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Fundamentals of Polymer Biodegradation Mechanisms

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2.1 Introduction

Plastics wastes can be removed from circulation mainly via mechanical recycling, thermal recycling, or chemical recycling. Each one of these recycling methods alone can face inherent limitations that may severely impact large-scale feasibility. Therefore, selection of one or multiple proper recycling methods largely depends on the chemical structure, purity, and the application of the specific plastic polymer. For example, thermomechanical recycling often results in a loss of the mechanical properties of the recycled polymer that cannot compare to those of the virgin polymer [1]. Mechanical recycling (e.g. grinding and microionization) is further complicated by the presence of additives and plasticizers within the plastic, and heating and remolding during the process cause chain scission to the degree that most plastics cannot be used beyond three recycled stages. Consequently, de novo polymer synthesis is preferred, leading to increasing accumulation of plastic polymers.

Thermal recycling typically includes incineration, gasification, pyrolysis, and plasma arc. Incineration can help produce energy from municipal waste, but it usually results in incomplete combustion, releasing toxic compounds into the atmosphere where it becomes a public health hazard [2]. Although new technologies have recently been introduced to clean up toxic exhausts, their full practical implementation is still far from a viable solution, partly due to political incompetency. In the past two decades, gasification of municipal waste has also been considered for waste disposal and energy recovery in the form of syngas, which is eventually converted into methanol, ethanol, or diesel. Although gasification appears to be a promising alternative for disposing municipal waste compared to

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other approaches such as landfill burial or incineration, its success in the long run depends on appropriate governmental policies. Despite the advantages mentioned, gasification cannot be applied to the disposal of plastic waste and microplastics that have already contaminated the oceans. Moreover, this technology cannot rescue us from the need for de novo synthesis of plastics from oil, which eventually adds more carbon on earth every year.

Chemical recycling can be achieved via abiotic and biotic methods. It is typically used to convert plastic wastes back to oil and, in some cases, can achieve full depolymerization, where the resulting monomers can be reused to resynthesize new polymers [3]. For example, polymers with carbon chain structures such as hydrocarbon polymers can be reverted back to oil, while hydrolysable polymers with heterologous chain structures including polyesters can be converted into raw monomers and oligomers via physiochemical processes such as glycolysis, amine decomposition, pyrolysis, supercritical decomposition, and enzyme-based hydrolysis [4]. Although abiotic methods are currently considered as the predominant approach for chemical recycling of plastic polymers, they can pose limitations in a few situations depending on the type of plastic waste. For example, these methods require nearly pure polymer feedstock, which is realistically impossible to obtain from plastics synthesized with additives or plasticizers and/or compounded by the contamination of plastics sourced from landfills or municipal waste. Moreover, every year, an increasing amount of plastic waste including polyester-based microplastics present in laundry products, for example, enters our oceans through sewage systems. In most cases, collection and disposal of these microplastics are not practical. Therefore, their degradation and assimilation by microbes is highly considered. Given these limitations, chemical recycling based on biodegradation methods can offer extra advantages for polymer waste disposal.

By its very nature, material degradation by microorganisms is a dirty process and benefits from mixed microbial communities. Therefore, biodegradable polymers exposed to the environment, compost, or landfill automatically benefit from exposure to as many microorganisms and weathering conditions as possible. Some polymers can be assimilated into microbial biomass and converted into energy (e.g. biogas) or other value-added bioproducts. These microbial processes usually require no or little energy inputs outside of the typical metabolism. The use of additives and plasticizers can sometimes inhibit microbial degradation but can also serve as an energy source for secondary metabolizers or even attract primary colonizers instead of the polymer itself. Finally, the scale of plastic accumulation in landfills and the environment is substantially high that recollection of all such plastics would be monumental. These facts suggest that widespread biodegradable plastic use in the future would at least enable plastic waste degradation on a wide scale while non-biodegradable plastics in general may require a more deliberate disposal process. It is important to note that biodegradation is a last resort.

This chapter will explore degradation of several types of common polymers grouped by the bonds that hold their monomer units together in polymer chains. More specifically, degradation of polysaccharides, polyamides, polyesters, hydrocarbon polymers, halogenated polymers, and polyethers will be discussed. Each section

will begin with a brief introduction to the chemical structure of a specific polymer, followed by a discussion about available abiotic and biotic degradation mechanisms. Next, secondary characteristics such as the polymer's crystallinity, melting and glass transition temperatures, the presence of plasticizers or additives, and conditions under which these polymers reach the end of their lifecycle will be explained. Moreover, we will explore how a combination of mechanical pretreatments and chemical recycling using engineered enzymes would enable depolymerization and repolymerization of the most abundant polyester, poly(ethylene terephthalate) (PET), in controlled industrial environment, introducing a path toward a full circular plastic economy. We will conclude the chapter by presenting applications of biodegradation and the current challenges and possible future directions in the management of plastic waste disposal.

2.2 Overall Scheme of Polymer Degradation

Polymer degradation can occur through two separate mechanisms: abiotic and biotic. Abiotic degradation results in fragmentation. This fragmentation usually comes in the form of pretreatments. Ultraviolet (UV) and gamma irradiation often produce radicals, which propagate throughout the polymer chain, thereby increasing the reactivity. The sun readily provides UV exposure from which photo-oxidation occurs by function of the intensity of electromagnetic radiation. This often occurs simultaneously with oxidation since oxygen is available during these pretreatments. Peroxides and hydroperoxides tend toward homolytic cleavages in their bonds from UV radiation in the 240–400 nm range [5]. Thermal pretreatments can also introduce hydrophilic functional groups allowing for more microbial contact, facilitating further biotic degradation. In addition to forming radicals, which can be generated through radiation, oxidative chemicals can also impact the polymer through depolymerization and inducing bulk physical changes, resulting in increased permeability and tensile strength [6]. However, the downsides to these reactions include the long time frames (up to two years) for any discernable effect to take place for buried samples. The most promising results, thus far, come through *in vitro* experiments on polyesters via gamma radiation [7].

Biotic degradation is assisted through microbial action. A more comprehensive degradation occurs through bioassimilation, in which polymer fragments are consumed by microorganisms and converted to products such as water, carbon dioxide, methane, and biomass depending on the presence or the absence of oxygen. The extent of this process depends on, but is not limited to, terrestrial or marine environments, the microorganism itself, the presence of additives in the polymer, and even the polymer's shape. Biotic degradation generally necessitates the formation of biofilms. A biofilm is a consortium of microorganisms that produce a network of extracellular polymeric substances that stabilizes the microorganisms and adheres them onto the target surface [8]. After this adherence, extracellular enzymes are secreted, which then hydrolyze the surface of the polymer, which have been primed via abiotic degradation. The released oligomers, dimers, and

monomers, as the hydrolysis products, are then used by microbes as nutrients [7, 8]. This process can become a function of surface area in that the biodegradability increases proportionally with biofilm colonization. Besides hydrolytic enzymes, microbes can release biosurfactants, which would reduce the surface tension at the interface between the biofilm and plastic. They are also amphiphilic compounds, which can increase the bioavailability and biodegradability of the polymer chain [6].

2.3 Biodegradation of Polysaccharides

2.3.1 Cellulose

As a naturally produced polymer, cellulose is a glucose molecule connected via β-1,4 linkages [9]. These covalent bonds are referred to as glycosidic bonds. There are two stable conformers of natural cellulose (cellulose I), one of which contains a bifurcated bond with the oxygen on the C-6 hydroxyl and the ring oxygen with the C-3 hydroxyl on the adjacent chain. The other variant consists of the ring oxygen bonding with the C-3 hydroxyl on the next residue (Figure 2.1a) [10]. Cellulose may contain one variant in surplus, while both variants found together in disordered configurations are typical of amorphous regions. Further polymorphs (cellulose II,

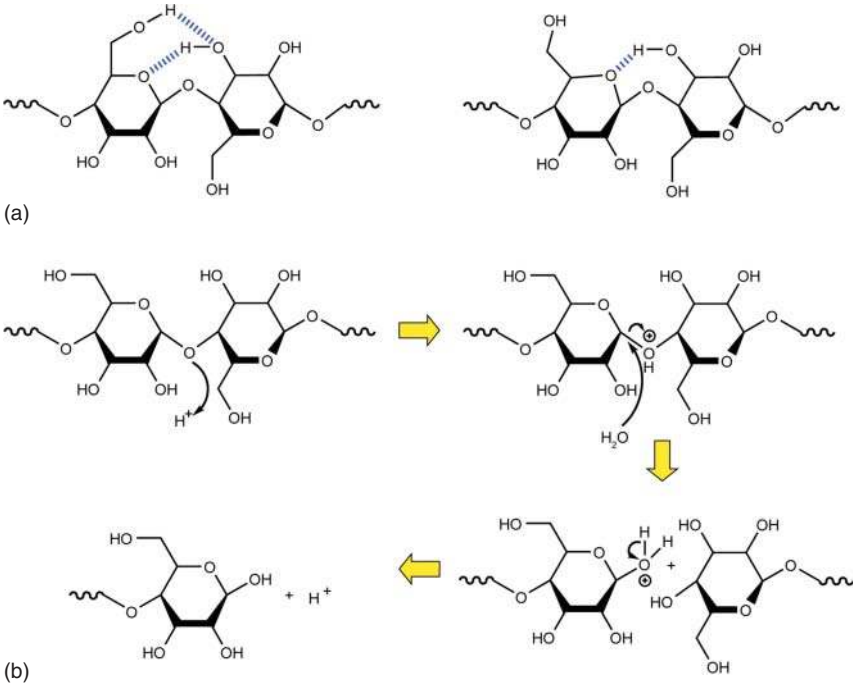

Figure 2.1 Biodegradation of cellulose. (a) Conformers of cellulose. (b) Cellulose hydrolysis mechanism.

III_I, III_{II}, IV_I, and IV_{II}) can be generated through various treatments such as with liquid ammonia [11]. Depending on the type of the microorganism, biodegradation often occurs in the amorphous parts of the polymer [12]. Depending on the chain length of the polymer, also known as the degree of polymerization, the bonds can be very strong due to London dispersion forces that increase proportionally with chain length. These chains are in the form of fibers that are further embedded in a hemicellulose and lignin-composed matrix. Because of this crystalline and inaccessible structure, penetration of enzymes to the fragment cellulose would be impaired [13].

As noted above, cellulose contains enough crystallinity to deter enzymatic hydrolysis. This barrier can only be overcome through a medley of enzymes that work in unison to degrade this natural polymer, which also requires up to a 100-fold enzyme concentration than that of starch [14]. Studies have shown that cellulose-hydrolyzing enzymes, cellulases, in particular, have larger catalytic active sites that can accommodate 5 to 6 units of glucose. These enzymes have considerable diversity but belong to a common class called cellulase, which contain endoglucanases, cellobiohydrolases, and cell biases [15]. Despite these differences, these enzymes can also contain a variety of similarities relating to folding patterns, catalytic residues, and reaction mechanisms. Other enzymes are involved in auxiliary functions such as assisting in degradative product recovery, focusing hydrolysis efforts, and securing enzymes along the surface of the polymer [16].

