branson sonifier, 20 kHz) was dipped into the beaker 1 cm below the upper liquid level. The treatment was carried out for total 30 minutes with specific condition (70% amplitude with pulse of 1s ON and 1s OFF). So, the total reaction time was 15 minutes. After reaction, the samples were removed from the solution and air dried over-night for the hydrolysis process.

3.2.3.3. Size Reduction

1x1 PET films were mechanically grinded to get smaller particle size. A grinder from our lab was used to grind the films into powders. Then they were separated using sieves of different mesh size ranging from 250 μm to 1 mm.

3.2.4. Hydrolysis of PET films

Both enzymatic and chemical hydrolysis of PET films were done for virgin (untreated) PET and pretreated PET. The methods for both experiments are as follows.

3.2.4.1 Enzymatic Hydrolysis

This method was adapted from (Carniel *et al*., 2016). Each 1x1 inch (~185 mg) PET films/ pretreated PET films were weighed and taken in Erlenmeyer flasks and 10 mL of 0.1 M tris-HCl was added to each of them. Then 4 g of immobilized cutinase enzyme per gram of PET samples was added into the flasks. The flasks were placed inside the rotary shaker at 60C ± 2C under agitation of 100 rpm for 48 hours. After 48 hours PET films were removed from the buffer and washed with tris-HCl. The films were then kept in -80 freezer before freeze drying (-53C, 0.200 mBar, 8h). Enzymes were separated from the liquid solution by vacuum filtration and the liquid solution was stored in the freezer for HPLC analysis. Blank reactions were performed with treated PET film under the same reaction condition but without the enzyme. The final weight of the samples was measured and gravimetric weight loss was calculated. All the experiments were done in triplicate.

3.2.4.2. Chemical Hydrolysis

This method was adapted from Bhogle et al, 2018. In each 125 mL Erlenmeyer flask 30 mL 2.5 M NaOH/ methanol solution and two 1x1 inch (~370 mg) PET films/pretreated PET films were added. The flasks were placed inside the rotary shaker at 50C ± 2C under agitation of 100 rpm for 1 hour. After 1 hour 30 mL of chilled distilled H_2O was added in each flask to stop the reaction. Unreacted PET was separated by vacuum filtration, washed with distilled H_2O and then dried in hot air oven at 100 C for an hour.

The final weight of the samples was measured and gravimetric weight loss was calculated. Pure H_2SO_4 was added to the liquid solution until the pH dropped to 7. The

white precipitation was separated by vacuum filtration and washed again with distilled water to get the undissolved precipitation which is known to be TPA. It was the dried in hot air oven at 100 C for an hour. The rest of the liquid solutions were kept in the freezer for HPLC analysis. Finally, the weight of the recovered TPA was measured and was added to the HPLC recovery to get the total recovery of monomers and dimers. All the experiments were done in triplicate.

3.2.5. Ultrasound Assisted Hydrolysis of PET film

Ultrasonic energy was incorporated during the enzymatic hydrolysis and chemical hydrolysis reaction. Both virgin PET and ozone pretreated PET were used in these experiments. The methods for both experiments are as follows.

3.2.5.1. Ultrasound Assisted Enzymatic Hydrolysis

This method was adapted from Pellis *et al.*, (2016). Each 1x1 inch (~185 mg) PET film/ozone pre-treated PET film was weighed and taken in Erlenmeyer flasks and 10 mL of 0.1 M tris-HCl was added to each of them. Then 4 g of immobilized cutinase enzyme per gram of PET samples was added into the flasks. The flasks were placed inside the ultrasonic bath for 0 to 15 minutes at 60C ± 2C. The flasks were removed from ultrasonic bath in intervals of 5 minutes and kept inside the rotary shaker at 60C ± 2C under agitation of 100 rpm for 48 hours. After 48 hours PET films were removed from the buffer and washed with tris-HCl. The films were then kept in -80 freezer before freeze drying (-53C, 0.200 mBar, 8h). Enzymes were separated from the liquid solution by vacuum filtration and the liquid solution was stored in the freezer for HPLC analysis. Blank reactions were performed with/without treated PET film under the same reaction condition but without the enzyme. The final weight of the samples was measured and gravimetric weight loss was calculated. All the experiments were done in triplicate.

3.2.5.2. Ultrasound Assisted Chemical Hydrolysis

This method was adapted from Bhogle et al, 2018. In each 125 mL Erlenmeyer flask 30 mL 2.5 M NaOH/ methanol solution and two 1x1 inch (~370 mg) PET films/ozone pretreated PET films were added. The flasks were placed inside the ultrasonic bath for 1 hour at 50C ± 2C. After 1 hour 30 mL of chilled distilled H_2O was added in each flask to

stop the reaction. Unreacted PET was separated by vacuum filtration, washed with distilled H_2O and then dried in hot air oven at 100 C for an hour. Blank reaction was performed in same reaction condition in a rotary shaker outside ultrasonic bath.

The final weight of the samples was measured and gravimetric weight loss was calculated. Pure H_2SO_4 was added to the liquid solution until the pH dropped to 7. The white precipitation was separated by vacuum filtration and washed again with distilled water to get the undissolved precipitation which is known to be TPA. It was the dried in hot air oven at 100 C for an hour. The rest of the liquid solutions were kept in the freezer for HPLC analysis. Finally, the weight of the recovered TPA was measured and was added to the HPLC recovery to get the total recovery of monomers and dimers. All the experiments were done in triplicate.

3.2.6. Chemical Hydrolysis of PET Powders

This method was similar to the chemical hydrolysis of PET films as mentioned in the last section. Here the difference was adding 370 mg of 250 μm PET powders to the 2.5 M NaOH/ methanol solution instead of 1x1 inche PET films.

3.2.7. HPLC Analysis

For TPA, EG, BHET and MHET quantification by HPLC, an Agilent system available in our lab was used. The column used was Agilent Poroshell 120, EC-C18 (2.7 μm, 4.6x100 mm) with a guard column. Detection was done using an UV detector at 299 nm. For standard curve a mixture in the range of 1 to 100 mM of standard EG, TPA, and BHET were prepared in tris-HCl buffer (pH 8). 50 mM of each solute was prepared and run through the HPLC to get the retention time. MHET was obtained by hydrolysis of BHET using HiC. Then $4g_{enzyme}/g_{BHET}$ enzyme was added to each centrifuge tube with 0.5 mL BHET solution and 0.7 mL of Tris-HCl.

The temperature was 60C ± 2C and the agitation was 180 rpm for 30 minutes. Then enzymes were separated and solution was run through HPLC. BHET, MHET and TPA was measured by using Poroshell 120 EC-C18 column. Injection volume was 1 μL at a flow rate of 0.75 mL/min and temperature was 40±0.8 C. Total time was 18 minutes including 7 minutes' post time. A gradient mixture of 0.1% (v/v) formic acid, methanol and water

was used as mobile phase at a ratio from 1:5:94 to 1:90:9. UV detection was done at 299 nm.

3.2.8. FTIR Analysis

FTIR analysis was performed using a Bruker Tensor 37 Fourier Transform Infrared Spectrophotometer available in the Lakehead University Center for Analytical Services (LUCAS) was used for this study. All spectra were obtained in the 600-4000 cm^{-1} spectral region with 32 scans and 4 cm^{-1} of resolution.

3.2.9. Data Analysis

For statistical analysis, both one-way and two-way ANOVA were performed followed by pairwise post-hoc Tukey test for all the replicates using a software (JASP, version 0.14.1.0). The level of significance was set at $p<0.05$.

3.2.10. Experimental Design

The overall experimental design is summarized in the figure 3.1.

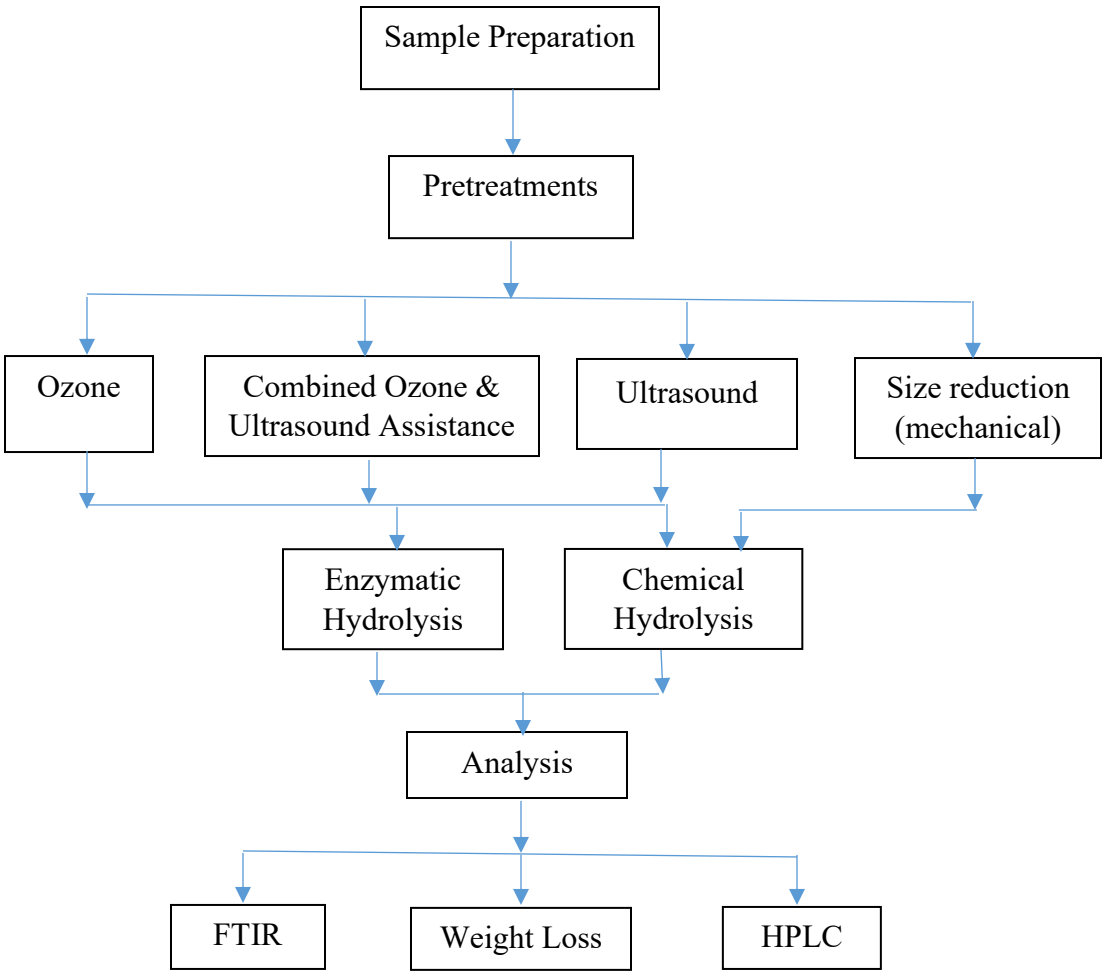

Figure 3.1: Systematic Illustration of Experimental Setup

Chapter 4

RESULTS & DISCUSSION

In this chapter, the results of all experiments carried out in the course of this study will be presented and discussed. The schematic in figure 3.1 provides and overview of the sequence of experiments conducted. All hydrolysis experiments were followed by estimation of weight loss, HPLC analysis and FTIR analysis of the PET sample.

4.1. Enzymatic Hydrolysis of Untreated PET film

The experiments initially carried out to check the effects of enzyme treatment on the original untreated PET films. The result and discussion of enzymatic hydrolysis of virgin PET films included measurement of weight loss, HPLC and FTIR analysis.

4.1.1. Weight loss

After 48 hours of enzymatic hydrolysis of 1x1 inch PET films by immobilized HiC, approximately 6.67% weight loss (WL) was found (figure 4.1). In order to investigate effect of time on PET hydrolysis by HiC activity, the process was further continued for 96 hours and 120 hours respectively. All experiments were carried out in triplicate. Figure 4.1 shows the WL of untreated PET films after different period of enzymatic hydrolysis.

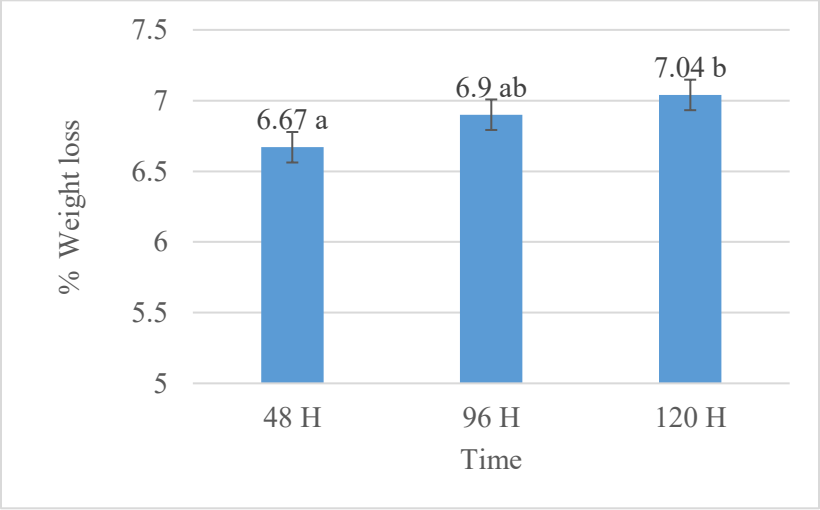

Figure 4.1: Weight loss (WL) of PET films at different time of enzymatic hydrolysis.

