Chapter 4

Enzymes Involved in Plastic Degradation

S.Z.Z. Cobongela^{1*}

¹Nanotechnology Innovation Centre, Advanced Materials Division, Mintek, Randburg, South Africa

snazozezethu@gmail.com

Abstract

The global increase in production of plastic and accumulation in the environment is becoming a major concern especially to the aquatic life. This is due to the natural resistance of plastic to both physical and chemical degradation. Lack of biodegradability of plastic polymers is linked to, amongst other factors, the mobility of the polymers in the crystalline part of the polyesters as they are responsible for enzyme interaction. There are significantly few catabolic enzymes that are active in breaking down polyesters which are the constituents of plastic. The synthetic polymers widely used in petroleum-based plastics include polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polyurethane (PUR), polystyrene (PS), polyamide (PA) and polyethylene terephthalate (PET) being the ones used mostly. Polymers with heteroatomic backbone such as PET and PUR are easier to degrade than the straight carbon-carbon backbone polymers such as PE, PP, PS and PVC.

Keywords

Polymers, Lipase, Cutinase, Esterase, Polyethylene Terephthalate, Polyurethane, Active Site, Protease, Enzymatic Degradation

Contents

Enzymes Involved in Plastic Degradation	95
1. Introduction.....	96
2. Enzymatic degradation of polyethylene terephthalate	97
3. Enzymatic degradation of polyurethane	100
4. Enzymatic degradation of polyethylene	101

5. Enzymatic degradation of polystyrene	103
6. Enzymatic degradation of polyvinylchloride (PVC)	103
7. Enzymatic degradation of polyvinylchloride polyamide (PA)	104
Conclusions	104
References	104

1. Introduction

Polymeric material can be degraded by enzymes secreted by microorganisms. They are degraded either by hydrolases or oxidation enzymes. The struggle in degrading these polymers is due to their low hydrophilicity. Their hydrophobic nature makes it challenging to wet them as they hinder water from penetrating the pores of the material. Alkali treatment is a conventional treatment commonly used by industries to hydrolyse the polyester bonds which requires high amounts of sodium hydroxide and high temperatures which in turn are a disadvantage in the environment. Therefore, there is a need for substances that improve surface hydrophilicity and increase hydrolysis of these polymers. The enzymes enhance water absorption ability of the synthetic fibres through increase in surface hydrophilicity as seen on treatments with alkaline treatment [1].

Enzymes have shown a potential in combating this problem. Examples of these surface modifying enzymes are several hydrolases, such as lipases, carboxylesterases, cutinases, and proteases. Some of these enzymes are selected based on their known primary activities e.g. cutinase from *Aspergillusoryzae* (*A. oryzae*) which hydrolyses a protective cutin lipid polyester matrix in plants [2]. The enzyme reaction with the polymers occur under mild conditions which require no additional chemicals or complex machinery. Temperature plays a huge role in the performance of the hydrolases. Most of the enzymes seem to have optimum activity between 50-80°C [3]. An ideal hydrolase should possess high hydrolytic activity to plastic polymers, show specificity and thermostability. One way that has been proven to increase thermostability is the presence of disulphide bond between cysteine residues [2,4]. Some of these enzymes are genetically modified to enhance their enzymatic activity while achieving industrial requirements [5]. Cutinase belongs to a group of enzymes that catalyse the hydrolysis of esters and triglycerides and has been ascertained as a biocatalyst for biotechnological and industrial bioprocesses. Cutinases generally display their active site forming a pocket-like structure rather than having the gate structure as observed in most lipases [6]. Polymerases prefer to degrade the amorphous region [3].

Most of these enzymes have a catalytic triad made up of serine, glutamic acid and histidine with an exception of carboxyesterases that replace glutamic acid with aspartic acid [6,7]. These amino acid residues have shown to play a major role in hydrolysis of the ester bond. Billing et al. [6] (2010) confirmed this by using some of the inhibitors that bind irreversible to these amino acids. Phenylmethylsulfonyl fluoride and tosyl-L-phenylalanine-chloromethyl ketone permanently bind and inactivate serine and histidine, respectively, on the active site. The enzymes lose catalytic activity in the presence of either of these inhibitors. In addition, substitution mutagenesis of glutamic acid leads to extensive decrease of the enzyme activity [6]. These observations suggest a major role played by the amino acid triad in catalytic mechanism of enzymes. Usually these enzymes contain a specific sequence around the serine characterised by G-X-S-X-G (G- glycine, X- any polar amino acid, S- serine) motif [7].