During cellulose hydrolysis, enzymes attack the β-1,4 glycosidic bonds. This process promotes fragmentation, resulting in glucose, cellobiose (a double-glucose molecule), and cellodextrins (cyclic oligomers of cellulose) (Figure 2.1b). Endo- and exolytic enzymes work synergistically to unzip various layers of cellulose by moving along the surface. For example, cellobiohydrolase acts to pull away chains from the strata of the substrate, thereby undoing the hydrogen bonds holding the lattice together [9]. β-Glucosidases wrap up hydrolysis by cleaving cellobiose units and extracting the glucose monomer from the non-reducing end – a group that has no free aldehyde or ketone to be oxidized [9, 17].

In the next step, cellulose fragments are consumed by microorganisms. It can be attacked by a myriad of microorganisms as its polymer constituents are natural metabolites. The high nutrient utilization by microbes highlights the cellulose's biodegradability. These microorganisms have cellulosomes (complexes on the cell surface) that adhere onto cellulose. The cellulosomes facilitate multiple cuts resulting in cellobiose, which is then bioassimilated [18]. *Clostridium thermocellum* and *Thermomonospora curvata* are examples of microbes that have this capability [19]. In fact, *C. thermocellum* (an anaerobe) can reach up to 93% utilization of cellulose at a rate of 2.9 g/l/h. during fermentation [20]. Although this organism shows the highest growth on cellulosic substrates, it does not produce tremendous amounts of enzymes [21]. Comparing burial and exposure methods shows that the consortium of microorganisms present in soil have a greater effect than the oxidative benefit from sunlight. In fact, soil burial boasted 65.7% molecular weight decrease in nine weeks, whereas cellulose hung outside merely lost 38.9% [22]. Many species from commonly recognized genera degrade cellulose such as *Aspergillus*, *Penicillium*, and

Trichoderma [19]. Overall, the entire process should result in carbon dioxide and water as by-products. The same by-products are present in anaerobic biodegradation in addition to methane, hydrogen sulfide, hydrogen gas, and ammonia [7].

2.3.2 Starch

Starch is another biodegradable polysaccharide. It consists of amylose and amylopectin. The former is a linear polymer, which contains α-1,4 linkages of glucose units. The latter is a branched polymer connected by α-1,6 bonds (Figure 2.2a). At least 10–20% of the material is composed of the linear amylose, while the remainder is branched amylopectin, which is responsible for the polymer's crystallinity [23]. Each iteration of the glycosidic bond in amylose proffers a natural twist, which is reminiscent of a helix. In the case of cellulose, however, the successive glycosidic bonds have a 180° rotation per monomer. Starch residues contain the hydroxyl groups on the external side of the helix, allowing for abundant hydrogen bonding with adjacent chains, other similar functional groups, and water molecules [24], which varies physical properties depending on extent of network formation. Little is known about the structure of amylopectin, but it is estimated to have a variety of chain lengths.

Starch can undergo hydrolysis. It comes in the form of granules, which are naturally formed by many plants to be used for storage. These granules are initially

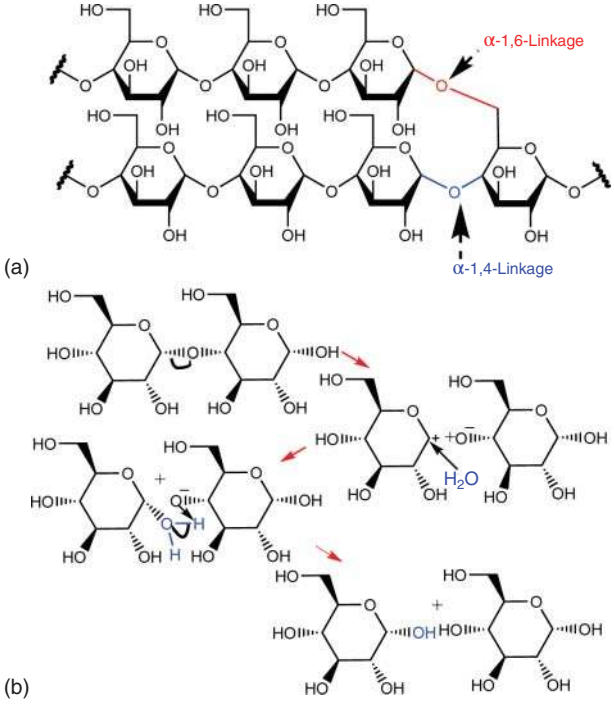

Figure 2.2 Hydrolysis of starch. (a) Two types of starch linkages. (b) Hydrolysis mechanism of starch.

degraded on the surface. Some starches have specific zones susceptible to corrosion from within. These holes or pits grow larger and form channels permeating through each stratum of starch [24]. Amylases are the main enzymes that degrade starch. Amylases were shown to disentangle the helix structure of amylose. As with other polymers, starch greatly benefits from pretreatment strategies, particularly gelatinization, in which heating a starch suspension allows for swelling within the amorphous regions of the polymer and opening the crystal structure. This process greatly improves the reactivity to hydrolytic enzymes. Exo- and endolytic enzymes play a role in starch degradation as well. For example, β-amylase and glucoamylase catalyze the non-reducing end of the polymer chain, which is the location of the anomeric carbon. This is where glycosidic bond cleavages occur whose product leaves a newly formed hydroxyl group in the C1 position. The ketal bond in the glucose ring is especially susceptible to breakage. The overall rate of hydrolysis is initially quick but slowly tapers off [25]. Glycosidic hydrolysis is very sensitive to ring shape and size. Formation of the enzyme–substrate complex allows for ring distortion, thereby decreasing the dihedral angle. This transition state is more thermodynamically favored toward hydrolysis [26]. Research has shown that *Aspergillus niger* can degrade starch completely in 20 days in both solid and liquid cultures without light and heat, which is faster than the case for cellulose [27].

Starch is often used in other polymers to promote biodegradation. Pure starch is brittle and moisture sensitive, enough to disintegrate in the presence of water [28]. Because of its sensitivity to humidity and temperature, starch is typically added to other polymers as a degradation enhancer, filler, and cross-linking agent [7]. Because of the availability and relative inexpensiveness of starch, there is a push to incorporate it into more traditional polymers. However, there are inherent limitations to this process because of starch's subpar mechanical properties [29]. Regardless, studies show that starch-infused plastics are more heat resistant with the added benefit of attracting more microbes for biodegradation and consequently improving the overall biodegradability [7]. This biodegradation comes through initial hydrolysis (Figure 2.2b).

Use of starch as a biodegradation inducer of other polymers has grown in the compost market because of its pairing with low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE) even though these blends are a long way from being considered fully biodegradable [29]. However, starch–PE blends have been shown to degrade quickly – initial starch degradation forms pores and holes in the composite film and facilitates high oxygen saturation and therefore oxidation and biodegradation of PE [7, 30]. There is a balance to achieve in that higher starch content does not yield higher biodegradability. In 10 weeks, 40% starch–LDPE samples had a weight loss of 5.24%, which is higher than that of 50% starch composites that had 2.75% in the compost [31]. This shows that there is an upper limit to starch blends that minimizes the amount of recalcitrant PE used in the composite polymer, which may be a better option compared to a pure hydrocarbon polymer. Although there is degradation of PE, this may also harm terrestrial and aquatic environments in that PE fragments may be ingested by animals and propagate through the food chain in the form of microplastics [32].

2.4 Biodegradation of Polyamides

Polyamides are also an important polymer coming in both natural and synthetic forms. The amide linkages combine various monomers of different types composed of aliphatic, semiaromatic, or aromatic molecules. It is known for its high crystallinity and strong interchain interactions [33]. The most common synthetic polyamide applications are in nylon and Kevlar. Natural polyamides such as silk are prevalent; however, scientists have not yet specified which microorganisms biodegrade the polymer [34]. There are bacteria that can degrade lower weight oligomers such as 6-aminohexanoic acids (cyclic and linear) typically found in waste streams in nylon production facilities [35]. The *Arthrobacter* sp. have been found using several hydrolases and aminotransferases that aid in the degradation and eventual bioassimilation [34]. The hydrolysis of amide bonds leads to the metabolism of 6-aminohexanoate to adipate semialdehyde. This is further catalyzed by adipate semialdehyde dehydrogenase to form adipate through the use of a NADP+ cofactor [36]. Some researchers have shown *Bacillus cereus*, *Bacillus sphaericus*, *Vibrio furnissii*, and *Brevundimonas vesicularis* to degrade nylon, although it has not demonstrated that these bacteria are not just degrading the polymer additives, which is a common issue in measuring biodegradability [37]. Other researchers have reported *Pseudomonas* sp. to utilize only 6-aminohexanoate dimers as its carbon and nitrogen source [38]. No enzymes or pathways are described for nylon degradation by bacteria but one has been identified as a manganese peroxidase isolated from a white-rot fungus. Alas, the evidence for its biodegrading capabilities is scant at best [39]. However, polyamide 4 (PA4), a common type of nylon, have been found to be degraded in activated sludge [40]. In addition to activated sludge, soil and seawater tests have been performed with seawater tests showing biodegradation tapering off at 50% in 12 days [41]. PA4 has not shown any discernable degradation *in vitro* when incubated at 37 °C in phosphate buffers [42].

2.5 Biodegradation of Polyesters

Polyesters are classified into three groups: aliphatic, aliphatic-co-aromatic, and aromatic. Because of their good material performance, there is a great interest of aliphatic-co-aromatic polyesters such as PET in various industries, which has led to accumulation of large amount of post-consumer polyesters in the environment. Therefore, recycling these polymers is absolutely necessary. Although aliphatic polyesters are generally susceptible to biodegradation, aliphatic-co-aromatic and aromatic polyesters are extremely resistant to enzymatic attack largely due to the presence of aromatic groups. Although a number of native and engineered enzymes have been shown to degrade these polyesters, no enzyme is still known to fully degrade high-crystalline variants of these polymers. Here, we first review the fundamentals of biodegradation of common aliphatic polyesters such as polylactic acid (PLA), poly(ε-caprolactone) (PCL), and polyhydroxyalkanoates (PHAs). Then, we present a more comprehensive discussion surrounding the biodegradation of PET, which has been investigated in greater detail by the scientific community compared to other polymers.