Although the observation of Figure 4.1 shows that there is slight increase of weight loss even after 120 hours, the result was not found to be significant. For further analyzing the effect of time on enzymatic hydrolysis, enzymatic hydrolysis was carried on for 60 days, which gave 24.76% WL was recorded.

Based on the preliminary results, a decision was made to carry on enzymatic hydrolysis for 48 hours for further experiments. As mentioned earlier Muller *et al.*, (2005) reported 14% WL of PET by TfH in 1 week and 50% of WL in 3 weeks. Similarly, Ronkvist *et al.*, (2009) reported WL 97% of lcPET film by HiC and 5% WL by FsC and PmC. However, in our study, the low WL is due to the use of immobilized HiC. As our enzyme was absorbed in polymer beads, the solid-solid reaction resulted in slower enzymatic hydrolysis process.

4.1.2. HPLC Analysis

After 48 hours of enzymatic hydrolysis of virgin PET, more dimers were found than the monomers. The monomers recovered were 0.5% TPA and 0.15% EG from the initial weight of the PET samples. On the other hand, the dimers recovered were 3.27% BHET and 2.63% MHET. This result was in agreement with previous studies (Carniel *et al.*, 2016; Castro *et al.*, 2017) that enzymatic hydrolysis of virgin PET generates more dimers than monomers. The total recovery of the monomers and dimers was 6.55% which is close to the WL 6.67%. There were no monomers or dimers present in the blank experiment that co-relates with our results obtained from the FTIR and WL. However, after 60 days of treatment the monomers and dimers recovery were 1.07% TPA, 3.81% EG, 0.71% BHET, 0.84% MHET. So, the total HPLC recovery was 6.42% which is a lot lower than the WL found. This could be due to drying up the buffer during the hydrolysis process.

4.1.3. FTIR Analysis

The major bonds present in polyethylene terephthalate (PET) are carboxylic C=O, aromatic and aliphatic C-H, hydroxyl O-H, ester C-O, C=C and phenyl ring. In the untreated PET film absorption peaks detected at 725 cm^{-1} and 873 cm^{-1} are for C-H bending mode of vibration out of plane of benzene ring (Prasad *et al.*, 2011; El-Saftawy *et al.*, 2014;

Ioakeimidis *et al.*, 2016; Dubelley *et al.*, 2017; Fadel *et al.*, 2020). Peak at 956 cm^{-1} is for C-O stretching mode of trans conformation of ethylene glycol (EG) (Dubelley *et al.*, 2017). Peak at 1016 cm^{-1} refers to C-H bending mode of vibration in plane of benzene ring (Prasad, *et al.*, 2011; Dubelley *et al.*, 2017; Fadel *et al.*, 2020).

Peak at 1093 cm^{-1} and 1242 cm^{-1} are related to C-O stretching of aliphatic and aromatic ester respectively (Prasad *et al.*, 2011; El-Saftawy *et al.*, 2014; Ioakeimidis *et al.*, 2016; Dubelley *et al.*, 2017). Strong peak at 1714 cm^{-1} is related to C=O stretching of carboxylic ester bond (Prasad *et al.*, 2011; Siddiqui *et al.*, 2012; El-Saftawy *et al.*, 2014; Ioakeimidis *et al.*, 2016; Dubelley *et al.*, 2017). Peak at 1407 cm^{-1} refers to aromatic C=C stretching and peak at 1575 cm^{-1} refers to C-H stretch of aromatic phenyl (Siddiqui et al., 2012; El-Saftawy et al., 2014). Absorption peak at 1452 cm^{-1} is for C-H bending of EG (Siddiqui et al., 2012; Dubelley *et al.*, 2017). Peaks at 2858 cm^{-1} and 2931 cm^{-1} refers to C=H stretching mode of vibration (Prasad et al., 2011; Fadel *et al.*, 2020). Figure 4.2 shows a typical FTIR spectrum of virgin PET.

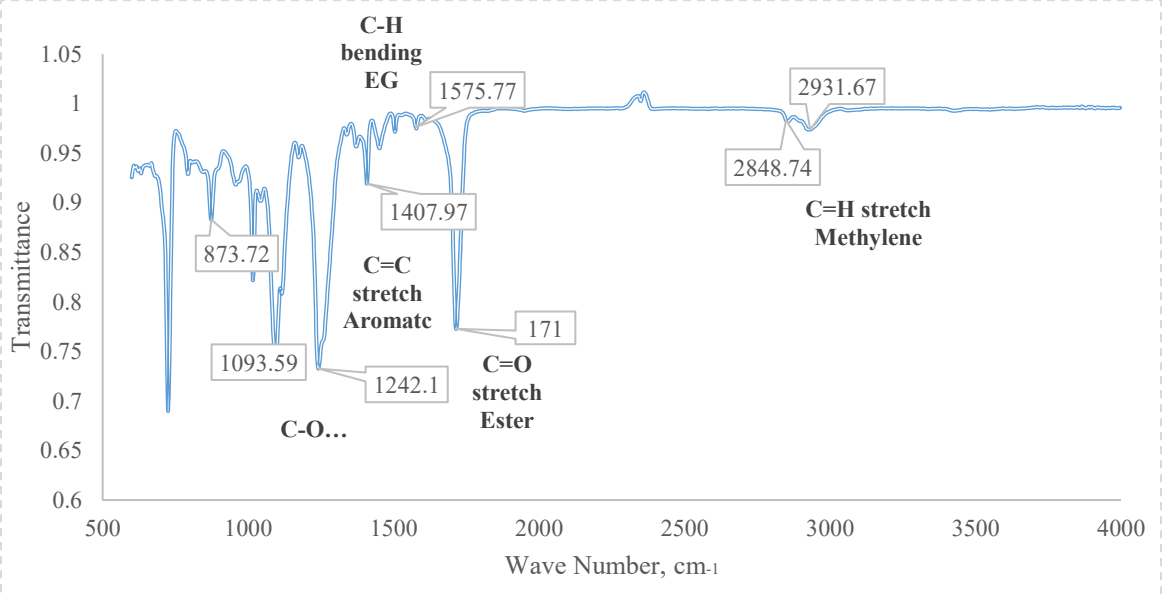

Figure 4.2: FTIR spectrum of virgin PET film.

Appearance or alteration of the bonds causes shift of the peak position, changes intensity or area. These changes indicate hydrolysis of the polymer. From figure 4.3 it was observed that there was appearance of new peak at 3184 cm^{-1} which can be assigned to O-

H stretch of hydroxyl group (El-Saftawy *et al*., 2014). Appearance of O-H group is a likely phenomenon in hydrolysis process. Figure 4.3 shows the changes of the FTIR spectrum of PET after enzymatic hydrolysis.

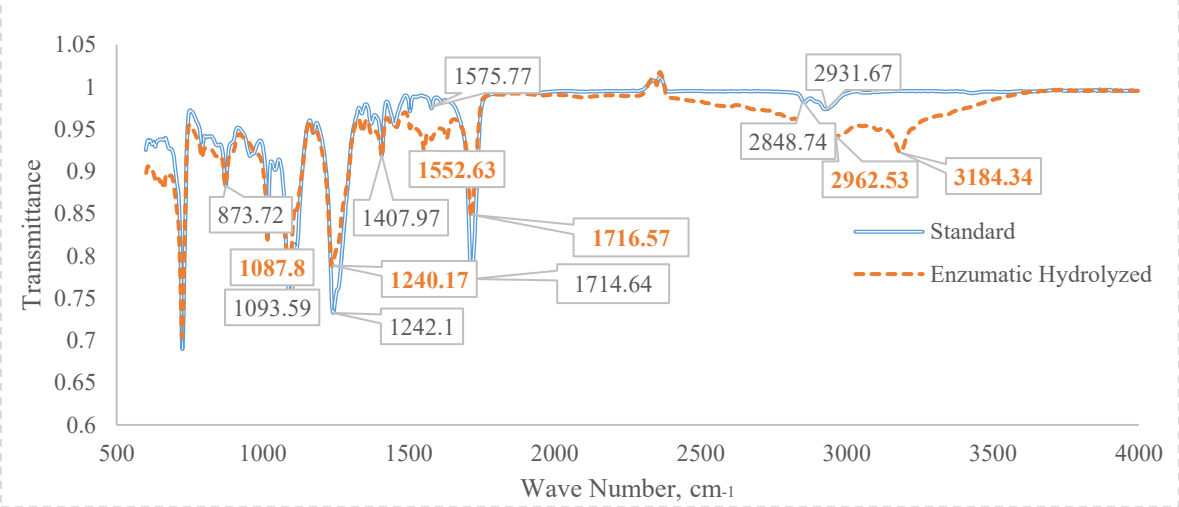

Figure 4.3: FTIR spectrum of enzymatically hydrolyzed PET

Besides, the decreased intensity and shift of strong C=O peak to 1716 cm^{-1} also indicated that these bond was hydrolyzed by HiC. Moreover, changes of C=H stretch, aromatic C-H stretch, aliphatic and aromatic C-O stretch was found after enzymatic hydrolysis. The changes are summarized in table 4.1.

Table 4.1: Changes of FTIR spectrum after enzymatic hydrolysis of PET.

Functional group and Bonds		**Original Peak (cm^{-1})**	**Shift in Peak (cm^{-1})**
Hydroxyl	O-H stretch	none	3184
Easter	C=O stretch	1714	1716
Methylene non-sym & sym	C=H stretch	2858 & 2931	Almost disappearance of 2858 and change of 2931 to 2962
Phenyl Aromatic	C-H stretch	1575	1552
Aliphatic & Aromatic	C-O stretch	1093 & 1242	1087 & 1240

Considering the small changes in the PET films as a result of enzymatic treatment, it is clear that the PET films need to be pretreated before it can be hydrolyzed to its monomeric form.

4.2. Enzymatic Hydrolysis of Ozone and Ultrasound Pretreated PET films

The result and discussion of enzymatic hydrolysis of both ozone and ultrasound pretreated PET films are given in the following sections.

4.2.1. Weight Loss

The WL of ultrasound pretreated PET films were found to be approximately 6.62% after 48 h. This result was similar to the result of virgin PET films (without pretreatment). Ultra-sonication causes formation of micro-cavities on polymer surface and damages the polymer by breaking weak bonds like hydrogen bonds and van der Walls bonds. As a result, more functional groups get exposed which help in enzymatic degradation. In our case, such results were not obtained as the HiC was in immobilized form. This might hinder the binding of active site of the enzyme with the available functional group. Figure 4.4 shows comparison between WL of enzymatically hydrolyzed untreated PET films and pretreated PET films.

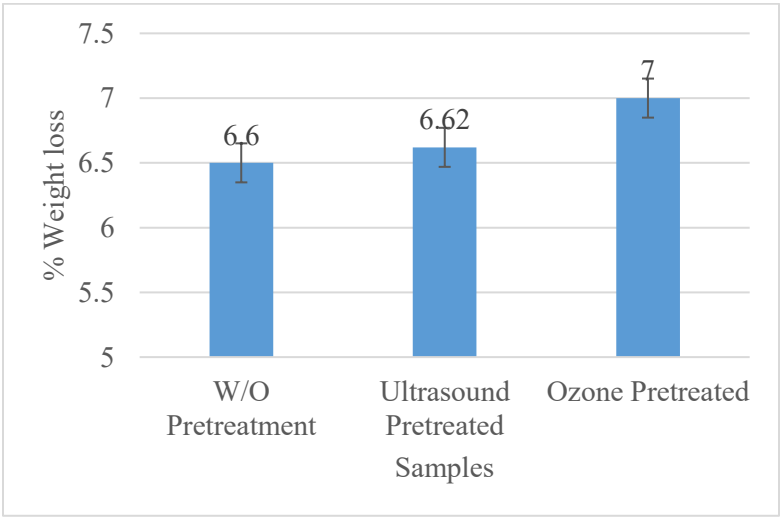

Figure 4.4: WL of enzymatic hydrolyzed pretreated PET films.

Similarly, when ozone pretreated PET films were used for enzymatic hydrolysis, the WL was 7% after 48 h. In this step no significant result was obtained after incorporation pretreatment prior to enzymatic hydrolysis.

4.2.2. FTIR Analysis

There was no change found in the FTIR spectrum after ozone and ultrasound pretreatment. However, when these pretreated films were enzymatically hydrolyzed, changes were observed in the spectrum. The result obtained was similar to the result from enzymatic hydrolysis of untreated PET. This indicated that the alteration of the bonds was due to the enzymatic hydrolysis.

As the results from FTIR and WL were not significant, we decided to use ultra-sonication during the start of enzymatic hydrolysis as suggested by Pellis *et al.*, (2016).

4.3. Ultrasound Assisted Enzymatic Hydrolysis of PET film

In the next set of experiments, the PET samples were taken in the ultrasonic unit along with the enzyme catalyst. This is different from previous set of experiments where samples were pretreated by ultrasound before enzyme treatment. The combination of sonication during hydrolysis is termed as “ultrasound-assisted” hydrolysis for the rest of this thesis. The result and discussion of ultrasound assisted enzymatic hydrolysis of PET films are given in the following sections.

4.3.1. Weight Loss

In this experiment we found that 10 minutes of ultra-sonication gave the best result which was 9.5% WL after 48h. The use of ultrasound during hydrolysis process significantly increased enzymatic activity which was in agreement with the study by Pellis *et al.*, (2016). The energy generated by ultrasound reduces the activation energy and the enthalpy of the enzymatic reaction, hence increased enzymatic hydrolysis was observed even when the sonication was stopped after a certain time and the enzymatic reaction was continued for 48 hours (Malani *et al.*, 2014). Figure 4.5 shows almost 1.3-fold increase of WL when compared to enzymatic hydrolysis alone. A decrease of WL was observed when longer time of ultra-sonication was applied. This could be because of partial inactivation

of the enzyme as a result of mechanical and chemical effects arising from the cavity formed by ultrasound.