2. Enzymatic degradation of polyethylene terephthalate

Polyethylene terephthalate (PET) is used in the manufacturing of bottles, foil and fibers used in the textile industry. It is characterized by polar and linear polymer with aromatic terephthalic acid and ethylene glycol. PET is a thermoplastic polymer and is partly crystalline which is made up of bis(2-hydroxyethyl) terephthalate (BHET) monomer [8,9]. There are relatively few known enzymes that degrade PET to oligomers or monomers and have all shown low turnover rates [10]. PET degrading enzymes are classified as serine hydrolases, e.g., cutinases, lipases and esterases [11]. Most of these enzymes are from a Gram-positive phylum *Actinobacteria* [12], possessing α/β hydrolase folds housing a catalytic triad that is composed of serine, histidine and aspartate residues [13,14]. Some of the enzymes contain cysteine residues which encourages disulfide bonds with the sulphur groups and that in turn promotes thermal stability and specific binding to PET [15]. The cutinase partially hydrolyzes the ester bond of PET on the surface to much more simpler components that are water soluble. The preference for the *A. oryzae* enzyme to hydrolyze longer chain substrates can be explained by the deep continuous groove extending across the active site, while that of *Fusarium solani* (*F. solani*) pisi favors short chain substrates due to the shallow and interrupted groove. This is due to the arrangement of the key hydrolysing residues on the active site. The distance between the “gatekeeper” residues determines the groove of the active site being narrow or wide. Wider opening of the active site is assumed to be related to rapid hydrolysis of longer chains such as PCL while the narrow active site result in slow hydrolysis and prefers shorter polymer chains [1]. The by products (oligomers and monomers) include terephthalic acid (TA), mono(2-hydroxyethyl) terephthalate (MHET), bis(2-hydroxyethyl) terephthalate (BHET), benzoic acid (BA) and 2-hydroxyethylbenzoate (HEB) [16,17].

An example of such enzymes is a PET-digesting enzyme (PETase) from *Ideonellasakaiensis* (*I. sakaiensis*) which also uses PET as the major source of carbon and energy source. The PETase from *I. sakaiensis* is assumed to be a lipase that shares about 51% amino acid residues with a hydrolase isolated from the actinomycete *Thermobifidafusca* (*T. fusca*) that also exhibit activity in PET hydrolysis [12]. In comparison with other hydrolases such as lipases, hydrolase from *T. fusca* known to be a cutinase depolymerizes the aromatic polyesters of PET at a higher rate [18]. Other cutinases are produced by *Humicola insolens*, *F. solani* pisi, *Fusarium oxysporum*, *Penicillium citrinum*, *Pseudomonas mendocina* and different species of *Thermobifida*. The genus *Thermobifida* has few other known and characterized cutinases that hydrolyzes PET.

Herrero-Acero [12] working with Ribitsch and others in 2011 compared two closely related cutinases from *Thermobifida Cellulosilytica* (*T. Cellulosilytica*) and *T. fusca* about the structure: function relationship. Their distinctive catalytic properties for both cutinases were distinctively different due to their structural difference. The study shows that all the cutinases cloned and tested hydrolyzed substrate bis(benzoyloxyethyl) terephthalate (3PET) more soluble products such as MHET, TA, benzoic acid (BA) and 2-hydroxyethyl benzoate (HEB) *Thermobifida Cellulosilytica* (*T. Cellulosilytica*) cutinase (Thc_Cut1) released more of the soluble products compared to the *T. fusca* (Tf42_Cut1) and *T. Cellulosilytica* (Thc_Cut2) [12]. The release of the PET oligomers and monomers is confirmation that all three enzymes were able to cleave the ester bonds. A linear increase of the by products from 3PET substrate hydrolyzed by cutinases from *T. Fusca* and *F. solani* pisi was observed on the study done by Eberl et al. [19] (2009). Both cutinases, like others, also proved to catalyze the conversion of HEB to BA as the increase in concentration of BA was observed while there was a decline in HEB. The same phenomenon was observed with the increase of TA against MHET. Other PET degrading enzymes such as lipases and polyesterases are not able to degrade the secondary components of PET [19,20].