2.5.1 Polylactic Acid

Polylactic acid or polylactide (PLA) is an aliphatic polyester synthesized from lactic acid and can be produced from entirely renewable resources, such as corn and sugar beets [43, 44]. PLA can be degraded back into its biologically benign monomers and is currently one of the most widely produced polymers in the biodegradable polymer market. The degradability of PLA has been mostly measured following chemical hydrolysis [45]. Much of PLA biodegradation has been studied under aerobic conditions such as composting [46]. For example, industrial composting at 58 °C can achieve 90% biodegradation within a maximum of six months [47]. Based on these studies, PLA can be considered sustainable under industrial composting conditions. However, only certain types of PLAs are anaerobically degradable under thermophilic (52 °C) and mesophilic (37 °C) conditions [48]. For example, anaerobic incubation of amorphous PLA at 35 °C for 180 days [49] and PLA cup at 58 °C for 56 days [50] resulted in 56% and 40% PLA degradation, respectively. These numbers are calculated based on the amount of methane released from 1 g of PLA during anaerobic digestion compared to that of theoretical yield (467 ml of CH_4/g of PLA) [49]. Finally, biodegradation of PLA in landfills is still not proven [47].

PLA polymer exists in three stereoforms: poly(L-lactide) (L-PLA), poly(D-lactide) (D-PLA), and poly(DL-lactide) (DL-PLA) [51]. The synthesis of pure L-PLA or pure D-PLA facilitates partial stacking of the polymer chains, yielding semicrystalline polymers. However, random polymerization of the two monomers, depending on the amount of D and L monomers, does not facilitate the formation of hydrogen bonds and therefore yields more amorphous polymer and less crystallinity overall [51]. Although pure L-PLA and D-PLA appear to have similar chemical hydrolysis rates, DL-PLA degrades much faster because of more amorphous structure. The blends of the two polymers (stereocomplexed) by contrast appear to facilitate the formation of crystal structures and therefore hydrolyze more slowly [52]. It is thought that increases in polymer crystallinity can inhibit water molecules from accessing the surface of the polymer chain, thereby retarding chemical hydrolysis overall. Similar to chemical hydrolysis, PLA biodegradability is also highly dependent on its level of crystallinity [53–55].

Similar to other aliphatic polyesters, PLA is susceptible to microbial degradation. However, the number of PLA-degrading microorganisms in the environment is limited compared to those involved in the degradation of PHA and PCL. PLA-degrading microorganisms mostly belong to actinomycetes, particularly *Pseudonocardiaceae* family, and are also found in bacteria and fungi. The PLA-degrading bacteria mainly belong to Firmicutes phylum. Fungal degradation of PLA has been reported for *Tritirachium album* (ATCC 22563), which was able to degrade L-PLA in liquid culture after 14 days in the presence of 0.1% (w/v) gelatin [56]. Gelatin, silk fibroin, elastin, a number of peptides, and amino acids are known to induce L-PLA degrading activity in several microorganisms by stimulating the production of PLA-degrading enzymes [57–59]. Most of these inducers are composed of the L-alanine unit, whose stereochemical chiral carbon is similar to that of the L-lactic acid unit of PLA.

Microbial degradation of PLA begins with secretion of extracellular PLA depolymerase by the PLA-degrading microbe, where production of extracellular depolymerase is often stimulated by some inducers, as noted above. The enzyme is

then absorbed on the surface of the polymer through its surface-binding domain and, in the next step, attacks intramolecular ester bonds in PLA. This process results in production of oligomers, dimers, and monomers, where the low molecular weight compounds are assimilated by the microbe and eventually converted into carbon dioxide, water, or methane by intercellular enzymes [60–62].

PLA-degrading enzymes are mainly proteases, lipases (esterases), and cutinases. Protease-type enzymes, such as proteinase K, show preference toward L-PLA, while lipase/cutinase-type enzymes show specificity toward D-PLA [63]. The activity of PLA-degrading enzymes is also affected by local pH, temperature, oxygen or water availability, and polymer crystallinity. For example, enzymatic hydrolysis of PLA amorphous regions is faster than its crystalline regions [63, 64].

Proteases and lipases/cutinases belong to serine protease and α/β hydrolase protein families, respectively. Although these enzymes share same amino acid residues (e.g. serine, histidine, and aspartate) in their catalytic active sites, the positioning of these residues in serine proteases is the mirror image of that in α/β hydrolases [65]. This structural difference may explain why these enzymes show different preferences toward L-PLA and D-PLA substrates [63].

A typical catalytic mechanism of proteinase K against ester bonds in L-PLA generally involves four steps: (i) binding of the enzyme to polymer surface, (ii) nucleophilic attack by the serine residue, (iii) protonation of substrate enzyme–substrate complex, and (iv) hydrolysis of ester bond [63, 66]. Briefly, the enzyme first interacts with L-PLA side chain through its substrate recognition site. Next, in the acylation half of the reaction, the catalytic serine residue attacks the carbonyl of the L-PLA substrate, which is mediated by the catalytic histidine acting as a general base. This reaction yields a tetrahedral intermediate and the protonated catalytic histidine (His-H^+). Here, the oxyanion of tetrahedral intermediate is stabilized by the main chain NHs of the oxyanion hole and His-H^+ is stabilized by the hydrogen bond to the catalytic aspartate. His-H^+ then mediates the collapse of tetrahedral intermediate by donating a proton, yielding the acyl enzyme intermediate and cleaved L-PLA^+. In the deacylation half of the reaction, water attacks the acyl enzyme, assisted by the catalytic histidine, yielding a second tetrahedral intermediate. Finally, the resulting intermediate collapses and forms the second product L-PLA^- with the carboxyl group and regenerates the catalytic serine (Figure 2.3).

Studies exploring the effect of UV irradiation on PLA degradation confirm that polymer chains are cleaved following exposure, although only to a partial extent [68]. Other studies reveal that UV irradiation, followed by exposure to PLA-degrading microorganisms can enhance overall polymer degradation rates to completion. Under environmental conditions, such as in landfill, the polymer is susceptible to chemical hydrolysis, UV exposure, and microbial action and can take a few weeks to a few years to degrade depending on the polymer's crystallinity [68]. Composting of PLA results in a faster degradation: elevated temperatures and humidity facilitate early chemical hydrolysis while enabling the growth of thermophilic organisms effective at degrading PLA [69]. The only environment in which PLA does not readily degrade is in marine waters [70]. Collectively, it appears that isolation and screening of new microbial strains from

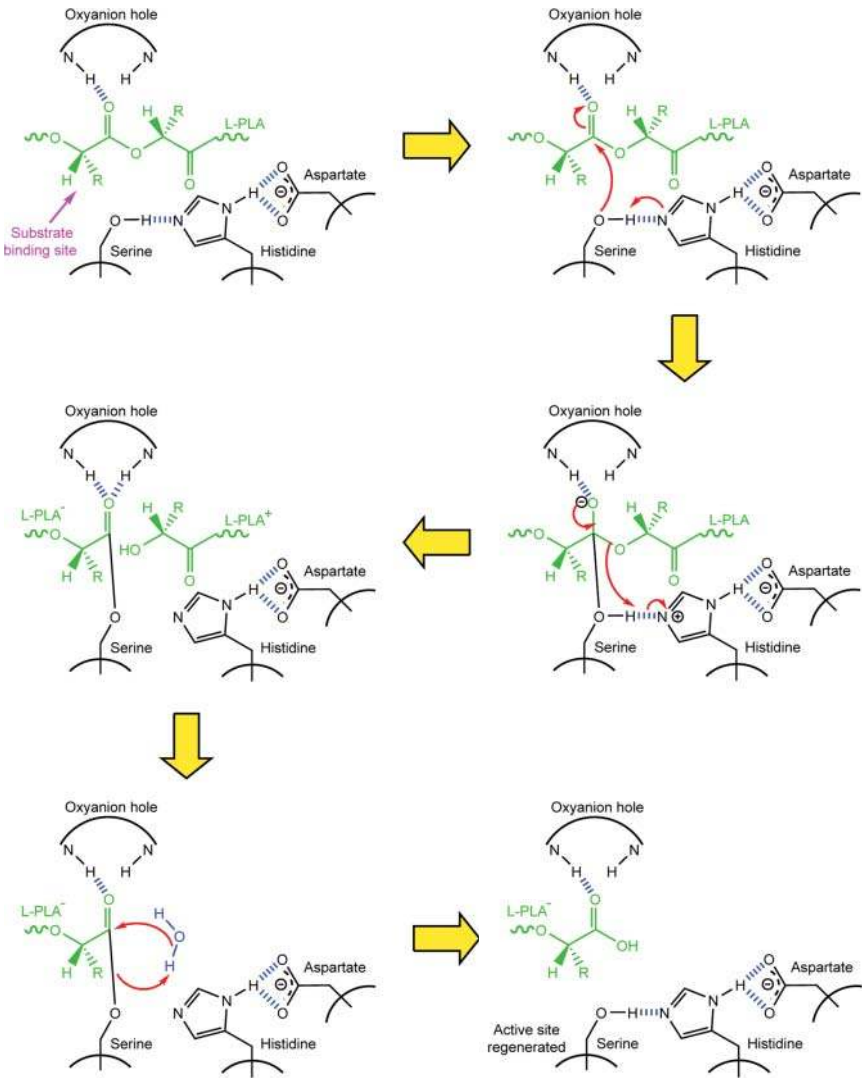

Figure 2.3 Hydrolysis mechanism of protease K against L-PLA. Source: The mechanism is adapted from Ref. [67].

the environment and identification of degrading enzymes are necessary to improve the overall degradation PLA in the industrial level.

2.5.2 Poly(ε-caprolactone)

PCL is polymerized from synthetic monomers but nevertheless degrades readily. Degradation typically occurs via chemical hydrolysis or enzymatic action, both of which target the polymer's ester bonds, and degradation can take a few weeks up to a year in the environment. Relatively less has been studied regarding polymer–enzyme

interactions in PCL degradation. Most enzymes that degrade PCL are lipases, cutinases, and esterases [71–73]. One molecular dynamics study suggests the presence of a typical α/β hydrolase fold and the classic serine, histidine, and aspartate catalytic triad. Similar to enzymatic hydrolysis of PLA (see Figure 2.3), the nucleophilic serine attacks the PCL substrate, followed by a concerted proton transfer and C—O bond formation between the serine and the substrate, which is then attacked by a water molecule and releases the degradation product 6-hydroxycaproic acid.

In the environments such as compost, sea, or pond, PCL can degrade over the course of a few weeks up to a year [74]. A variety of bacteria and fungi have been isolated degrading the polymer [71–73]), and the typical degradation product, 6-hydroxycaproic acid, can easily be absorbed by cells and incorporated into their metabolisms [75]. Degradation is enhanced under compost conditions, where elevated temperatures and humidity facilitate early hydrolysis of the polymer backbone and make it more accessible to biological attack [76].