At the beginning of sonication, high intensity ultrasonic wave creates small vacuum bubbles containing low pressure in the molecule. After reaching a certain volume, the bubbles can no longer absorb energy. As a result, these micro-cavities generate shock waves from extreme heat and pressure which helps in breaking the weak bonds as mention earlier. At the same time, this energy negatively affects the enzyme activity on prolonged exposure (He *et al.*, 2006; Pellis *et al.*, 2016). So, it can be concluded that ultrasound assistance increases enzymatic hydrolysis of PET, but ultra-sonication should be done for limited periods as it can affect enzyme activity as well.

Figure 4.5 shows WL of PET films after addition of different times of sonication during enzymatic hydrolysis.

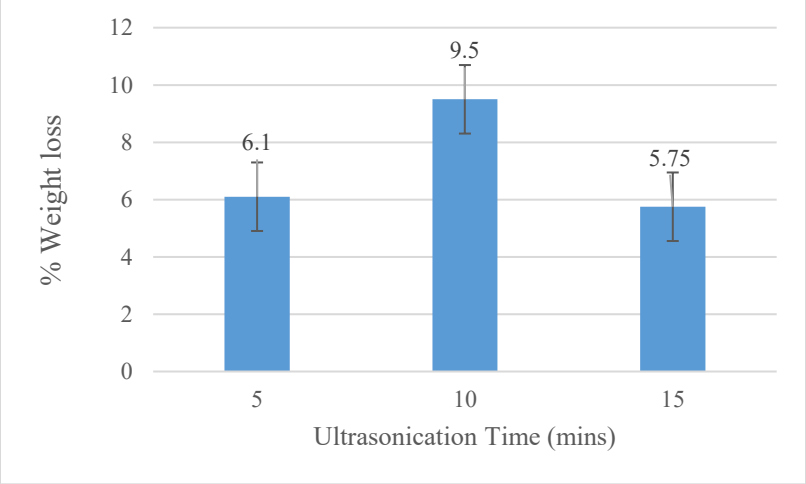

Figure 4.5: Ultrasound Assisted Enzymatic Hydrolysis of PET Films

4.3.2. HPLC Analysis

The HPLC results showed that 2.99% TPA, 0.89% EG, 2.9% BHET and 2.4% MHET was obtained when 10 minutes of sonication was added during hydrolysis process. Total recovery of monomers and dimers was 9.18% that is close to the WL value of 9.5% obtained. The HPLC result co-relates with the result of WL. Moreover, 10 minutes of sonication generated higher level of TPA which is 5.9-fold increase form without

ultrasound assistance. Table 4.2 depicts the percent of monomer and dimers recovered after using different sonication time during enzymatic hydrolysis

Table 4.2: HPLC recovery of PET monomers (TPA and EG) and dimers (BHET and MHET) after ultrasound assisted enzymatic hydrolysis.

Ultra-sonication (minutes)	% TPA	% EG	% BHET	% MHET	% Total Recovery	% weight Loss
5	1.02	0.3	2.6	2.11	6.03	6.19
10	2.99	0.89	2.9	2.4	9.18	9.5
15	0.72	0.22	2.55	2.05	5.54	5.75

It can be observed that increasing the sonication time decreased the TPA recovery. This again supports our explanation given earlier that the increased shock waves generated from the micro-cavity during sonication results in inactivation of the enzyme. Hence, the conversion of monomer decreased.

4.3.3. FTIR Analysis

To investigate the effect of ultrasound further, different time ranging from 5 minutes to 15 minutes of ultra-sonication was applied during the enzymatic hydrolysis process. The changes in FTIR spectrum were similar to previous experiments. The additional peak that we found here was at 1089 cm^{-1} which can be assigned to C-O stretch of ester according to the report by Dubelley *et al.*, (2017). This peak was the deformation of original C-O peak at 1093 cm^{-1}. The deformed stretch might be resulted from the micro-cavities formed during sonication and contributed to hydrolysis process. The slight decrease of transmittance also suggested that the bond might be hydrolyzed by HiC. Figure 4.6 shows the deformation of C-O stretch due to ultrasound treatment during enzymatic reaction.

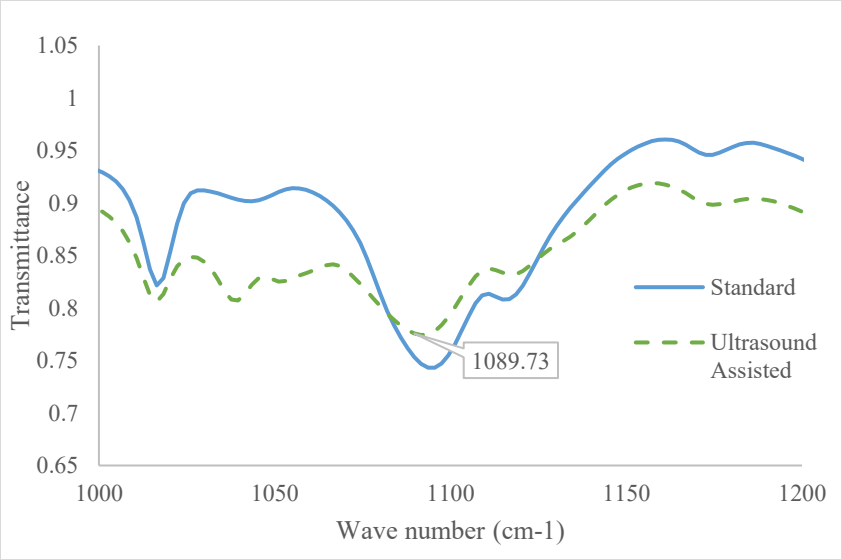

Figure 4.6: FTIR spectra of deformation of C-O due to ultrasound assisted enzymatic hydrolysis of PET.

Finally, we decided to investigate the additional effect of ultrasound on enzymatic hydrolysis of ozone pretreated PET films.

4.4. Ultrasound Assisted Enzymatic Hydrolysis of Ozone Pretreated PET film

A combination experiment of ozone pretreatment and ultrasound assistance on enzymatic hydrolysis of PET film was investigated. As mentioned earlier, ozone can oxidize the PET surface by forming hydroxyl radical (*OH) that eventually forms carbonyl groups (Tian *et al.*, 2017). Different amount of ozone was exposed to the PET film to check the dosage effect. The result and discussion of ultrasound assisted enzymatic hydrolysis of ozone pretreated PET films are given in the following sections.

4.4.1. Weight Loss

From the WL analysis it was observed that, ozone pre-treatment in combination with ultra-sonication of PET film enzymatic hydrolysis did not significantly increase the WL as expected. This could be due to formation of low *OH radicals (Asaithambi *et al.*, 2017). Moreover, the use of immobilized form of enzyme could another reason for lower WL.

But, from the Figure 4.7 it is evident that 10 minutes of ultra-sonication enhanced enzymatic degradation of the PET film in all combination of ozone dosage.

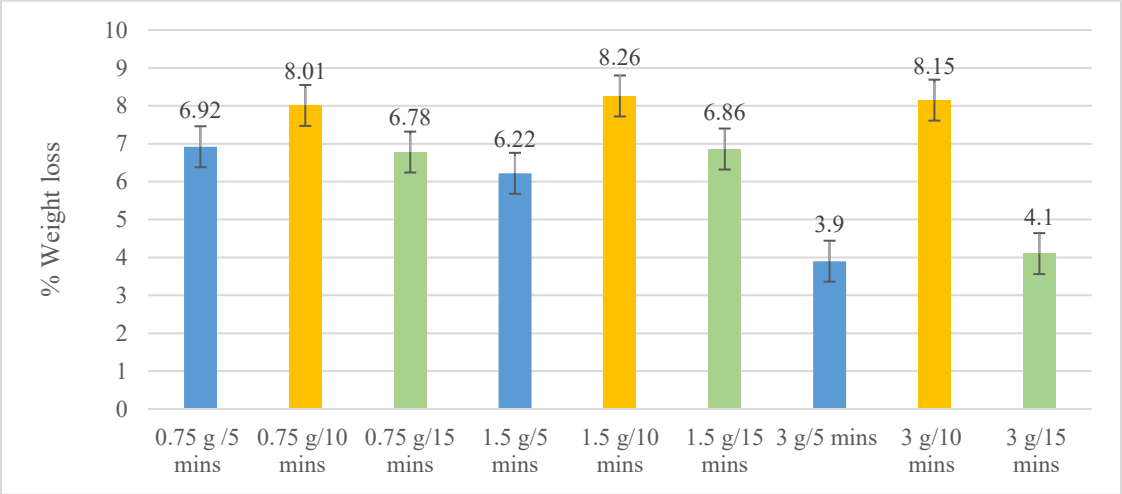

Figure 4.7: WL of ultrasound assisted enzymatic hydrolysis of ozone pretreated PET films (the yellow bar shows highest WL at 10 minutes of ultra-sonication; blue bar shows WL after 5 minutes of sonication; green bar shows WL after 15 minutes of sonication for different ozone dosage).

Figure 4.8 summarizes the statistical analysis of different combination of ozone pretreatment with ultrasound assisted enzymatic hydrolysis of PET films.

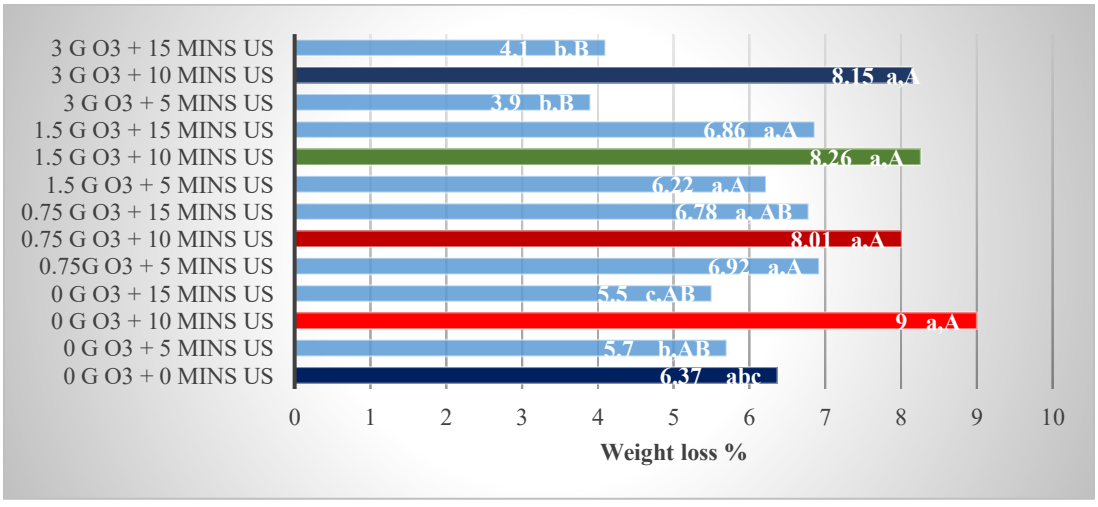

Figure 4.8: Statistical Analysis of Ultrasound Assisted Enzymatic Hydrolysis of Ozone Pretreated PET (lower case different letters mean significant difference in the ozone dose. Upper case different letters mean significant difference in the ultrasound treatment).

From this analysis, it was observed that increasing ozone exposure beyond 10 minutes decreases the enzyme activity. The reasonable explanation could be the increase of ozone fused benzene rings inhibits hydrolysis. Similar phenomenon was found in Nam and Kukor (2000) and Olewnik *et al.* (2013) where increased ozonation caused formation of charge transfer between phenyl rings and ozone molecule that resulted in tightening the ring structure of organic polymer.

4.4.2. HPLC Analysis

Similar to ultrasound assisted enzymatic hydrolysis of untreated PET, 10 minutes of sonication during enzymatic hydrolysis gave the best result in terms of monomer recovery of ozone pretreated PET films. Moreover, different amount of monomers and dimers were recovered from different ozone dosage. It was observed that 1.5g ozone/ sample resulted in 4.92% of TPA recovery which was the highest TPA recovered among all the experiments with enzymatic hydrolysis. Table 4.3 shows all the data of monomers and dimers recovery from different combination of ozone dosage and ultra-sonication time.

Table 4.3: HPLC recovery of PET monomers and dimers after ultrasound assisted enzymatic hydrolysis of ozone pretreated PET films.

O_3 - Ultra-sonication (g-mins)	**% TPA**	**% EG**	**% BHET**	**% MHET**	**% Total Recovery**	**% Weight Loss**
0.75-5	1.88	0.56	2.24	2.1	6.78	6.88
0.75-10	3.29	0.99	1.62	1.47	7.37	8.01
0.75-15	1.89	0.56	2.26	1.92	6.63	7.16
1.5-5	2.79	0.84	1.81	1.28	6.72	6.88
1.5-10	4.92	1.47	0.77	0.54	7.7	8.26
1.5-15	3.02	0.9	1.78	1.17	6.87	7.16
3-5	1.11	0.33	1.41	1.17	4.02	4.16
3-10	3.18	0.96	1.64	1.35	7.13	8.15
3-15	1.29	0.39	1.68	1.3	4.66	6.86

Statistical analysis showed that both combination of 0.75g ozone per sample with 10 minutes ultra-sonication and 1.5 g ozone per sample with 10 minutes ultra-sonication gave significant result. We decided to take 1.5 g ozone/sample with 10 minutes ultra-sonication as our best result as the monomer recovery is the highest at this combination.