In 2012, Ribitsch et al. [21] cloned a cutinase (Tha_Cut1) from *Thermobifida alba* (*T. alba*) for hydrolysis of PET. Data obtained from modeling reveals that Tha_Cut1 is almost identical to a cutinase produced by *T. cellulosilytica* with only four different amino acids found outside the active site [21]. Despite the identical active site, Tha_Cut1 had higher activity on PET surface hydrolysis compared to *T. Cellulosilytica* cutinase due to the ability of the cutinase to increase the hydrophilicity of the polymer. Hydrophilicity is measured by the variation in water contact angle such that decrease in water contact angle equates increase hydrophilicity and that in turn increases polymer hydrolysis. Treatment with cutinase results in a large number of hydroxyl end groups at the surface of the polymer [22]. The process of hydrolysing the ester bond of PET to polyethylene glycol and

terephthalic generates hydroxyl and carbonyl groups [23,24]. Interestingly, an esterase isolated from *Thermobifida halotolerans* (*T. halotolerans*) is a 262 amino acid with a mass of 28.7 kDa sharing about 85–87% homology to an esterase from *T. alba*. It hydrolyses PET and polylactic Acid (PLA) by increasing their hydrophilicity measured by water contact angle from 90.8° and 75.5° to 50.4°, respectively [21].

Another Actinomycete *Thermomonospora curvata* (*T. curvata*) is phylogenetically related and sharing about 61% sequence identity with *T. fusca* enzymes, produces extracellular hydrolases which can degrade synthetic polymers [13]. Tcur1278 and Tcur0390 are two polyester serine hydrolases coded by *T. curvata*. The study compared the two hydrolases catalytic activity on PET nanoparticles with varying temperatures. Tcur0390 showed higher activity at temperatures lower than 50°C while Tcur1278 showed to thrive in higher temperatures between 55°C to 60°C. It is suggested that the difference in thermal stability and hydrolytic activity is due to the difference in their molecular dynamics simulation. Tcur1278 would be ideal in codegradation with the thermal degradation of PET process. Agitation and pH control between pH 6 to 8 during reaction is necessary to increase the enzyme catalyses hydrolysis [23,25]. Calcium ions and has been shown to activate cutinases [26]. Table 1 [1–3, 6, 12, 17, 19–31] contains a list of enzymes involved in PET degradation.

Table 1 Polyethylene terephthalate degrading enzymes [1–3, 6, 12, 17, 19–31]

Enzyme	Species	Substrate	Optimum temperature	References
Lipases	<i>Candida antarctica</i>	PET		[27]
	<i>Thermomyces lanuginosus</i>	PET		[19]
	<i>Burkholderia spp.</i>	PET		[28]
	<i>Triticum aestivum</i>	PET		[29]
Cutinases from fungi	<i>Aspergillus oryzae</i>			[3]
	<i>Humicola insolens</i>	PET to terephthalic acid and ethylene glycol	70-89 °C	[3]
	<i>Aspergillus oryzae</i>	PET and poly(ϵ -caprolactone) (PCL)		[2]

	<i>Penicillium citrinum</i>			[20]
	<i>Fusarium solani pisi</i>	PET	50°C	[1,23]
Cutinases from actinomycetes	<i>Thermomonospora fusca</i>	PET		[17,22]
	<i>Thermobifida cellulosilytica</i>	PET		[12]
	<i>Thermobifida alba</i>	PET		[21]
	<i>Saccharomonospora viridis</i>	PET	50°C	[24]
Cutinase	Unidentified	Cyclic PET	60°C	[25]
Esteraseserine hydrolases	<i>Thermobifida halotolerans</i>	PET and PLA to lactic acid		[30]
Carboxylesterases		PET	60°C	[6]
Protease		Improves hydrophilicity of the polyester fabrics		[31]
Polyesterase	<i>Saccharomonospora viridis</i>	PET		[26]

3. Enzymatic degradation of polyurethane

Polyurethane(PUR)is a polymer made up of polyether/ polyester (polyisocyanate) polyols connected by intra-molecular carbamate ester bond. Correspondingly, both fungi and bacteria have been shown to secrete enzymes capable of degrading PUR. The enzymatic degradation of polyether PUR and polyester PUR is quite distinct and specific to different enzymes [32]. PUR is degraded by oxidative enzymes (oxidases) such as horseradish peroxidase, catalase, and xanthine oxidase [33].