2.5.3 Polyhydroxyalkanoates

PHAs are a family of aliphatic polyesters derived from hydroxyalkanoic acids. The variety of their mechanical properties comes from differing chain lengths and the isomeric positions of the hydroxyl groups present in the chemical structure. PHAs are thermoplastic and are produced via a single-step fermentation derived from sugars, starches, fatty acids, or other renewable resources [28]. Poly(3-hydroxybutyrate) (PHB), a common PHA variant, is produced by methanotrophs that utilize methane *in vivo* [77]. PHAs, in general, are made via microorganisms that use it as a carbon and energy source [78]. In fact, up to 80% of the microorganisms' dry cell mass can be accounted for by the various PHAs [79].

The biodegradation mechanism begins with hydrolytic cleavages at ester linkages that result in carboxyl and hydroxyl groups leading to smaller fragments [80]. This is a surface area operation, and while the enzymes are released superficially, the bulk properties of the polymer remain unaffected. It has a synergistic effect with physical or chemical pretreatments that bolster the subsequent biotic degradation [81]. However, in marine environments, plastics such as PLA and PHA are often submerged, where typical physical conditions such as UV exposure and heat are limited, which prevent further abiotic degradation. So far, the current biodegradation of a PHA film takes up to 20 days in laboratory compost for full disintegration. As biodegradation occurs, the formation of pores and holes on the polymer surface increases its surface area and promotes overall bulk degradation [82].

The entire process for polyesters can be broken down to three important steps: biofilm formation, hydrolysis, and bioassimilation. Biofilms adhere onto surfaces and release extracellular enzymes that oxidize and fragment the substrate, which can then be used as food. The surface increases in porosity, which allows for further degradation that compromises the overall properties of the polymer [83]. PHAs can be degraded by many bacteria and fungi that are present in most environments such as in compost and ocean surfaces which degrade the polymer both aerobically and anaerobically [84].

PHAs are denser than water and tend to sink in marine environments. In addition, they are more likely to settle in sediment, thus limiting UV exposure and, consequently, photo-oxidation rates [85]. PHAs present in deeper sediment layers are privy to a wider consortium of bacteria but a lower dissolved oxygen content. Those that are closer to the shore experience warmer temperatures and sunlight that can permeate shallow waters with more active bacterial populations. PHA biodegradation is therefore more effective along shallow shorelines than the open ocean [8].

2.5.4 Polyethylene Terephthalate

PET belongs to aliphatic-co-aromatic polyesters, which is formed from terephthalic acid (TPA) and ethylene glycol (EG). PET is a semicrystalline polymer with a glass transition temperature (T_g) around 70–80 °C (assumed lower in water) [86]. In its purest form, PET is an amorphous polymer. However, it develops crystallinity upon addition of modifying additives or heat treatment of the polymer melt [87]. The crystallinity rate varies among PET-based products. For example, PET bottles and textiles have 30–40% crystallinity, while PET used for packaging has 8% crystallinity [88].

Unlike aliphatic polyesters, which can be hydrolyzed at ambient temperatures by various ester bond hydrolases, the presence of repeating aromatic terephthalate units in the PET backbone results in slow hydrolysis of PET. Several other factors are also reported to hamper enzymatic hydrolysis of PET surface and its inner building blocks. For example, high surface hydrophobicity and low surface area can significantly reduce enzyme access to the polymer, resulting in lower degradation overall [89]. In this case, generating hydrophilic carboxylic acid or hydroxy residues on PET surface using PET surface-modifying enzymes and increasing the polymer surface area through mechanical micrionization can greatly improve the subsequent enzymatic hydrolysis [90]. High crystallinity also poses additional inhibition to PET biodegradation, likely due to limited mobility and fluctuation of molecular chains in crystalline regions of PET, which reduces the accessibility of enzymes to the polymer inner blocks. Using high reaction temperatures (e.g. 65–70 °C) can significantly improve hydrolysis of crystalline PET in aqueous solutions. The likely reason is that the polymer has higher chain mobility and flexibility at temperatures near PET's T_g, which consequently exposes crystalline regions of PET to hydrolases. In this regard, thermophilic PET hydrolases with high melting temperatures ($T_{m,e} > 70$ °C) are absolutely required to achieve noticeable PET degradation.

Various aromatic polyesterases from fungal and bacterial species have been reported to degrade PET to different levels (see Figure 2.A.1 and Table 2.A.1 in Appendix 2.A for species and enzymes). Significant PET degradation has been mostly observed with hydrolases, which belong to cutinase family (EC 3.1.1.74). First discovered in phytopathogenic fungi, cutinases are generally known to initiate fungal invasion of plants by degrading the waxy polyester cutin of plant surface cuticle layer [109]. In the case of PET hydrolysis, for example, notable degradation was observed when a commercialized thermophilic cutinase (HiC), purified from the thermophilic fungus *Humicola insolens*, was incubated with amorphous PET (PET-GF) at 70 °C. Several other thermophilic cutinases capable

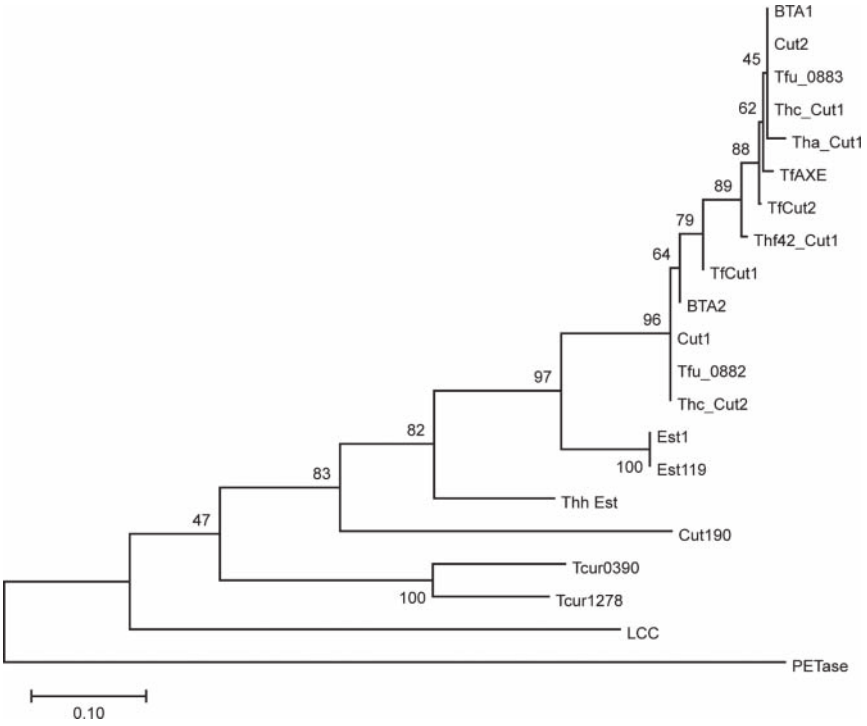

Figure 2.A.1 Phylogenetic tree for amino acid sequences of PET hydrolases reported in Table 2.A.1. The amino acid alignments were performed using MUSCLE (v-3.8.31) [108]. The unrooted tree was generated using the maximum likelihood method. Numbers on the nodes are the bootstrap values calculated from 1000 simulations and represent the reliability of the nodes on the tree.

of PET hydrolysis have also been identified from thermophilic actinomycetes, namely, BTA-1 (TfH) from *Thermobifida fusca* DSM43793 [91] and TfCut2 from *T. fusca* KW3 [110]. In addition, leaf-branch compost cutinase (LCC) identified using metagenomic approaches [102] and a cutinase-type polyesterase (Cut190) from *Saccharomonospora viridis* AHK190 [97] have been shown to degrade PET extensively at high temperatures (e.g. 50–70 °C).

In a recent study, a mesophilic bacterium *Ideonella sakaiensis* was isolated, which can grow on a low-crystallinity (1.9%) PET film [88] and possesses a mesophilic PET hydrolase (lsPETase). In this study, IsPETase could hydrolyze the amorphous PET film after 18 hours of incubation at 30 °C. However, it appeared to hydrolyze only the PET film surface, leaving the polymer inner blocks nearly intact. Additionally, the total PET degradation level induced by IsPETase was significantly lower than those of cutinases (1% at 30 °C with IsPETase versus 40% at 70 °C with LCC). IsPETase's PET hydrolysis activity also dropped dramatically over a relatively long time, and the enzyme lost almost its entire hydrolytic activity after 24 hours at 37 °C. These behaviors are likely attributed to IsPETase's low thermostability ($T_{m,e} \sim 40$ °C) even though its crystal structure is highly similar to those of actinomycetes thermophilic

Table 2.A.1 PET hydrolase homologs.

Name	NCBI accession number	Source organism	References
BTA-1 (TfH)	AJ810119.1	*Thermobifida fusca* DSM43793	[91]
BTA-2	AJ810119.1	*Thermobifida fusca* DSM43793	[92]
Tfu_0882	AAZ54920.1	*Thermobifida fusca* YX	[93]
Tfu_0883	AAZ54921.1	*Thermobifida fusca* WSH03-11	[94]
TfCut1	CBY05529.1	*Thermobifida fusca* KW3	[95]
TfCut2	CBY05530.1	*Thermobifida fusca* KW3	[95]
Est1	BAI99230.2	*Thermobifida alba*	[96]
Est119	BAK48590.1	*Thermobifida alba* AHK119	[97, 98]
Thc_Cut1	ADV92526.1	*Thermobifida cellulosilytica* DSM44535	[95]
Thc_Cut2	ADV92527.1	*Thermobifida cellulosilytica* DSM44535	[95]
Thf42_Cut1	ADV92528.1	*Thermobifida fusca* DSM44342	[95]
Tha_Cut1	ADV92525.1	*Thermobifida alba* DSM43185	[99]
Thh_Est	AFA45122.1	*Thermobifida halotolerans* DSM44931	[100]
TfAXE	ADM47605.1	*Thermobifida fusca* NTU22	[101]
LC-cutinase	AEV21261.1	Metagenome from leaf-branch compost	[102]
Cut1	JN129499.1	*Thermobifida fusca* NRRL B-8184	[103]
Cut2	JN129500.1	*Thermobifida fusca* NRRL B-8184	[103]
Tcur1278	ACY96861.1	*Thermomonospora curvata* DSM43183	[104]
Tcur0390	ACY95991.1	*Thermomonospora curvata* DSM43183	[105]
Cut190	AB728484	*Saccharomonospora viridis* AHK190	[106]
PETase	GAP38373.1	*Ideonella Sakaiensis* 201-F6	[88]
HiC	4OYY_A	*Humicola insolens*	[107]

cutinases, such as TfH and LCC. In addition to IsPETase, there have been reports of several other bacterial PET-degrading enzymes, such as esterases from *Bacillus subtilis* [111] and *Clostridium botulinum* [112] and a cutinase from *Pseudomonas putida* [113]. Again, these enzymes are only capable of hydrolyzing the PET surface.