4.4.3. FTIR Analysis

FTIR analysis showed different alteration of peaks in ozone pretreated and ultrasound assisted enzymatic hydrolysis of PET. From figure 4.9 overall changes of the spectrum was observed. Figure 4.9 (a, b, c, d, e and f) shows alteration of different functional groups of ozone pretreated and ultrasound assisted enzymatic hydrolysis of PET.

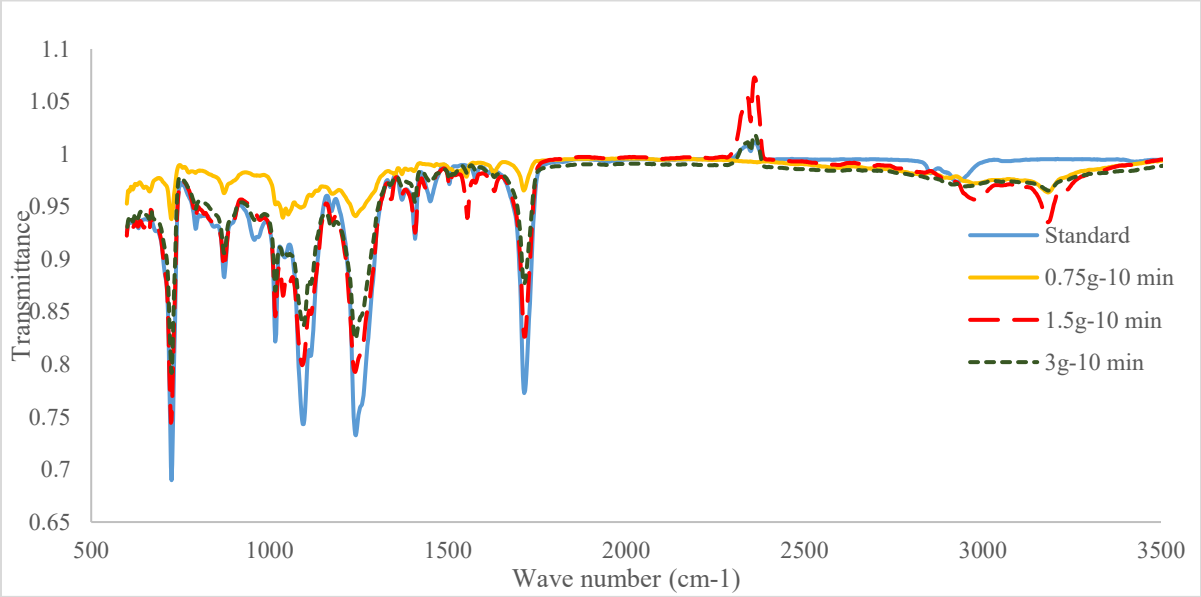

Figure 4.9 (a)

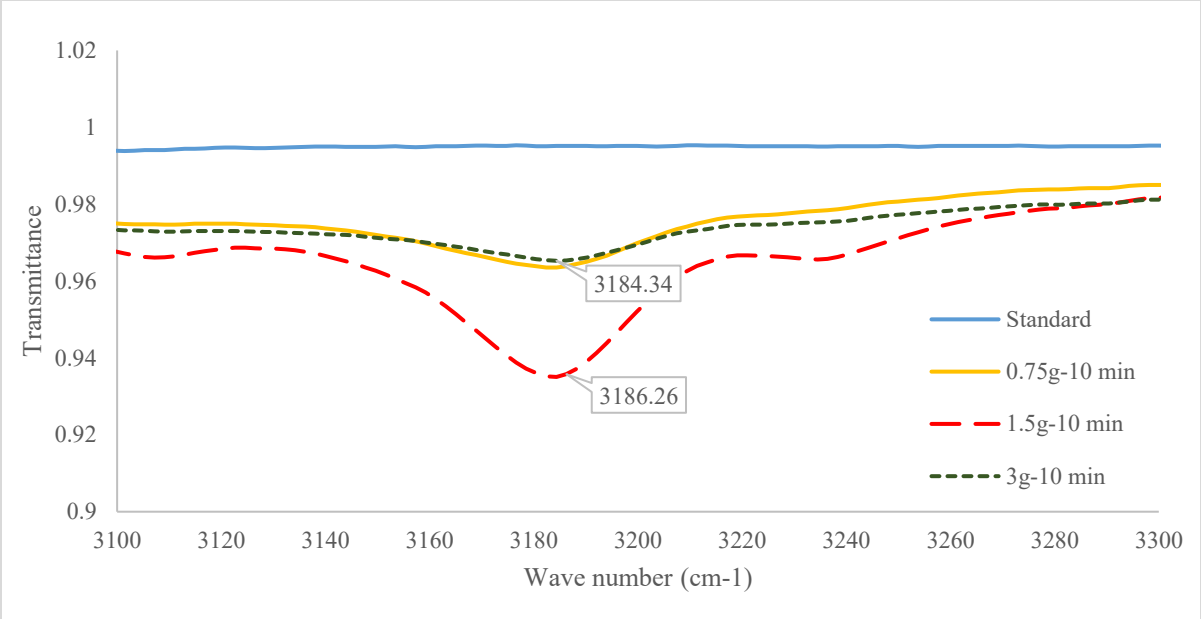

Figure 4.9 (b)

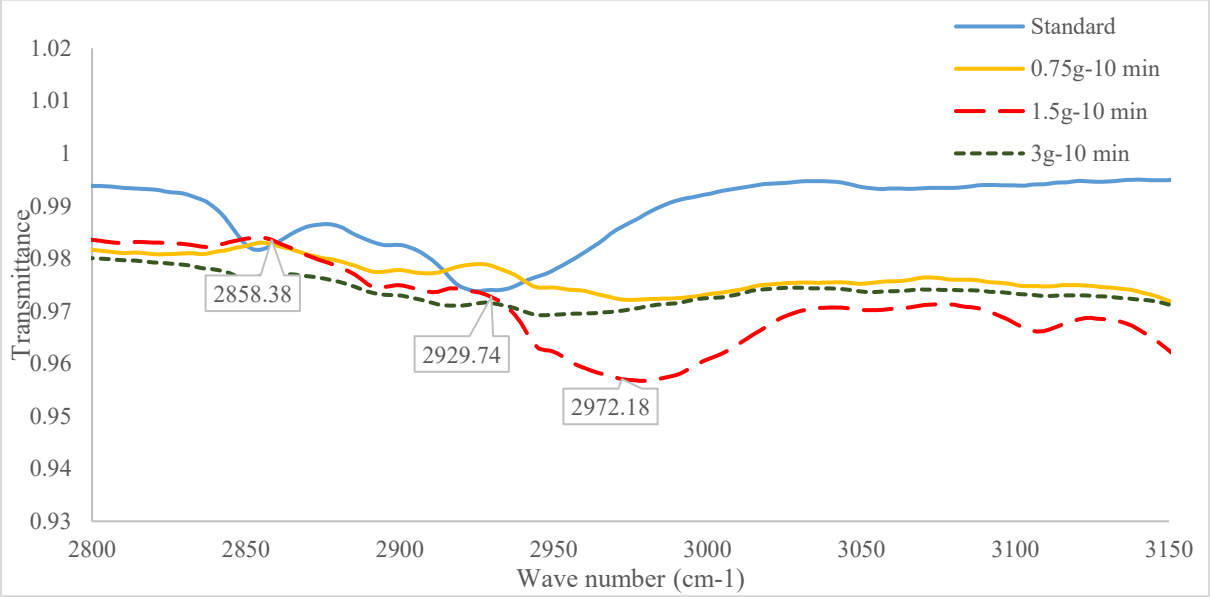

Figure 4.9 (c)

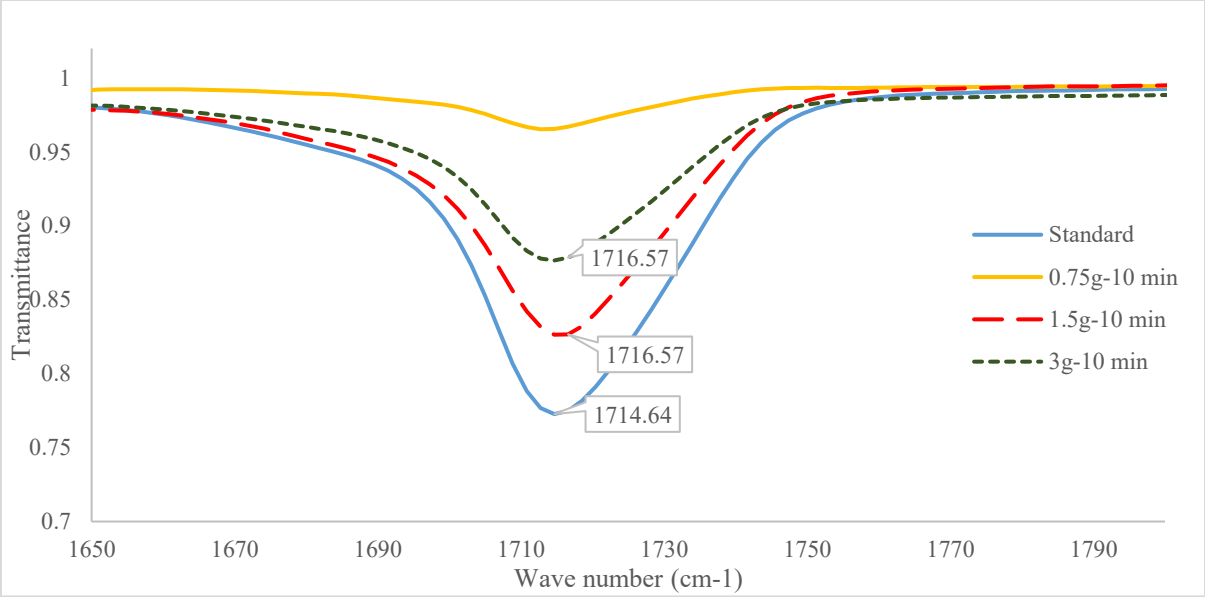

Figure 4.9 (d)

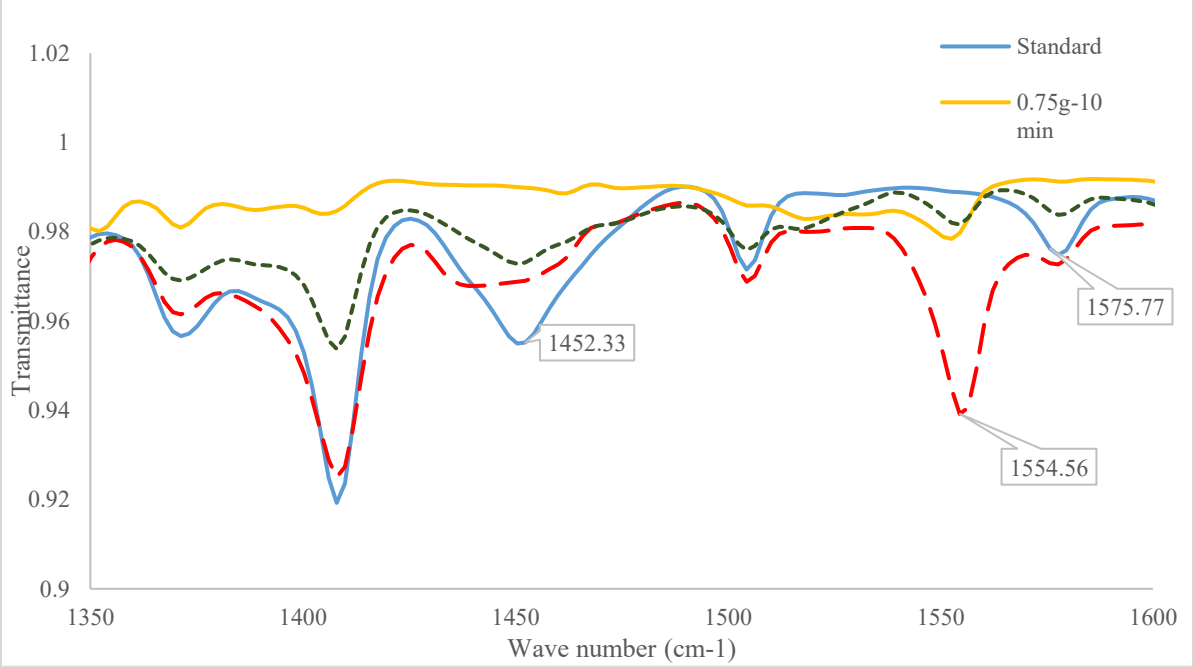

Figure 4.9 (e)

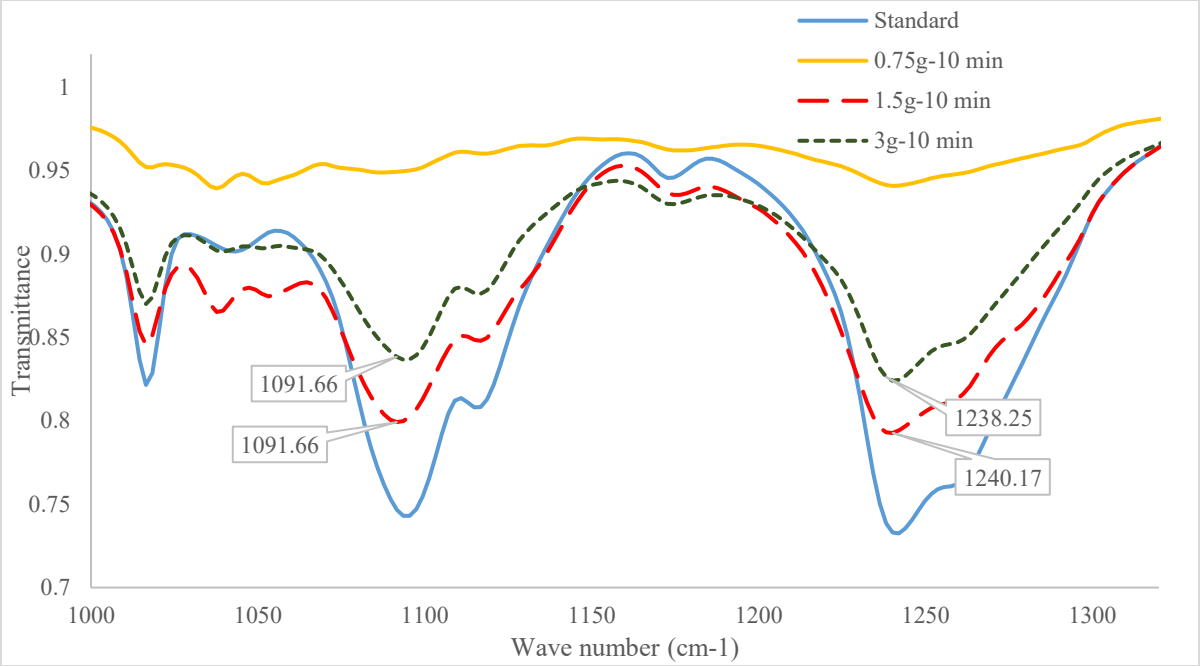

Figure 4.9 (f)

Figure 4.9: a) FTIR spectra of ozone pretreated and ultrasound assisted enzymatically hydrolyzed PET films. b) changes of O-H stretch c) changes of C=H stretch d) changes of C=O stretch e) changes of C=C stretch and C-H stretch & f) changes of C-O stretch.

It was observed from figure 4.9 that ozone pretreatment followed by additional ultra-sonication during enzymatic hydrolysis significantly affected the bonds. Disappearance of C=H stretch at 2858 cm^{-1} indicated they had undergone complete hydrolysis. New O-H peak at 3184 and 3186 cm^{-1} indicated addition of O-H group during hydrolysis. Similarly, changes of peak position, decrease and increase of their intensity were associated with hydrolysis. Disappearance of C-H stretch at 1454 cm^{-1} was also found. Table 7 summarizes the alterations of functional groups found in this experiment. Most alterations were associated with enzymatic hydrolysis. However, shift of O-H at 3186 cm^{-1}, disappearance of C-H at 1454 cm^{-1} and shift of C-O at 1238 cm^{-1} could be due to ozone pretreatment as these changes were not observed in previous experiments. The possible explanation cloud be formation of ozonated intermediate compounds that aided in the enzymatic hydrolysis process (Olewnik *et al.* 2013). The changes are summarized in table 4.4.

Table 4.4: Changes of FTIR spectrum after ultrasound assisted enzymatic hydrolysis of ozone pretreated PET films.

Functional group and Bonds		**Original Peak (cm^{-1})**	**Shift in Peak (cm^{-1})**
Hydroxyl	O-H stretch	none	3184 & 3186
Easter	C=O stretch	1714	1716
Methylene non-sym & sym	C=H stretch	2858 & 2931	Disappearance of 2858 and change of 2931 to 2972
Phenyl Aromatic	C-H stretch	1454 & 1575	Disappearance of 1454 and change of 1575 to 1554 and increased intensity
Aliphatic & Aromatic	C-O stretch	1093 & 1242	Decrease of intensity and shit at 1091 & 1240-1238

From analyzing all the results, we can conclude that ultrasound assistance during enzymatic hydrolysis enhance the reaction. Although, pretreated samples did not show significant difference in enzymatic hydrolysis, it certainly increased the monomer recovery when combined with ultra-sonication. Moreover, the percent of WL is close to the percent of total recovery suggesting that the PET that was hydrolyzed, was successfully converting into monomers and dimers. Hence, there is no reason to worry about generating micro plastics or short chain polymer. Recovering monomers was our initial aim so that we can use the process in circular bio-economy. However, the low breakdown rates and long reaction time required, prompted us to look for alternative chemically catalyzed methods.

4.5. Chemical Hydrolysis of Untreated PET film

In this method, methanolic NaOH solution was used for chemical hydrolysis. As mentioned earlier, PET has poor resistance against alkaline hence NaOH could be used as

chemical catalyst (Bhogle and Pandit, 2018). The result and discussion of chemical hydrolysis of virgin PET films are given in the following sections.

4.5.1. Weight Loss

Unlike enzymatic hydrolysis, chemical hydrolysis showed better results in terms of WL of PET films. One hour of chemical hydrolysis by methanolic NaOH solution resulted in 49% WL of PET films which is many times higher than the results obtained by enzyme hydrolysis.

Bhogle and Pandit (2018), Paliwal and Mungray (2013) reported significant improvement of hydrolysis while using non-aqueous hydrolysis rather than aqueous hydrolysis. Both of the studies reported that using non-aqueous solution decreased reaction time and temperature. For alkaline hydrolysis, PET should be able to react with NaOH molecule which is easily soluble in aqueous solution. Although PET has poor resistivity against alkaline (Kamaruzamal, 2014), the highly polar water molecule associated with NaOH in aqueous solution is repelled at the hydrophobic PET surface. The polarity of methanol is 0.762 compared to water which is 1. As methanol is less polar than water, it faces less repellent force. Hence, methanolic NaOH resulted in better hydrolysis (Bhogle and Pandit, 2018).

4.5.2. HPLC Analysis

As observed from HPLC analysis, monomer recovery seemed was much lower. Although peaks of all the monomers and dimers were found by HPLC the quantity obtained was lower than expected. 0.13% TPA, 0.46% EG, 1.77% BHET and 1.57% MHET was obtained from HPLC recovery. So, the HPLC recovery was 3.93% and the total recovery was 16.94% including TPA precipitated by adding H_2SO_4. There was a significant difference between the WL and total recovery. The possible explanation could be PET underwent partial hydrolysis during chemical hydrolysis process. The partially hydrolyzed molecules were solubilized in the chemical solution thus did not break down to monomers and dimers completely. Apparently, the generated partially hydrolyzed molecules were filtered out before HPLC analysis as there were no additional peaks found in the HPLC results besides the monomeric and dimeric compounds.

4.5.3. FTIR Analysis

Like enzymatically hydrolyzed PET films, chemically hydrolyzed PET showed alteration of similar bonds in FTIR spectrum. However, the peak intensity of few bonds differed from the enzymatic hydrolyzed PET. A new peak at 3195 cm^{-1} was found that is assigned for O-H stretch but the peak intensity was very low. Peaks at 2856 and 2925 cm^{-1} for both symmetrical and asymmetrical stretch of C=H disappeared in chemical hydrolysis. C=O stretch at 1714 cm^{1} and C-O stretch at 1093 and 1242 cm^{-1} decreased in intensity significantly. Besides, peak at 1093 cm^{-1} also shifted to 1095 cm^{-1}. Increased intensity and shift of peak position from 1575 cm^{-1} to 1554 cm^{-1} was also observed. These alterations indicated chemical hydrolysis of PET. Figure 4.10 shows the changes in FTIR spectrum of PET after chemical hydrolysis.

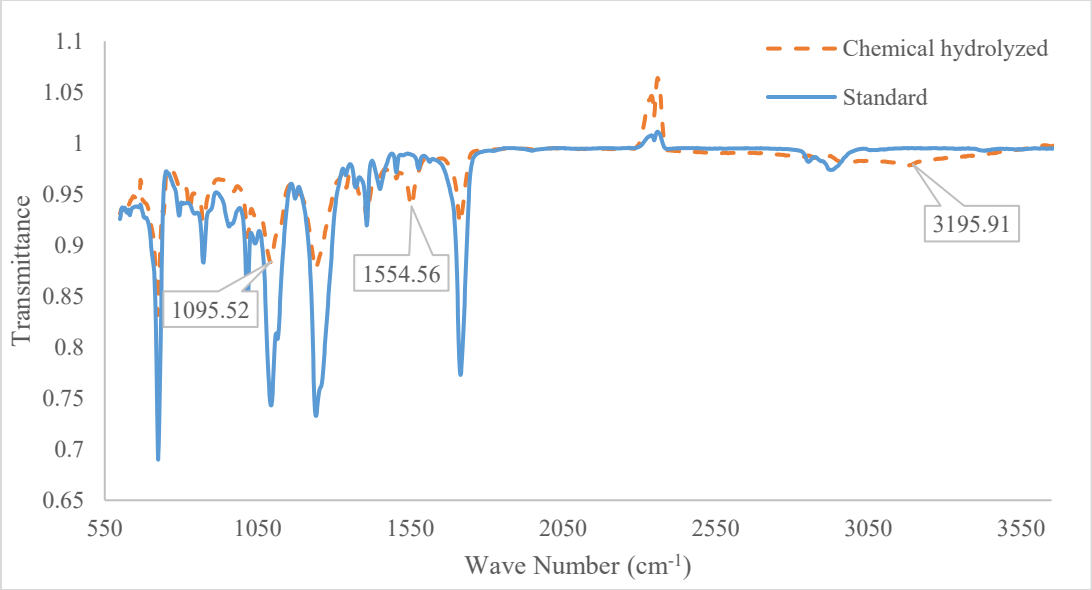

Figure 4.10: FTIR spectrum of chemically hydrolyzed PET film.

4.6. Chemical Hydrolysis of Ozone and Ultrasound Pretreated PET film

Next, the effects of chemical hydrolysis on ozone and ultrasound pretreated PET films were investigated. The results obtained from this experiment are discussed in the following section.

4.6.1. Weight Loss

Ultrasonic pretreatment followed by chemical hydrolysis resulted in 60% WL whereas, in ozone pretreatment followed by chemical hydrolysis 65% WL was recorded. Both results showed increase of WL in the pretreated films compared to the virgin PET (without any pretreatment). These results are in agreement with the explanation given earlier in the enzymatic section. The micro-cavity formed during ultra-sonication and the oxidation of PET by ozone enhanced chemical hydrolysis in their respective experiments. This result also indicates why enzymatic hydrolysis did not show any significant difference. It was the immobilized form of enzyme that hindered the hydrolysis earlier. Figure 4.11 depicts the effect of WL after using pretreatments.

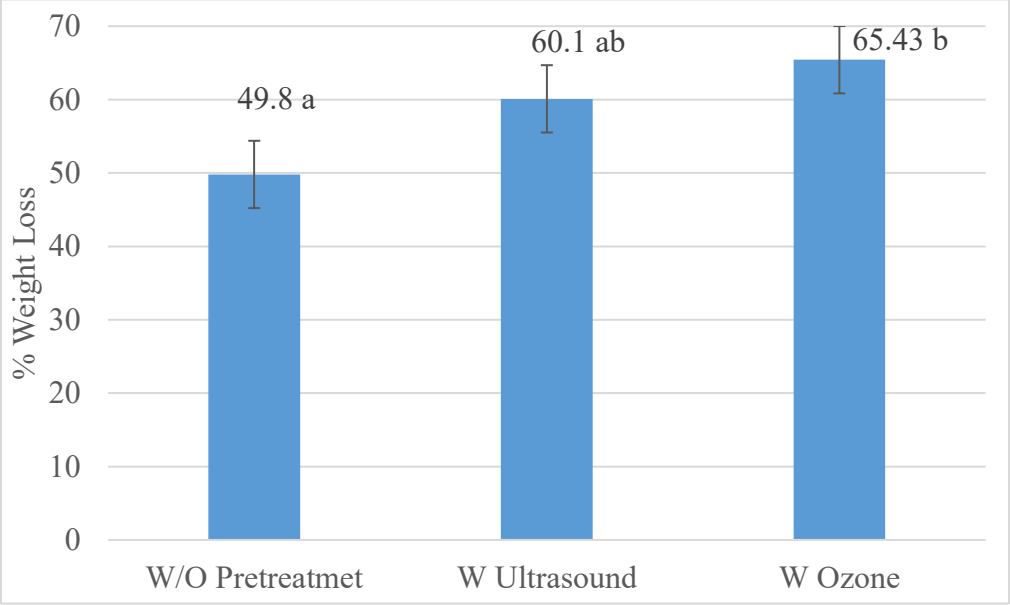

Figure 4.11: WL of chemical hydrolysis of pretreated PET films.

4.6.2. HPLC Analysis

Similar to the WL results, more monomers and dimers were recovered when ozone and ultrasound pretreated PET films underwent chemical hydrolysis. For ultrasound pretreatment, the HPLC recovery was 13.54% including 0.73% TPA, 6.98% EG, 0.81% BHET and 5.02% MHET. The total recovery was 38.3% including 24.76% TPA precipitated by adding H_2SO_4. The effect of ozone dosage also affected monomer recovery.

It was found when samples were exposed to 1.5 g ozone, they generated more monomers after chemical hydrolysis. 3.02% TPA, 3.02% EG, 0.78% BHET and 1.09% MHET was obtained from HPLC recovery. So the HPLC recovery was 7.91% and the total recovery was 44.53% including TPA precipitated by adding H_2SO_4. This also indicated that ozone pretreatment resulted in better TPA precipitation after chemical hydrolysis. Furthermore, the difference between total recovery and WL also decreased compared to untreated PET films. This suggested that, oxidation by ozone helped in better hydrolysis of PET. Hence higher amount of high molecular weight PET was solubilized. Table 4.5 summarizes the HPLC recovery of monomers and dimers from chemical hydrolysis of ozone (different dosage) pretreated films.

Table 4.5: HPLC results of filtrate after filtering out neutralized monomers. Recovery values are based on a summation of PET monomers and dimers obtained by weight of solids filtered out and HPLC values after chemical hydrolysis of ozone pretreated PET.