Taken these observations together, the PET-degrading enzymes can be classified into two groups of PET hydrolases and PET surface-modifying enzymes [4]. PET hydrolases must be able to hydrolyze the main body of PET, preferably at temperatures above the polymer T_g (60–70 °C in aqueous solution), resulting in PET surface erosion visible in SEM and releasing millimolar level (>10% of initial PET) of degradation products. As of now, only five cutinases – TfH from *T. fusca* DSM43793 [91], a variant of TfCut2 from *T. fusca* KW3 [114], a variant of Cut190 from *S. viridis* AHK190 [106], LCC from uncultured bacteria [115], and HiC from the fungus *Humicola insolens* [107] – meet these criteria and are qualified as PET hydrolases [4]. On the other hand, PET surface-modifying enzymes are expected to

hydrolyze only the cyclic trimer of mono-(2-hydoxyethyl)terephthalic acid (MHET) present inside and outside of PET, releasing micromolar level (<1–2% of initial PET) of degradation products. IsPETase and most mesophilic bacterial polyesterases belong to this group.

Although hydrolytic activity against PET varies immensely among PET-degrading enzymes, they share highly similar crystal structures. In particular, they all belong to α/β hydrolase superfamily with nine β strands surrounded by seven to eight α helices depending on the enzyme [116]. Fungal cutinases such as HiC generally possess smaller structures compared to those from actinomycetes and bacteria (Figure 2.4a) [113, 117]. Notably, three amino acid residues constituting the catalytic site are almost identical among all PET-degrading cutinases (Figure 2.4b), implying that a similar mechanism is likely employed by these enzymes to cleave the PET scissile ester bonds (Figure 2.4c). It is noteworthy that the mechanism for enzymatic cleavage of the ester bonds is highly similar among different polyesters including PLA, PHA, and PET (see Figure 2.3). In the case of PET, however, the presence of aromatic group seems to slow this process, causing PET hydrolysis more challenging compared to aliphatic polyesters (see Appendix 2.A).

The activities and thermal stabilities of the cutinases were shown to increase in the presence of Mg^{2+} and Ca^{2+}. Molecular dynamics simulations indicate that binding of Ca^{2+} to the hydrolase decreases fluctuation in its structure, leading to higher thermal stability [118, 119]. For example, the $T_{m,e}$ values of *T. fusca* cutinases were increased by 10–14 °C in the presence of 10 mM Ca^{2+} or Mg^{2+}, which consequently led to significant degradation of PET-GF (supplied by Goodfellow Cambridge, Ltd) at 65 °C [98]. Similar to catalytic sites, the Ca^{2+} binding sites on PET hydrolases are also highly conserved [4], highlighting their important role in improving thermal stability and PET hydrolysis activity of the enzymes. Interestingly, introduction of new disulfide bonds via cysteine residues at the Ca^{2+} binding sites of TfCut2, Cut190, and LCC, for example, resulted in higher $T_{m,e}$ values and improved durability of the enzymes at high reaction temperatures [106, 120]. This method indeed would eliminate high costs associated with purification of Ca^{2+} salts following large-scale PET hydrolysis reaction [120] and would enable direct use of microbes for the treatment of PET waste where high salt concentrations pose toxicity to cells.

Finally, unlike enzymes such as cellulase, PET hydrolases lack a specific substrate-binding site. Docking calculations supported by mutagenesis analyses suggest that the PET surface initially binds a long, shallow hydrophobic cleft on the surface of the PET hydrolases. These analyses further demonstrate that the binding cleft should be relatively wide to accommodate bulky aromatic residues of PET molecular chains [121]. Remarkably, all the four main PET hydrolases have wider binding cleft compared to those of PET surface-modifying enzymes, namely, IsPETase [4].

Although studies of PET biodegradation have mainly focused on the characterization of efficient PET hydrolases, not much is known about assimilation of PET by microorganisms. As noted earlier, one group recently isolated *I. sakaiensis* bacterium, which can degrade the amorphous PET film and metabolize the degradation products near ambient temperature [88]. This discovery, since then, has encouraged

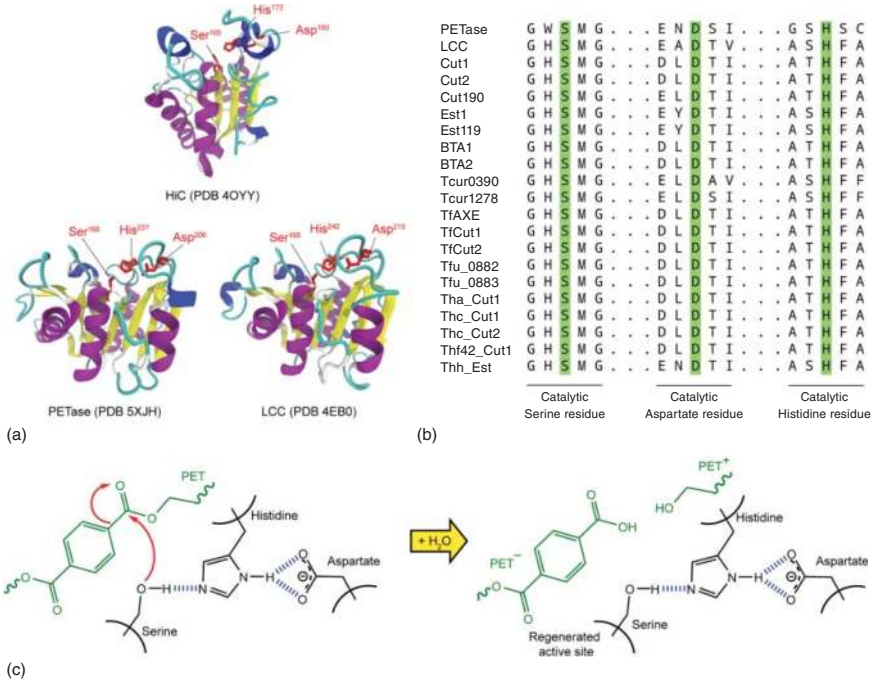

Figure 2.4 **PET hydrolases**. (a) Crystal structures of PET hydrolases; HiC: PDB 4OYY [114], PETase: PDB 5XJH [117], and LCC: PDB 4EBO (to be published). (b) Conservation of catalytic triad residues on different PET hydrolases. (c) Overall mechanism of PET hydrolysis.

the scientific community to attempt to isolate new microbes from nature or engineer mutants, which can efficiently degrade and metabolize PET waste.

To illustrate how PET chains can be metabolized by bacteria, we briefly describe the assimilation pathway of PET film products in *I. sakaiensis*. Using transcriptomic analysis of the cells cultured on maltose, sodium terephthalate BHET, or PET film, Yoshida et al. found that genes encoding PETase, a MHET hydrolase (later termed lsMHETase), and a cluster of genes involved in the TPA catabolism pathway were significantly upregulated in the presence of BHET or PET film [88]. Additionally, they suggested that PETase expression was mainly induced by the PET film itself and/or some degradation products other than TPA, EG, MHET, and BHET. Using bioinformatics analysis, they next predicted that PETase is a secretory protein, while MHETase is a lipoprotein localized in the outer membrane. MHETase was also found to hydrolyze MHET to TPA and EG, showing no hydrolytic activity against PET itself.

Based on these results, the following pathway was proposed for the metabolism of the PET film by *I. sakaiensis*. Briefly, when cells are cultured on amorphous PET film, they secrete out PETase to the medium. PETase then hydrolyzes PET chains on the film surface, producing MHET and minor amount of TPA. MHETase then hydrolyzes MHET to TPA and EG at the outer membrane, where the resultant TPA is transported into cytosol through the TPA transporter (TPATP) and is converted to pyroglutamic acid (PCA) through terephthalic acid 1,2 dioxygenase (TPADO) and 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate dehydrogenase (DCDDH), respectively. Finally, the ring opening reaction of the resultant PCA is catalyzed by PCA 3,4-dioxygenase (Pca34) (Figure 2.5). Although the authors were unable to find

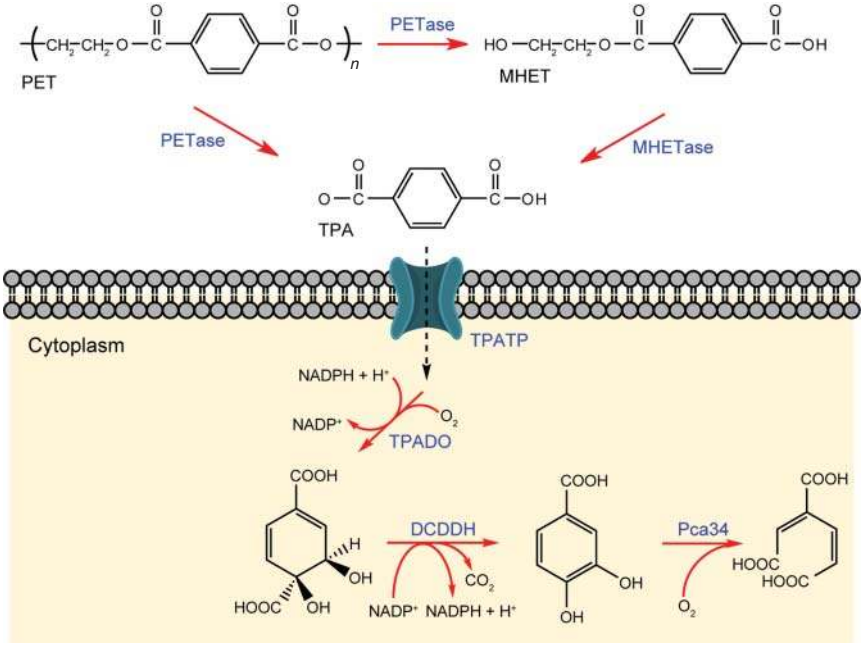

Figure 2.5 PET metabolism pathway by *I. sakaiensis*. Source: Adapted from Ref. [83].

the complete set of genes involved in PET metabolism using fully sequence genomes at the time, they found that of 92 microorganisms possess MHETase homologs and 33 had homologs for both TPA and PCA dioxygenases. These observations suggest that many microorganisms are capable of metabolizing MHET and its analogs.

Efficient degradation of PET-based plastics, particularly high-crystallinity PET film and bottles, requires high reaction temperatures, ideally near PET glass temperature (e.g. 65–75 °C). Unfortunately, the thermal stability of the PET-degrading enzymes is generally low and is known as a major limiting factor in PET degradation. The most obvious example is mesophilic IsPETase with $T_{m,e}$ around 46–49 °C, which is reported to lose its hydrolytic activity against amorphous PET after 24 hours at 37 °C, likely because the enzyme denatures over time. Even thermophilic cutinases with $T_{m,e}$ ranging from 56 to 85 °C show reduced hydrolytic activity against PET over time, resulting in partial depolymerization [120]. Over the past few years, researchers have employed rational and computational based enzyme engineering techniques to create variants of hydrolases with improved thermal stability and enhanced PET hydrolysis activity [98, 120]. For example, using a computational-based protein engineering approach, Cui et al. were able to create an IsPETase variant, which exhibited an increased $T_{m,e}$ by 31 °C and an improved degradation performance by more than 100-fold against semicrystalline (23%) PET films at mild temperatures [122].