Ozone (g)	% TPA	% EG	% BHET	% MHET	% HPLC Recovery	% Weight of washed filtrate after neutralization	% Total Recovery	% Weight Loss
0.75	1.95	6.93	3.37	3.06	15.32	5.23	20.55	65.43
1.5	3.02	10.69	0.78	1.09	15.59	28.94	44.53	52.97
3	1.52	5.4	2.34	2.4	11.73	21.85	33.59	55.62

4.6.3. FTIR Analysis

From hydrolysis of ozone pretreated PET films, the appearance of O-H stretch was found at 3197 cm^{-1}. Disappearance of C=H stretch at 2856 and 2925 cm^{-1} was observed. Aromatic C=C stretch was shifted from 1407 to 1409 cm^{-1} which is likely because of ozone pretreatment. Shift of C-O stretch at 1244 cm^{-1} was also observed. The rest changes were similar to chemical hydrolysis of untreated PET films. Figure 4.12 shows the changes in FTIR spectrum of pretreated PET after chemical hydrolysis.

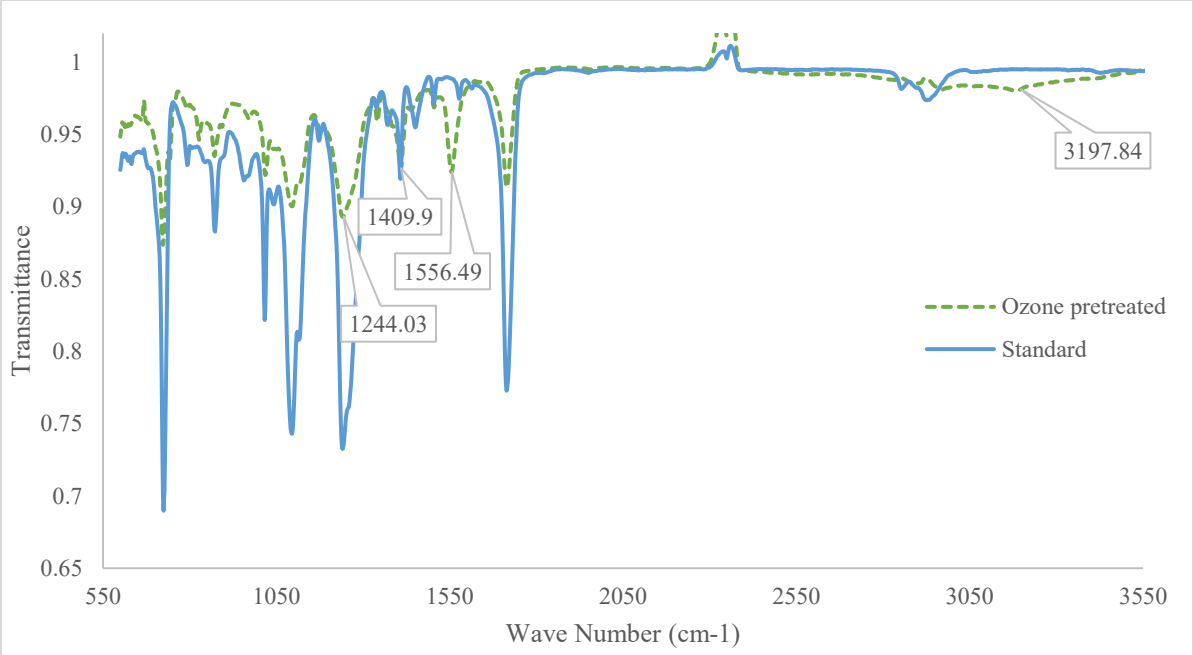

Figure 4.12: FTIR spectrum of chemically hydrolyzed pretreated PET film

4.7. Ultrasound Assisted Chemical Hydrolysis of Untreated PET film

In order to investigate the additional effect of ultrasound similar to the enzymatic study, sonication was incorporated during chemical hydrolysis of PET. Here, by ultrasound assisted we mean sonication and hydrolysis was done together. The results obtained from this experiment are discussed in the following section.

4.7.1. Weight Loss (WL)

68% WL was observed in ultrasound assisted chemical hydrolysis of PET. Similarly, higher rate of surface reaction was report by Paliwal and Mungray (2013) and Bhogle and Pandit (2018) while using ultrasound assistance during hydrolysis. The use of higher concentration of NaOH (2.5M) facilitates the process as it serves as nuclei for cavity formation during ultra-sonication.

4.7.2. HPLC Analysis

Although the additional effect of ultrasound on chemical hydrolysis of PET increased the WL, the HPLC result did not come as expected. 0.07% TPA, 0.26% EG, 5.79% BHET and 5.01% MHET was obtained from HPLC recovery. So the HPLC

recovery of all the monomers and dimers were 11.13% and the total recovery was 25.2% including the TPA precipitated by addition of H_2SO_4. It was observed that ultrasound assistance during chemical hydrolysis increased the dimer generation but complete hydrolysis to monomer was poor. Besides, significant difference between the WL and total recovery was recorded co-relating our explanation given earlier in the chemical hydrolysis of untreated PET.

4.7.3. FTIR Analysis

Ultrasound assisted chemical hydrolysis of PET showed bond alteration similar to previous experiment. One significant change was found for C=O stretch which decreased intensity drastically and also shifted the peak position from 1714 to 1716 cm^{-1}. In both chemical hydrolysis of untreated and pretreated PET, the only change found for C=O stretch was decreased intensity. Similarly, for C-O stretch, even further shift of peaks was observed. Peak at 1093 cm^{-1}shifted to 1091 cm^{-1} and 1242 cm^{-1} shifted to 1259 cm^{-1}. Figure 4.13 shows the changes in FTIR spectrum of ultrasound assisted chemical hydrolysis of PET film.

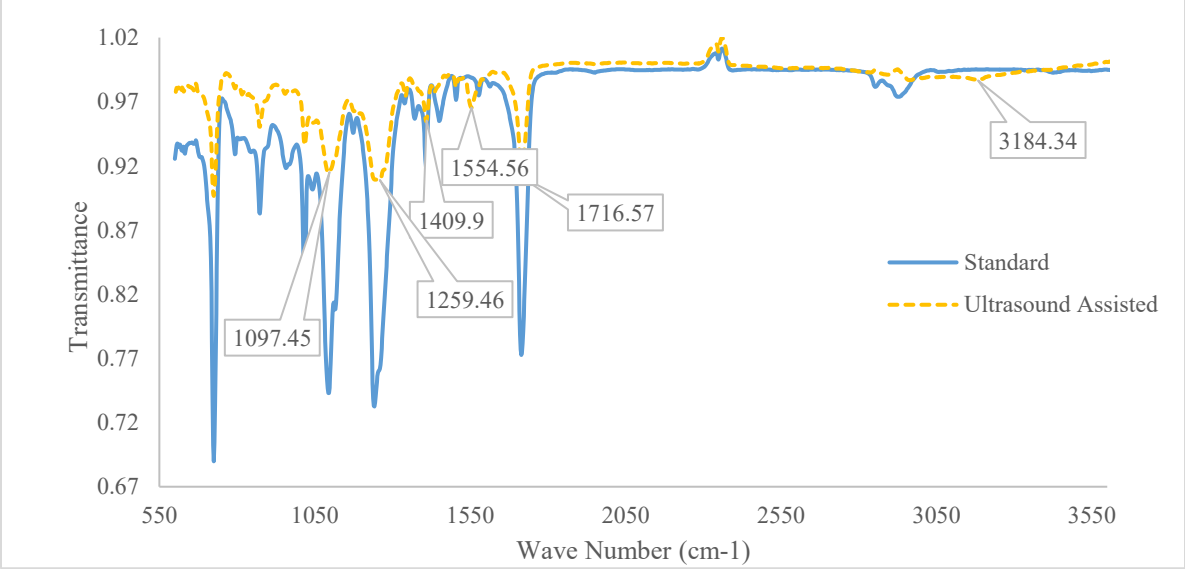

Figure 4.13: FTIR spectrum of ultrasound assisted chemically hydrolyzed PET film.

4.8. Ultrasound Assisted Chemical Hydrolysis of Ozone Pretreated PET film:

Finally, a combination experiment of ozone pretreatment and ultrasound assistance on chemical hydrolysis of PET film was investigated. The results obtained from this experiment are discussed in the following section.

4.8.1. Weight Loss

The combined effect of ultrasound during chemical hydrolysis showed ~80% WL. Figure 4.14 shows the effect of ozone dosage on ultrasound assisted chemical hydrolysis. It was observed that the dosage of ozone had no significant impact on the process. Figure 4.14 summarizes the statistical analysis of WL of ultrasound assisted chemical hydrolysis of ozone pretreated PET films.

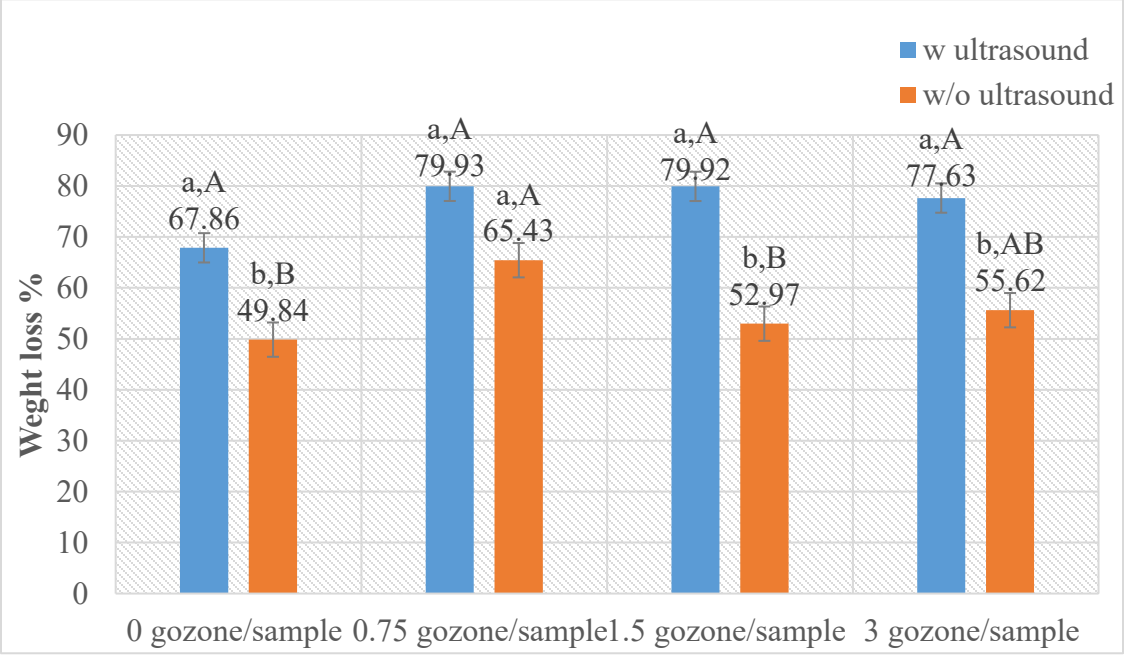

Figure 4.14: Statistical analysis of ultrasound assisted chemical hydrolysis of ozone pretreated PET films (lower case different letters mean significant difference in the ozone dose. Upper case different letters mean significant difference in the ultrasound assistance)

From figure 4.14 it was observed that there was no significant difference of ultrasonication on ozone pretreated PET films. However, there was a significant difference when 0.75g_{ozone}/sample was used for chemical hydrolysis.

4.8.2. HPLC Analysis

Combination of ultrasound energy during chemical hydrolysis of ozone pretreated PET increased the monomer recovery compared to ultrasound aided chemical hydrolysis of untreated PET. From this experiment we found 1.5 g ozone dosage per sample gave the significant result. The increase of monomers was most like due to ozone pretreatment that co-relates our result from the chemical hydrolysis of ozone pretreated film. Table 4.6 summarizes the HPLC recovery of monomers and dimers from chemical hydrolysis of ozone (different dosage) pretreated films.

Table 4.6: HPLC results of filtrate after filtering out neutralized monomers. Recovery values are based on a summation of PET monomers and dimers obtained by weight of solids filtered out and HPLC values after ultrasound assisted chemical hydrolysis of ozone pretreated PET.

Ozone (g)	% TPA	% EG	% BHET	% MHET	% HPLC Recovery	% Weight of washed filtrate after neutralization	% Total Recovery	% Weight Loss
0.75	3.29	11.68	1.92	2.3	19.19	0.16	19.3	79.93
1.5	5.55	19.71	0.82	1.37	27.45	25.44	52.9	79.92
3	3.33	11.81	2.19	2.19	19.92	37.86	57.78	77.63

Unlike enzymatic hydrolysis, the higher difference between WL and total recovery was found in most of the chemical hydrolysis process. Although Bhogle and Pandit (2018) reported significant amount of TPA recovery after ultrasound assisted chemical hydrolysis, our results did not show such significance. But it showed a little improved recovery than without ultrasound assisted chemical hydrolysis. Even though, ultrasound assistance

increased WL, monomer recovery was quite low compared to WL. This indicated that PET was not hydrolyzed completely rather solubilized.

4.8.3. FTIR Analysis

Significant changes were observed in the FTIR spectrum of ultrasound assisted chemical hydrolysis of ozone pretreated PET films. Figure 4.15 shows the overall changes in FTIR spectrum and the changes in each bond.

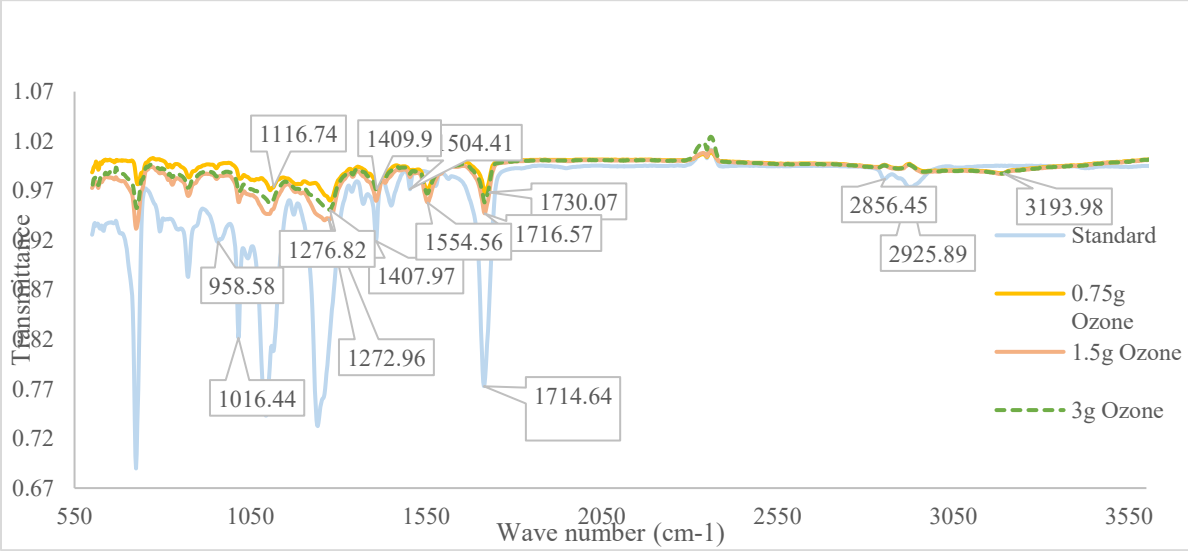

Figure 4.15 (a)

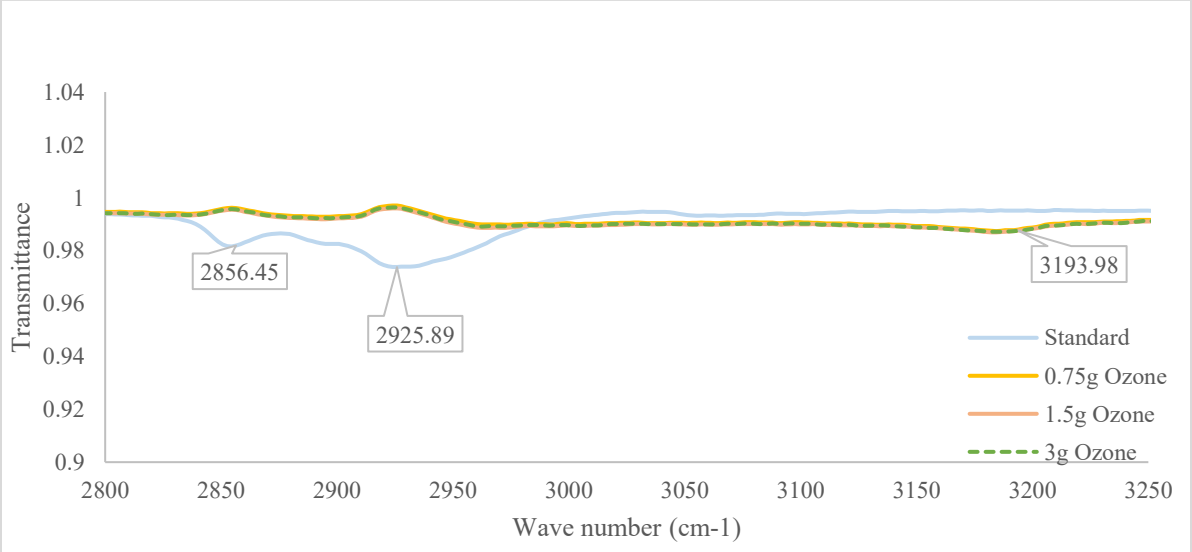

Figure 4.15 (b)

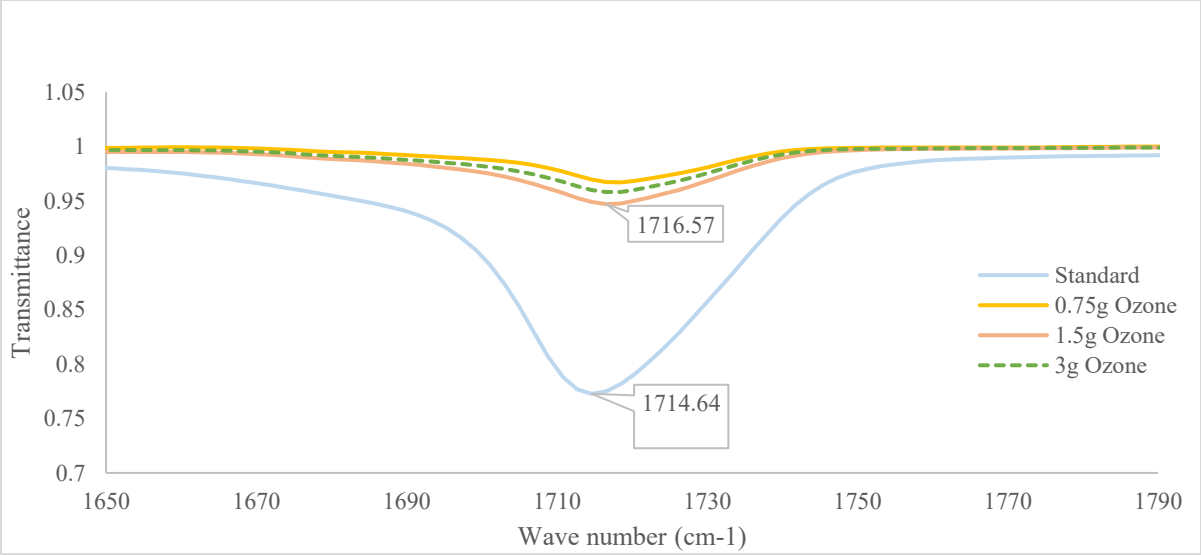

Figure 4.15 (c)

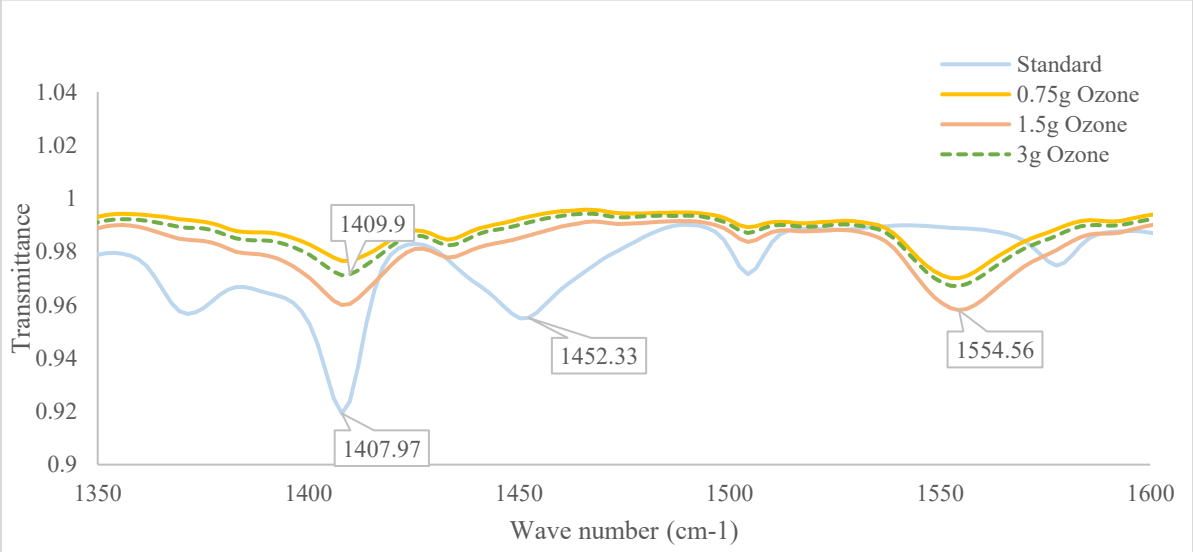

Figure 4.15 (d)

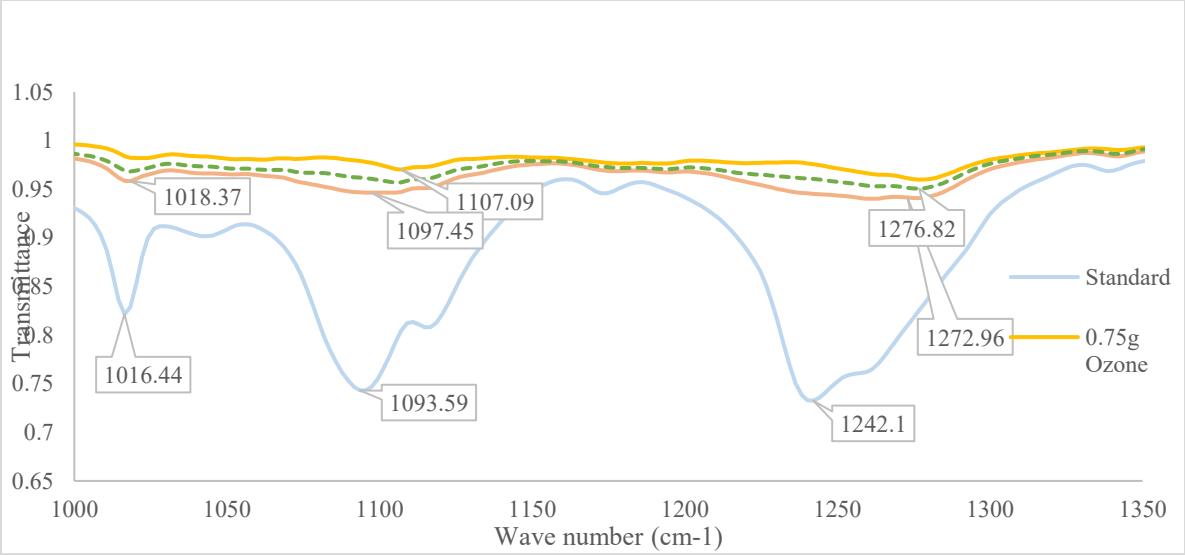

Figure 4.15 (e)

Figure 4.15: a) FTIR spectrum of ultrasound assisted chemically hydrolyzed pretreated PET film. b) changes of O-H stretch and C=H stretch c) changes of C=O stretch d) changes of C=C stretch and C-H stretch & e) changes of C-O stretch.

From these spectrums it was observed that the intensity of a few bonds decreased drastically. Disappearance of C=H stretch at 2858 cm^{-1} and 2925 cm^{-1} indicated they had undergone complete hydrolysis. New O-H peak at 3193 cm^{-1} indicated addition of O-H group during hydrolysis. Similarly, changes of peak position, decrease and increase of their intensity were associated with hydrolysis. Disappearance of C-H stretch at 1452 cm^{-1} was also found. Most alterations were associated with chemical hydrolysis. However, further decreased intensity of C-O stretch and their shift from 1093 to 1097-1107 cm^{-1} and from 1242 to 1272-1276 cm^{-1} could be due to ozone pretreatment as these changes were not observed in previous experiments. Similarly, C=O stretch at 1714 cm^{-1} shifted to 1716 cm^{-1} with drastic decrease of intensity. The possible explanation cloud be formation of ozonated intermediate compounds that aided in the chemical hydrolysis process similar to enzymatic hydrolysis (Olewnik *et al*. 2013).

4.9. Size reduction and Chemical Hydrolysis

As the chemical hydrolysis gave such high breakdown, it was decided to investigate the effect of mechanical pretreatment as size reduction on chemical hydrolysis of PET. The

PET samples were broken down to particle size 250µm using a grinder. Then the powdered samples were used for chemical hydrolysis. This resulted in 90% WL of the initial samples. However, no peak was found in HPLC for monomers and dimers. The precipitation found after neutralization by acid was 44.38% of initial weight of the sample which is known to be TPA by Bhogle and Pandit (2018). Despite giving better WL compared to the films, mechanical grinding pretreatment is a very tedious process and consumes a lot of time and energy and may not be economically feasible (Kim *et al.*, 2019). However, we can conclude here that physicochemical pretreatments enhance the hydrolysis of PET.

4.10. Discussion

Enzymatic hydrolysis by HiC of untreated PET after 48 hours gave 6.6% WL and 6.55% monomers and dimers of which 5.9% were the dimers, BHET and MHET. As mentioned before in a study by Ronkvist *et al.*, (2009) 5% WL was reported after 96 hours using FsC and PmC. Besides, they reported 97% WL of lcPET by HiC after 96 hours. In our study, WL increased to 24.76% after 60 days of hydrolysis. The slower hydrolysis rate is because we used an immobilized form of the enzyme. The solid-solid reaction rate is expectedly very slow. In order to increase the hydrolysis rate, ozone and ultrasounds pretreated films were used for enzymatic hydrolysis. Here the overall WL of the films did not increase significantly. This could be because of insufficient ultra-sonication or oxidation by ozone.