Moreover, using enzyme engineering based on *in silico* docking calculations, Tournier et al. were able to generate a LCC variant F243I/D238C/S283C/Y127G (ICCG) with an improved $T_{m,e}$ of +9.3 °C and a hydrolytic activity comparable to that of wild type [120]. Remarkably, when tested against pretreated (extruded and micronized) post-consumer colored flake PET waste (PcW-PEt) with 14.6% crystallinity level in optimal bioreactor conditions, ICCG variant achieved 90% depolymerization conversion in only 10.5 hour with a mean productivity of 16.7 g_{TA}/l/h. Later molecular calculations suggested that the significant improvement in the hydrolytic activity of ICCG variant was likely due to catalytically favorable binding of the mutant enzyme to the substrate and improved enzyme–substrate affinity compared with the parental LCC. It is important to note that the ICCG variant currently outperforms all PET hydrolases reported so far, and its productivity rate is considerably higher than that of starch degradation (4 g/l/h), which is a common biodegradable polymer [120, 123].

Finally, using optimized ICCG variant in a 150-l-pilot scale, the same authors were able to produce terephthalate acid monomers at 99.8% purity, which was subsequently reused to synthesize virgin PET. The newly made PET was ultimately blown into bottles where their physical and mechanical properties were comparable to those synthesized using petrochemical TPA. Overall, this work illustrates how computer-aided enzyme engineering can enable efficient conversion of post-consumer PET plastics into their building monomers and back to commercial plastics, paving the way toward circular economy in the plastic industry. It is noteworthy that these results are relatively comparable to those obtained from chemical hydrolysis. In general, chemical hydrolysis of PET can be achieved by acidic, neutral, and alkaline hydrolysis [124]. Acidic hydrolysis typically produces

high yields of TPA monomer, but high amounts of acid would decrease the purity of EG monomer, increase cost of the process, and raise environmental concerns [125]. On the other hand, neutral hydrolysis is eco-friendly, but all mechanical impurities present in the polymer remain in the TPA, resulting in lower TPA purity, overall [126]. Alkaline hydrolysis appears to be a better alternative. One study showed that the two-step aqueous alkaline hydrolysis of PET resulted in the highest yield (~95%) at 80 °C with the initial PET particle size lower than 500 μm in a solution containing 60 : 40 vol% EtOH:H_2O and 5 wt% NaOH in 20 minutes [127]. Moreover, among the real post-consumer PET waste samples including multilayer and monolayer PET trays and films and bottles tested in the same study, the highest PET conversion (~90%) was obtained with monolayer PET films at the smallest particle size (<500 μm). The conversion rate was diminished for monolayer PET trays, bottles, and multilayer PET samples. Especially with PET bottles and multilayer trays, the degradation yield did not exceed 70% [127].

2.6 Biodegradation of Hydrocarbons

2.6.1 Polyethylene

PE is the number one produced plastic, in volume, in the world. It comes in a variety of grades, for example, LLDPE, LDPE, and HDPE (high-density polyethylene). Ultrahigh molecular weight polyethylene (UHMWPE) is another PE variant with a high degree of polymerization (36000+) [128]. Each grade has its own unique applications, mainly because of the type and degrees of branching and degree of polymerization. Higher branching with longer side chains leads to less molecular packing because of higher steric hindrance within the polymer chains [129]. Similar to having high molecular weight, high branching also impairs biodegradation.

A number of studies have reported on biodegradation of PE grades, where oxidases, peroxidases, monooxygenases, etc., produced by aerobic microflora could cleave polymer chains to lower weight fragments [130, 131]. These resulting fragments were also shown to be taken up by bacteria and yeasts and converted into energy through β-oxidation pathways [130, 132]. For example, *Arthrobacter parraffineus* was shown to degrade LDPE after three years via β-oxidation [133]. Despite this, biodegradation can still take years because bioassimilation often being the rate-limiting step.

Biodegradation of PE can be enhanced through various pretreatment strategies such as thermal and UV exposure. UV oxidation produces free radicals from (C–H) and (C–C) homolytic bond cleavages. These radicals in the presence of oxygen form peroxy radicals, which abstract a hydrogen from the polymer backbone leaving behind another radical in its wake [134]. This process is continuous and leads to many hydrophilic functional groups, including ketones, carboxylic acids, esters, and alcohols, which are favored by many hydrolases. Indeed, an increase of UV exposure correlates with an increase of PE biodegradation [128]. For example, UV exposure via lamps led to a 25% increase in the biodegradation of LDPE. Thermal

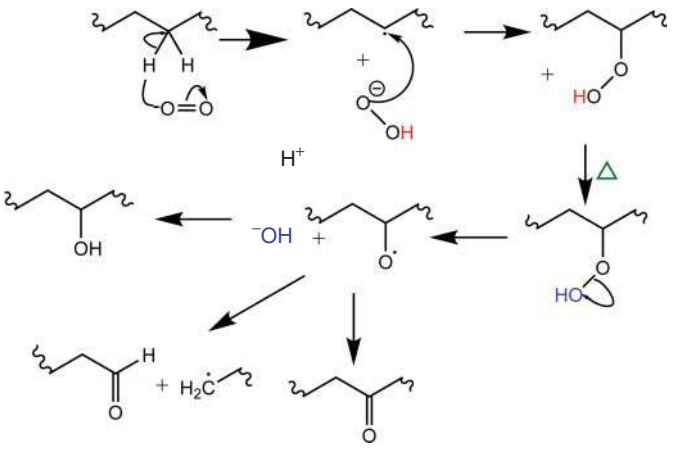

Figure 2.6 Thermal oxidation of PE. Source: The mechanism is adapted from Refs. [85, 135].

oxidation also works similarly (Figure 2.6). The following oxidation products are then primed for further bioassimilation. Research behind LDPE shows a 7% higher weight loss for thermally pretreated (80 °C for 10 days) polymers as compared to control via aerobic microbes (e.g. *Bacillus* sp.) [6].

Along with thermal and UV exposure, oxidative chemicals and surfactants may be used to pretreat recalcitrant polymers. Oxidative chemicals include nitric or sulfuric acid, which promote hydrophilicity in the polymer. Surfactants reduce the surface tension of a substrate. Some of these surfactants have very low critical micelle concentrations (CMCs) and therefore have a higher ability to solubilize and biodegrade the hydrocarbon polymer. Surfactants can be utilized at higher concentrations than their CMCs to get even more solubilizations, which may lead to higher levels of degradation on the PE surface. Other surfactants such as sodium dodecyl sulfate (SDS) oxidizes PE and sodium dodecyl benzene sulfonate (SDBS) hydrolyzes it [132]. Additionally, biosurfactants produced by *Lysinibacillus fusiformis*, for example, have the ability to solubilize the hydrocarbons because they are amphiphilic. In essence, these biosurfactants ensure smoother and easier microbial adhesion. Although it should be noted that the surfactants themselves must be biodegradable, otherwise, they would defeat the purpose [6, 132].

Additives can be used to increase the oxidation rate. Normal PE is recalcitrant. However, PE fitted with oxidation catalysts (i.e. iron complexes) can undergo abiotic transformation and subsequently support microbial growth [136]. Oxo-degradable additives may help pretreatment oxidization (e.g. photo oxidation) via UV radiation [137]. The additive rapidly reduces the molecular weight of the polymer, making it more susceptible to the second step of the process – the consumption of oligomers and monomers by microbes [137].

Although the pretreatment strategies discussed above were shown to promote biodegradation of the PE variants, microbial hydrolysis of UHMWPE is still quite challenging. In theory, UHMWPE should still be susceptible to biodegradation

only if the molecular chains can be fragmented enough for bacterial or fungal consumption. However, because these chains are extremely long (high DP) and the scission mechanism operates on a stochastic basis, it will take far longer for any pretreatment strategy to oxidize and cleave the polymer into an appropriate size for bioassimilation. In addition, the high crystallinity of this PE variant prevents it from extracellular enzyme activity as these enzymes typically target the amorphous regions. Coupled with its chemical inertness, it is highly unlikely that UHMWPE can sustain biodegradation. Because UHMWPE is used in such highly specialized applications, it is not part of the overarching plastic pollution problem.

2.6.2 Polypropylene

PP is a vinyl polymer with methyl groups on alternating carbons. Polymerization yields three variants based on the orientation of the methyl branch in the polymer chain. These isomers can either be isotactic (same orientation), syndiotactic (alternating pendent groups), or atactic (random orientation of pendent groups) [138]. These methyl branches add a layer of steric hindrance which further disallows biodegradation. Typically, PP waste exposed to natural conditions degrade over very long time scales [139].

Pretreatments are also required for PP degradation because of its high molecular weight, chemical inertness, and lack of hydrophilicity on its chains, PP must be subjected through similar pretreatment strategies to be oxidized and fragmented. In the PP chain, there are three positional carbons (e.g. primary, secondary, and tertiary), where the radical can form in the presence of oxygen to promote oxidation [131]. This oxidation introduces ester, carboxyl, and carbonyl functional groups [139]. These functional groups promote further microbial attachment to polymer surface in the form of biofilm. For example, thermal pretreatment promotes formation of radicals that propagate to form hydrophilic groups, resulting in chain scission [131]. This fragmentation allows for greater weight loss through microbial bioassimilation. One study showed that the thermally pretreated PP (80 °C for 10 days in hot air oven) exhibited 7.1% weight loss in six months through aerobic microbes, while a similar untreated sample showed only a dismal 0.42% degradation by soil consortia [6]. When comparing LDPE and PP with the same pretreatment conditions (i.e. 100 KGy of gamma radiation), PP had a 1.2% mass loss after fungal inoculation, whereas LDPE had no mass loss, whatsoever [140].

Irradiation is a promising pretreatment. UV exposure of PP works similar to thermal exposure as in the case for PE. For example, UV-treated PP is more biodegradable than LDPE [133]. Other techniques such as gamma irradiation can induce photo-oxidation, which is a form of pretreatment before the microorganisms begin their role by embrittling the polymer and introducing polar groups to promote hydrophilicity. However, dosages are important as lower doses of gamma rays can sterilize plastics, while higher doses serve to allow more microbial adhesion onto the plastic films [140].