Subsequently, the effect of ultra-sonication was investigated during enzymatic hydrolysis as ultra-sonication increases enzymatic activity by reducing activation energy and enthalpy of the enzyme reaction (Pellis *et al.*, 2016). The WL increased 1.3-fold as well as 5.9-fold increase was found in TPA recovery. The result suggested increase in HiC activity as more TPA and EG were convertd from BHET and MHET. This result is in agreement with a study by Pellis *et al.*, (2016) where they reported 5.2-fold increase of TPA release by incorporating ultrasound during enzymatic hydrolysis by the enzyme Thc_cut 1. Moreover, it was found that ultrasonication beyond 10 minutes decreased the WL. As mentioned earlier, the extreame shock waves from micro cavities during sonication inactivates the enzyme and hence reduces conversion.

Furthermore, effect of ultra-sonication during enzymatic hydrolysis of ozone pretreated films was investigated in our study as well. 9.8-fold increase of TPA recovery was recorded. This suggested that, combination of ozone pretreatment and ultra-sonication during enzymatic hydrolysis gave the highest monomer recovery in enzymatic hydrolysis. This is because the oxidation by ozone caused increase of carbonyl groups that are prone to hydrolytic cleavage by HiC and the sonication both increased the enzyme activity and made microbubbles on the surface of PET. To the best of our knowledge, our study is the first report of the combined effect of ozone and ultrasound on PET hydrolysis by HiC.

Later on, chemical catalyst was used for PET hydrolysis as this process was faster than enzymatic hydrolysis. An one hour of chemical hydrolysis of PET in methanolic NaOH gave 49% WL. This result is in agreement with Bhogle and Pandit (2018) who reported 42% WL of PET in non-aqueous alkaline hydrolysis. The total recovery from the coversion was 16.94% including 3.93% from HPLC recovery and 13.01% from precipitated TPA after nutralization. As mentioned earlier, the generated short chain polyolefins which were dissolved in the solution was not recovered. WL increased to 60% in ultrasound pretreated films and 65% in ozone pretreated films. this result suggested that ultrasound and ozone can increase PET hydrolysis. In case of monomer recovery, 38.3% was recovered when ultrasound pretreated films were used and 44.53% when ozone pretreated films were used for chemical hydrolysis. These result again proved that ultrasound and ozone pretreatments increased the functional groups, weekened the bonds of the PET films thus increased the hydrolysis.

Ultrasound assistance during chemical hydrolysis of untreated PET increased WL from 49% to 68% WL. This is similar to the report of Bhogle and Pandit (2018) who reported WL increase from 42% to 53%. But the total recovery was 25.2% including 11.13% HPLC recovery and 14.07% TPA precipitated after neutralization. This is because ultra-sonication helped in solubilizing the PET by forming short chain polyolefins, but the monomer recovery was lower. The WL and total monomer recovery increased to 80% and 52.9% respectively when ultra-sonication was used during chemical hydrolysis of ozone pretreated films. In this case the monomer recovery increased 2-fold from ultrasound assisted chemical hydrolysis of untreated film and 3.1-fold from chemical hydrolysis of

untreated film. Similar to enzymatic hydrolysis, combination of ozone pretreatment and ultra-sonication during chemical hydrolysis gave the highest WL and monomer recovery suggestion the positive effect of the combination in PET hydrolysis.

The highest WL of 90% was found using chemical hydrolysis of PET powders (250μ particle size) and 44.38% total recover of monomers and dimers were obtaind. However, mechanical size reduction precess is not energy efficient as 20 minutes of grinding generates approximately 100 mg of PET powder and consumes 7.5 Kw/hr energy. Moreover, the difference between WL and total recovey is high in most cases of chemical hydrolysis. This suggested the presensce of shorter chain polyolefins that might become difficult to recover.

One conclusion we can draw from this is that enhancing chemical hydrolysis process we can either incorporate ozone pretreatment or ultrasound assistance. Further studies are need to make the processes more economical and implementable on a larger scale. The basic aim of this work was to obtain monomers or dimers, so that they can be reused. Our results showed that while the enzymatic hydrolysis produced mostly monomers or dimers, the total yields were low. This was attributed to use of immobilized enzymes with an insoluble substrate. On the other hand, chemical hydrolysis resulted in higher weight loss (more than 90%) but the number of monomers and dimers produced were small. From a practical point of view, we suggest that an enzyme in solution that can react with the plastic surface (enhanced by pretreatment) and produces monomers in higher quantities faster. The products can be separated and reused for PET production and will be the best environmentally sustainable way forward.

Chapter 5

CONCLUSION & FUTURE WORK

5.1. Conclusion

Given its positive characteristics for a wide variety of application, it is very unlikely that the use of PET will be reduced substantially in the near future. The problems associated with post-consumer PET waste disposal cannot be solved overnight as the same characteristics make them difficult to degrade. Hence, these wastes will increase at an alarming rate posing a great threat to our environment and ecosystem. In addition, these wastes are contributing to generate Micro-plastics and Nano-plastics that are eventually consumed by us through food and water. To close loop of end of life of PETs and other plastics, implementing circular bio-economy seems a sustainable approach. This will require monomer recovery as an essential target. The recovered monomers have the potential to be reused to make the plastic again. This route will solve the difficulties of contamination associated with other recycling options.

Physicochemical pretreatment will certainly enhance degradation of polymers to its monomeric form. Ultrasonication and ozone pretreatment evaluated in this study enhanced the hydrolysis of PET and resulted in the accumulation and recovery of the monomers TPA and EG. The following conclusion can be drawn from our study-

- Enzymatic hydrolysis of untreated PET using immobilized HiC yielded lower degradation and lower TPA and EG recovery.
- Ultra-sonication for 10 minutes during enzymatic hydrolysis increases the activity of the process and increased breakdown of PET.
- Although a combination of ozone pretreatment followed by ultrasound treatment during enzymatic hydrolysis did not increase the WL of PET, it certainly increased TPA and EG recovery.
- In enzymatic hydrolysis the overall degradation is very slow compared to chemical hydrolysis, but the recovery of TPA and EG was great. This can be attributed to the immobilized form of the enzyme used.

- Chemical hydrolysis has significantly higher degradation rate than enzymatic hydrolysis.
- In terms of degradation of PET, ozone pretreatment (1.5g) followed by ultrasound assisted treatment during chemical hydrolysis gave 80% weight loss and 52.9% total recovery of monomers and dimers.
- Chemical hydrolysis leads to breakdown to monomers, dimers, and other shorter chain polymers. The difference between weight loss and total monomer-dimer quantities are accounted for by the short chain solubilized PET.
- Among pretreatments, size reduction prior to chemical hydrolysis gave the highest degradation which is ~90%. However, the process is not cost effective.

5.2. Future Work

To implement green route for PET degradation, more works need to focus on improving the enzymatic hydrolysis processes. The production of cutinase enzyme can be enhanced by using different inducers. Use of these enzymes in soluble form, will certainly result in higher biodegradation. Besides, the micro-organism can be genetically modified to produce more cutinase that can breakdown the pretreated PET into TPA and EG and increase the recovery yield. Further studies are required for the separation of the monomers obtained after hydrolysis. Furthermore, work needs to be done to remake PET using the recovered monomers in order to a circular bio-economy.

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APPENDIX

A. Calculations

- Weight loss (WL), % = $\left(\frac{Wpet,i,s - Wpet,f,s}{Wpet,i,s}\right) \times 100$

  Where *Wpet,i* is initial weight of pre-treated PET film before hydrolysis and *Wpet,f* is the final weight of PET film after hydrolysis.

- % monomer/dimer = $\left(\frac{mass\ (mg)}{Initial\ weight\ (mg)}\right) \times 100$

- Total Recovery = $\left(\frac{HPLC\ recovery + TPA\ precipitate}{Initial\ weight\ (mg)}\right) \times 100$

B. pNP Standard curve

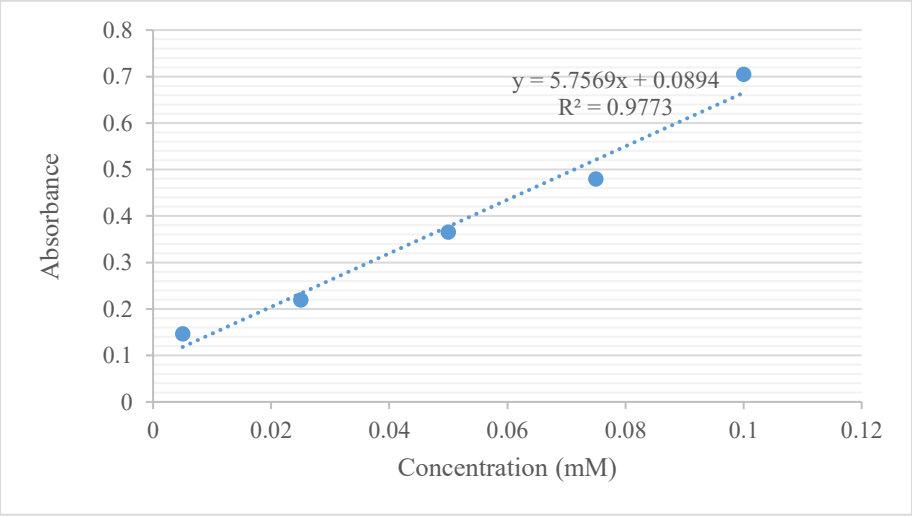

Figure: Standard curve of p-Nitrophenol at 405nm.

C. Ozone production

Mass with Syringe (g)		Mass (g)	Density (g/L)	Mass fraction	Ozone production (g/min)	Ozone Production (g/h)	Production rate from data sheet (g/h)
Oxygen	27.4383	0.0582	1.21200426				
Setting 2, Ozone	27.4391	0.059	1.22866411	0.005498282	0.040533249	2.431994937	2.6
Setting 5, Ozone	27.4394	0.0593	1.23491155	0.015120275	0.112033213	6.721992786	6.25
Setting 9, Ozone	27.4401	0.06	1.24948892	0.024742268	0.185491139	11.12946836	11.8
Dry empty	27.3801						

D. Retention time in HPLC:

- TPA = 8.8 min
- EG = 11.5 min
- BHET = 10.9 min
- MHET = 7.3 min

E. HPLC spectrum

- HPLC peaks are shown in the following figures.

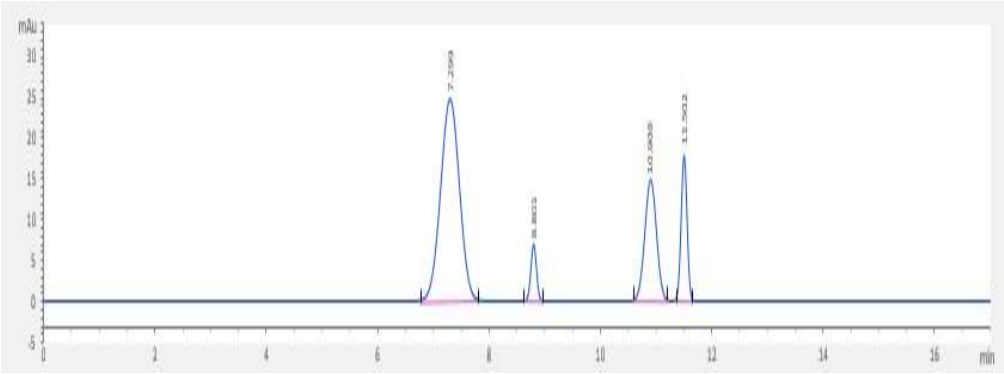

Figure a: Standard PET monomers and dimers

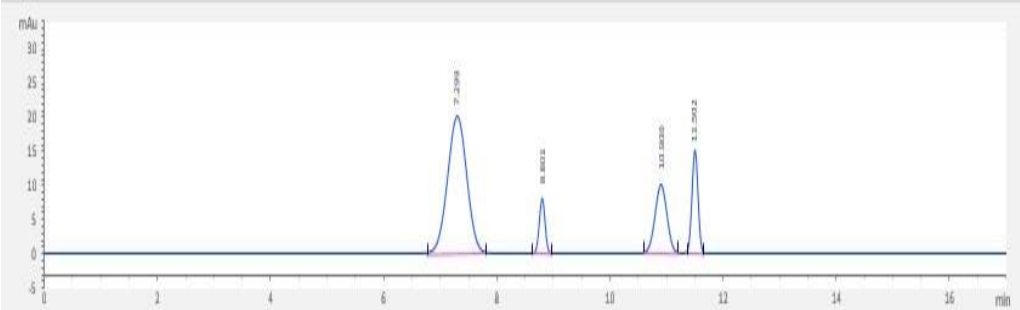

Figure b: Hydrolysis of untreated PET

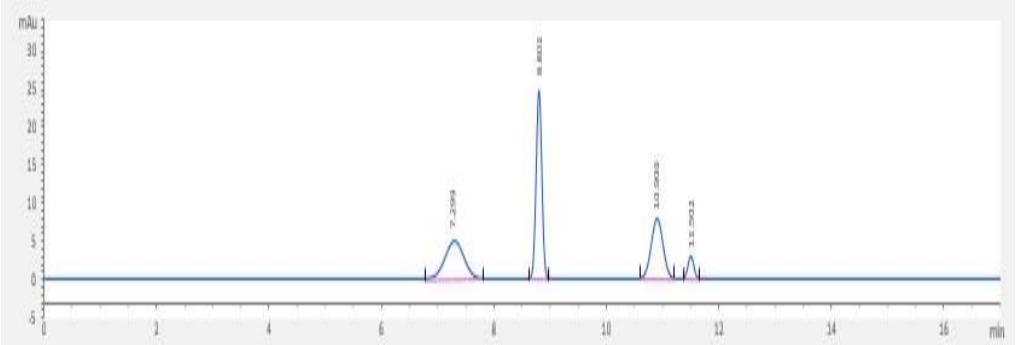

Figure c: Combined ozone and ultrasound treated Hydrolyzed PET