Bacteria are generally known to be less efficient in degrading PP. This is likely because the continuously repeating methylene units in PP increase hydrophobicity

on the polymer's surface, which hampers biofilm formation by bacteria [131]. Despite this, a number of bacterial species such as *Pseudomonas azotoformans* and *B. subtilis* were shown to produce biofilms on pretreated PP films and enhance the polymer degradation, which further bolsters the idea behind synergistic effects between pretreatment and microbial degradation. In addition, *Bacillus flexus* was reported to produce the highest CFU/ml (colony forming units), regardless of any pretreatments in a 12 month period in minimal media, thereby indicating that is growth was directly attributable to PP biodegradation [141]. In addition to degrading pretreated PP, it also oxidized untreated PP, which shows the microorganism's promise as a candidate for further exploration on practical large-scale approaches.

On the other hand, fungi are promising biodegradation candidates because of their surplus of degrading enzymes in addition to their ability to survive in harsher ecosystems under low nutrient and moisture conditions. They also release plastic-degrading enzymes such as laccase, which is a phenol oxidase and part of a family of multi-copper enzymes [142]. For example, *Lasiodiplodia theobromae* and *Psychotria flavida* could degrade the PP film that was irradiated with different doses of gamma radiation. Moreover, fungi can adhere onto the surface of PP. *P. flavida* and *Humboldtia brunonis* are two known endophytic fungi that grow very well on plastic surfaces [140]. Endophytic fungi can extend their hyphae into holes and crevices.

2.6.3 Polystyrene

Polystyrene is a widely used polymer because of its low cost in materials and processing, its variety of forms tailored for versatile applications. In addition to its stability, moisture, and thermal resistance, it is also very hard to biodegrade primarily because of its aromaticity. Efforts to engage the polymer in this process have yielded extremely long time scales – a single PS sheet buried in soil lasted up to 32 years with no sign of significant decay. The bulky nature of the polymer does not allow for flexible polymer chains that can engage with extracellular enzymes released by bacteria in the biofilm. In fact, microorganisms have a difficult time attaching to the polymer surface because of the hydrophobicity of PS. Any enzyme release acts only superficially as the enzymes themselves are too large to penetrate the polymer network. However, it has been recently shown that microbes can catabolize styrene without further breakdown. It is well understood that the oxidation of styrene is performed through a series of steps with styrene monooxygenase and styrene oxide isomerase, to name a few. The vinyl group in styrene is first converted to an oxide, which is then oxidized further into an aldehyde. After more oxidation, the resulting carboxylate functional group then bonds with the phenylacetyl coenzyme A ligase and is then brought into the Krebs cycle (Figure 2.7) [143].

PS must also be oxidized to improve its hydrophilicity. Similar to the pretreatment strategies of other two polyolefins, PS oxidation is accomplished after thermal or UV exposure. The addition of oxidation catalysts such as metal ions such as cobalt, nickel, and manganese can improve the oxidation rates as a pretreatment step that ultimately improves the hydrophilicity of the polymer surface [143]. Chemical catalysis is the most common method for abiotic degradation yielding 70–90%

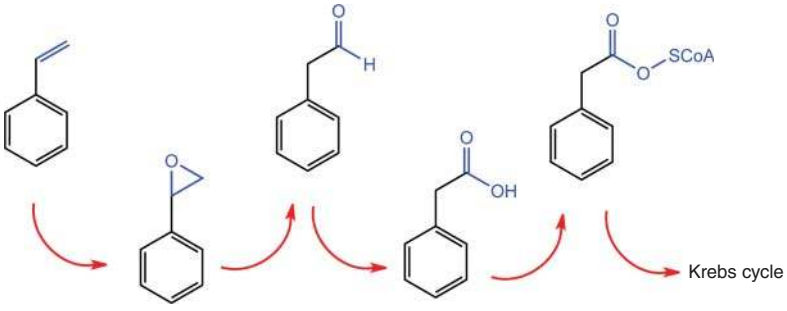

Figure 2.7 The metabolism pathway of styrene. Source: The mechanism is adapted from Ref. [136].

conversion, although liquefying PS at 240 °C does degrade the polymer into its monomers and, often, oligomers [144].

Combining pretreatment with bioassimilation is the best strategy**.** As with most non-biodegradable polymers, a synergistic effect must be achieved between the breakdown step and the following microbial assimilation of polymer fragments. In fact, this was exploited by Savoldelli et al. when implementing a two-step strategy involving liquefying and, consequently, degrading PS into its constituents and cooled down to allow for microbial inoculation that would further break the monomer down. *P. putida* and *Salmonella* have been effective in this process especially because of the increased surface area of the well-mixed liquid system. The temperature used in the first step was 240 °C, which is just around the melting point of PS but not quite high enough for the thermal degradation of PS. If it were, then the biodegradation step would be redundant and unnecessary all the while being more energy intensive. This method showed 100% degradation in a matter of days [144].

Typically, most evaluation of plastic degradation occurs on the lab scale. Regardless, there are some records of the extent of littering begotten by the population [145]. This may benefit abiotic processes as plastics are more exposed to oxygen and UV radiation, both of which enhance oxidation. However, if not under controlled laboratory conditions, the harmful chemical hazards emanating from the degradation process may harm the environment [85]. Plastics are also found in marine environments and are typically hindered in oxidation. Those exposed to seawater faced lower degradation rates than those of land [146]. The complete degradation may also be affected because of the varying conditions of the range of marine environments, which can lead to decades of persistence in deep water regions [147].

2.7 Biodegradation of Halogenated Polymers

Two of the most common halogenated polymers are polyvinyl chloride (PVC) and polytetrafluoroethylene (PTFE). PVC is a vinyl polymer with chlorine groups attached to every other carbon. PTFE, often known by its trade name Teflon, is also a vinyl polymer but is composed entirely of a (C–C) chain and attached fluorine atoms. Both polymers are extremely difficult to degrade enzymatically.

2.7.1 Polyvinyl Chloride

PVC can harbor bacterial and fungal surface colonization, which typically require a few weeks to become established [148–150] and can persist after the addition of germicides [151]. These observations may motivate the future studies of PVC disposal. A number of studies described weight loss of plasticized PVC films because of the action of microorganisms. However, it is not clear if this weight loss is attributed to PVC or the plasticizers [152, 153]. For example, in one study, researchers exposed plasticized polyvinyl chloride (pPVC) to the atmosphere for two years, where various bacteria and fungi were discovered colonizing its surface. Three fungi, namely, *Aureobasidium pullulans*, *Rhodotorula aurantiaca*, and *Kluyveromyces* spp., could grow independently on pieces of pPVC containing plasticizers dioctyl phthalate and dioctyl adipate (DOA). They showed that these fungi can degrade DOA and survive on it as its sole carbon source. However, no bacterial colonization was observed [149]. Although it is unclear if microorganisms are capable of degrading PVC, there are certainly microorganisms that consume its monomer vinyl chloride (VC). For example, aerobic cultures of bacteria isolated from contaminated groundwater and soil have been observed to dechlorinate VC to ethylene or assimilating it directly into their biomass [154, 155], while another bacterium, *Dehalococcoides ethenogenes*, can use VC as a terminal electron acceptor instead of oxygen in anaerobic cultures [156]. Thus far, there are no published studies exposing these bacterial species to the polymerized form of the substrate, but their current abilities may be reassuring for the future of PVC biodegradation.

2.7.2 Polytetrafluoroethylene

PTFE facilitates little to no biofilm formation and is frequently used to protect surfaces from microbial colonization, such as on stainless steel used in food processing [157] or biomedical implants [158]. This resistance to microbial surface colonization directly contrasts with PVC, possibly because of PTFE's hydrophobicity and slippery surface properties. One study targeted modified PTFE as a scaffold for bacterial immobilization. In that study, by creating a thin PTFE film via sputtered deposition onto polyethylene, researchers induced the formation of chemical functional groups in the PTFE surface such as hydroxyl, carboxyl, or amine groups, which facilitated bacterial biofilm formation [159]. Among identified bacteria forming biofilm on the surface of PTFE, none of them could degrade the material.

2.8 Biodegradation of Polyethers

2.8.1 Polyethylene Glycol

Polyethylene glycol (PEG) perhaps is the most commonly known polyether. PEG is susceptible to oxidation via free radicals and its degradation is hindered through antioxidant additions. Activation energy for hydrogen abstraction is reduced and formation and propagation of radicals are induced. The resulting radicals then form carboxylates with reactive oxygen species such as hydrogen peroxide or hydroxyl

groups. Heating PEG at 80 °C in aerobic environments leads to similar random chain scission events and the lowering of the molecular weight distribution, the melting point, and the heat of fusion. In fact, biodegradation is stifled when antioxidants have been incorporated into the polymer [160]. In addition, PEG fragments more in excess air because of higher degrees of oxidation from radical additions with peroxides. This fragmentation results in formic esters from terminal cleavage.

Fragmentation can also occur through enzymatic action. This process begins with enzymes oxidizing the terminal alcohols from PEG fragments via PEG alcohol dehydrogenase [161]. The resulting aldehyde is further oxidized to a carboxylate by PEG aldehyde dehydrogenase [162]. Alternatively, these enzymes act to oxidize and cleave the polymer into smaller fragments in the same manner as the aforementioned abiotic degradation. Certain strains can grow on PEG depending on the degree of polymerization, mostly in the form of activated sludges. Some of the most prominent genera of PEG-degrading bacteria are *Pseudomonas*, *Sphingomonas*, and *Gluconobacter*, and some methanogens, from which several enzymes capable of degrading the PEG chains have been purified [161]. For example, a PEG dehydrogenase was found in PEG-utilizing sphingomonads, which show high growth on PEG 6000 and 20,000. Moreover, PEG-degrading microbes can metabolize PEG fragments in periplasmic space or in the cytoplasm, which indicates transport of these oligomers past cell walls. Despite this, PEG metabolites have no close relatives in nature [163].

2.8.2 Polyurethane

With the advent of polyurethane (PU) synthesis beginning in the 1950s, its versatile uses in the furniture and automotive industries have prompted a manufacturing boon. Thus, various strategies have been implemented to mitigate the burgeoning problem of PU waste accumulation. On the industrial scale, up to 30% of PU foams is ground and incorporated into new foams [164]. Consequently, the microionization of these foams allows for better chemical hydrolysis similar to PET. However, it cannot treat waste generated from the resulting PU disposal [164]. Although this class of polymers are defined by the urethane bond, it only accounts for a small proportion of the bonds in the polymer to, sometimes, none at all [165]. These bonds act as linkages connecting polyisocyanates and polyols whose ratio of "hard" and "soft" segments can be modulated in order to generate myriad characteristics such as elasticity and tensile strength [166]. In fact, PU can be classified as either polyester or polyether depending on the polyol used. Polyethers are more represented industrially and are typically used for thermal insulation [164]. Hard segments lend PU its insolubility in water or commercial solvents and thermal resistance. Therefore, common pretreatment strategies cannot be employed to the same extent. A common polyol, or "soft" segment (as it is often referred), is poly(propylene glycol) and typically undergoes increased chain extension through the use of additives [167]. It itself is biodegradable by myriad microorganisms and subject to the same hindrances such as lack of nutrient availability [168]. In fact, biodegradation may actually endanger the environment through the release of toxic chemicals depending on the additives present in the polymer [164].

Initial studies conducted in the late 1960s demonstrated great resistance to abiotic and biotic degradation conditions [164]. However, sunlight may promote photolysis [167]. Further research has focused on accentuating abiotic conditions that would stimulate microbial growth *in natura* [164]. Although polyester-based PUs have been shown to degrade in aerobic conditions [167], polyether-based PU has shown no discernable breakdown in either aerobic or anaerobic conditions [164]. Similarly, in laboratory conditions, polyester-based PU has shown remarkable degradation ability from a variety of organisms such as *Delftia acidovorans*, which can degrade 2–3 g of PU every four to six days [164, 169]. Alternatively, many microbes struggle to grow on polyether-based PU with only *Dietzia maris* and *Exophiala jeanselmei* degrading the polymer to any meaningful extent. In fact, less than a third of PU foam was degraded during a month of incubation [170]. Although there is a mismatch in biodegradation results between polyester and polyether-based PU, the biochemical pathways to degradation are suspected to be similar despite the inherent recalcitrance of the ether bond [164].

Enzymes comprise the first wave of attack in biodegradation but are hard to narrow down [168, 171]. The membrane-bound polyurethanase adheres to the PU surface and hydrolyzes the urethane linkage, thereby releasing the polymer constituents [172]. The resulting monomers are still attached to the membrane-bound enzymes, which allow for polyurethanases released to grind the PU surface, which augments the contact surface, thereby improving microbial adhesion [171]. PU's increasing waste accumulation is promulgated by its inherent difficulties in biodegradation. This can be mitigated somewhat by further exploration into degrading microorganisms especially for polyether-based PU.

2.9 Application of Biodegradation

We earlier discussed how enzymatic degradation can be exploited and used along mechanical pretreatments to enable near-full depolymerization and repolymerization of PET waste within industrial setting. This approach can also be employed for other hydrolysable polymers such as PLA once more efficient enzymes are characterized through engineering or novel discoveries in nature. However, it is important to note that more efficient technologies should also be established for recovery and purification of monomers from the rest of the components in the bioreactor.

Biodegradation can also be used for energy recovery. Coupled with microfiltration, wastewater streams from many industries can be treated to separate value-rich products such as microbial lipids that can be used for energy consumption [173]. Hydrogen and methane can be produced via anaerobic digestion from organic materials. One- and two-stage systems both yielded around 14.20 kJ/kg of organic material [174]. Aerobic bioreactors also produce biogas at 85% and often out-compete its counterpart, which has a 10% less methane yield at the same residence time. In fact, the combination of the two could result in a more potent energy recovery system reaching up to 140 MJ/m^3 of wastewater [175]. Although both treatment types are found concurrently, their mechanism differs. Namely, aerobic treatments proceed through organic waste oxidation, whereas anaerobic

treatments occur through hydrolysis, fermentation, and methanogenesis [176]. Methanogenesis can also occur through biodegradation in oil fields because of the preponderance of anaerobes in low-nutrient environments. Anaerobic cultures degraded *n*-alkanes completely at 30 °C in 208 days. On the other hand, aerobic microcosms could fully degrade shorter chain *n*-alkanes (up to C33) in a tenth of the time [177].

In the case of upcycling, studies have shown that various forms of PHA have been found in municipal waste sludge. In fact, the synthesis of PHAs was observed in the context of wastewater treatment [178]. Although fermented food waste was used to yield an extrapolated 63.5 tons of PHA per day, the economic cost would be too high to implement this strategy [179]. The argument can be made that PHA, whose monomers are natural metabolites of bacteria present in various consortia, can be used to promote true upcycling processes. This is only possible because of the inherent biodegradability of PHAs. As other polymers, such as PE and PP, accumulate in waste streams, difficulties arise in verifying whether these fragments are bioassimilated via PHA production. Despite the many efforts of filtering out plastic contaminants, it still persists in the environment [180].

2.10 Current Challenges and Future Prospects for Biodegradation of Plastics Wastes

Additional polymers with continuous C–C polymer chain, namely, PE, PP, and PS, are highly recalcitrant to enzymatic degradation. Their surface hydrophobicity hinders the growth of biofilms that is needed for biodegradation. Hydrolysis requires oxidation of the chain, for example, by air or alternative oxidant, eventually assisted by UV pretreatment or oxidation catalysts/enzymes. Although a number of enzymes have been identified to partly contribute to hydrolysis of these polymers, complete mechanisms are still unknown.

On the other hand, condensation polymers including natural polymers (e.g. cellulose and starch) and polyesters are susceptible to enzymatic degradation. Among polyesters, however, PET is a challenging target for enzymatic attack. Despite the numerous studies of PET biodegradation, most accomplishments have been limited to degradation of amorphous or low-crystalline (<15%) PET products and yields for hydrolysis of high-crystalline PET bottles and textiles have been very slow. Given these challenges, it is therefore necessary to identify and characterize new efficient enzymes from the environment to tackle these limitations. Additionally, rational or semirational enzyme engineering should be highly considered by researchers to create variants of the existing PET hydrolases and new identified enzymes, which can exhibit enhanced thermostability and depolymerization activity against high-crystalline PET products. Finally, the application of thermomechanical pretreatments, such as extrusion and microionization, and UV radiation on polymers appear to help enhance their overall biodegradation as we showcased one example in the PET depolymerization case study (see Section 2.5.4).

It is important to note that biodegradability is not always desirable. Yet, for certain applications, such as single-use bags and mulch films, it can be enhanced to offset some of the more apathetic behaviors from people. For instance, designing LDPE bags with pro-oxidants (e.g. TiO_2) improve biofilm formation, albeit, at a rather slow pace. It would be better to phase out polyolefins in general in favor of polymers with constituents that are natural metabolites for a wide array of microbes, thereby reducing the time it spends as garbage. This would have obvious limitations, however, for products designed for longevity such as plastic containers (e.g. Tupperware) and sewer piping. If scientists can solve the cost-effectiveness problem of producing polyesters on the same scale as hydrocarbons, it can overtake PE and PP and mitigate further plastic accumulation.

The growth rate of biodegradable polymers is expected to be around 15% per annum [28]. However, there could be a catch-22 in that promulgating biodegradable plastics may actually lead to increased littering as consumers often incur less responsibility because they overestimate deterioration rates [84]. One could also make the argument that promoting further biodegradation may upset the balance of nature by further augmenting CO_2 levels [181]. In other cases, such as food packaging, biodegradability might not be desired. Food packaging, in part, is designed to prevent degradative changes in food and separate microbiota present within and outside the package. Any contamination, and subsequent bioassimilation of the plastic, will defeat the purpose of the packaging [182].

Designing the ideal biodegradable polymer depends on the desired time of functionality. Shorter time scales can benefit from the incorporation of starch with PLA or PHA that can readily hydrolyze and bioassimilate the monomers via the plethora of microbes available. Longer functional times would require aliphatic polyesters in the form of polymer alloys, which can compensate for the subpar mechanical properties found in each polymer [183].

2.A Detailed Mechanism of PET Hydrolysis

Catalytic mechanism of PET hydrolysis. Polyesters are almost always hydrolyzed by endo-type hydrolases. Likewise, PET degradation is thought to occur through endolytic cleavage of its internal ester bonds. The product of PET hydrolysis reaction is mainly mono(2-hydoxyethyl)terephthalate (MHET) along with minor amounts of TPA and bis(2-hydroxyethyl)terephthalate (BHET). The ratio of these products, however, can vary based on incubation temperature, reaction length, and enzyme concentration [88]. Most PET-degrading enzymes are also able to degrade BHET to MHET to EG and TPA. As noted earlier, thermophilic cutinases from actinomycetes (e.g. TfH, Cut190, and LCC) and HiC from *H. insolens* fungus are the main known PET hydrolases. These enzymes can significantly degrade the main body of amorphous and low-crystallinity PET chains at temperatures above PET's T_g.

Despite their high hydrolysis activity, no detailed molecular mechanism has been proposed for these enzymes. Instead, most attention has been drawn toward lsPETase, partly because lsPETase shows very potent activity against PET near

room temperature compared to cutinases and its host bacterium, *I. sakaiensis*, can assimilate the degraded PET chains.

Although PET hydrolysis ability of IsPETase and PET hydrolases differ extensively in their optimal conditions, the structures of the enzymes are very similar. In particular, the catalytic triad in these enzymes are almost identical, and the residues constituting the hydrophobic cleft, known as the PET binding site, are highly conserved (see Figure 2.A.4b), suggesting that similar PET hydrolysis mechanisms may be employed by these enzymes. One group recently proposed a detailed mechanism for degradation of PET by IsPETase [121]. Although this mechanism was primarily suggested for a minor PET-degrading enzyme, it can hint at some molecular aspects of PET hydrolysis by main PET hydrolases. Using *in silico* docking experiments with 2-hydroxyethyl-(mono-hydroxyethyl terephthalate)$_4$ (2-HE(MHET)$_4$), regarded as a model substrate for PET chain, and mutagenesis analysis, authors showed that the PET chain binds at two sites along the IsPETase flat hydrophobic surface – one MHET moiety is bound to subsite I and three adjacent MHET moieties are bound to subsite II. According to their model, once MHET moieties are positioned at these two subsites, degradation is initiated by cleavage of the scissile ester bond located between subsite I and subsite II near the catalytic Ser^{160} residue (see Figure 2.A.4a,c). This hydrolysis reaction releases one PET chain with TPA terminal (^{TPA}PET) from subsite I and another one with HE terminal (^{HE}PET) from subsite II.

In the next step, two released PET chains are successively broken down into smaller molecules via two partially different mechanisms. In the first mechanism, the scissile ester bonds in both chains are cleaved as described above, producing one MHET monomer from the ^{HE}PET-terminal chain and one TPA molecule from the ^{TPA}PET-terminal chain along with $^{HE}PET_{n-1}$ from both chains. Degradation of the resultant $^{HE}PET_{n-1}$ continues following the same process. In the second mechanism, which is thought to be less efficient than the previous one, ^{HE}PET and ^{TPA}PET chains and the enzyme interact in the reverse direction. Here, one or two MHET moieties are positioned at subsite II instead of subsite I, and the cleavage of the sessile ester bond located between subsites I and II is catalyzed with help of Ser^{160} residue. Continuation of this process then produces various sizes of PET monomers and dimers, such as 2-HE(MHET)$_2$, (MHET)$_2$, MHET, and BHET. Moreover, BHET can be broken down into MHET, TPA, and EG, and eventually, three molecules, MHET, TPA, and EG, are accumulated as the result of PET degradation.

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