One of polyurethase (PURase) is a 48 kDa esterase, extracted from *Pseudomonas fluorescens* (*P. Fluorescens*)in 1998, cloned and expressed in *Escherichia coli*. The *P. fluorescens*utilises PUR as sole carbon source. This enzymes resembles a typical serine protease catalytic triad with G-X-S-X-G motif [34]. Stern and Howard cloned a gene (pueA) that codes for a polyester PURase (lipase) from *Pseudomonas chlororaphis* (*P. chlororaphis*) in the year 2000. The active site for polyurethases is conserved like the other hydrolases mentioned in this text [35]. PUR esterase is highly hydrophobic which is

thought to play a huge role on the catalytic activity. The hydrophobicity increases the chance of the enzyme binding to PUR surface domain while helping with PUR binding on the catalytic site. There are two types of PURase, membrane bound and extracellular, degrading PUR in two distinct ways. Membrane bound PURase encourages cell mediated contact with the substrate [36] while the extracellular PURase binds to the surface of the substrate [34]. PUR esterase degrades PUR by hydrolysing the ester bonds releasing diethylene glycol and adipic acid at neutral pH [36]. In 2017, Schmidt and colleagues [32] tested a few bacterial polyester hydrolase on degradation of polyester PUR [32]. Some of these actinomycete enzymes were previously reported to be hydrolysing PET. These enzymes include hydrolases from *T. Alba* [21], *T. Fusca* [19,24], *Saccharomonospora viridis* (*S. viridis*) [24] and others. These enzymes proved to be hydrolysing the PUR ester bonds while the urethane bond resisted hydrolysis [32]. This is in agreement with what was reported by Akutsu et al. [36], 1998 and Nakajima-Kambe et al. [37] 1995 that degradation of PUR is due to cleavage of ester bonds. *Pestalotiopsis microspora*, a fungus species has also shown to secrete a metallo-hydrolase that robustly degrades PUR under anaerobic conditions using PUR as the main carbon source [38].

Table 2 Polyurethane degrading enzymes [34–36,38,40–43]

Enzyme	Species	Optimum Temperature	References
Esterase	<i>Curvularia Senegalensis</i>	100°C	[42]
	<i>Pseudomonas Fluorescens</i>		[34,43]
	<i>Comamonas acidovorans</i> TB -35	45°C	[36]
Esterase/ Protease	<i>Pseudomonas chlororaphis</i>	100°C	[40]
Lipase	<i>Pseudomonas chlororaphis</i> (pueA)		[35]
	<i>Pseudomonas chlororaphis</i> (pueB)		[41]
Metallo-hydrolase	<i>Pestalotiopsis microspora</i>	60-80°C	[38]

Cellulase is an enzyme that has the hydrophobic PUR surface binding domain containing a hydrophobic domain, a flexible hinge region and most importantly the C-terminus involved

in substrate binding [39]. Cellulases are widely known to hydrolyze the internal bond (β -1,4glycosidic) and terminal ends of cellulose. PURases show to be hydrolysing the ester bond; however, there is no clear view on how these enzymes breakdown the carbamate bond. On the other hand, it is unclear whether the urethane bond is hydrolyzed by the PUR esterase, because there is no evidence of the degradation products derived from polyisocyanate segments of the PUR were not detected [36]. The list of enzymes responsible for the degradation of PUR are listed in table 2 [34–36,38,40–43].

4. Enzymatic degradation of polyethylene

Polyethylene (PE) is considered the most durable polymer consisting of carbon-carbon backbone long chain of ethylene consisting of low density or high density. There is very limited research done on the enzymes capable of degrading PE. Some of the PE degrading enzymes are screened by degrading a protective polymer layers that are also made up of carbon-carbon bond linkages found in plant cell walls [44–46]. One of these enzymes is laccase produced by an Actinomycete *Rhodococcus ruber* (*R. ruber*) [47,48]. It is the first enzyme known to degrade PE. The activity of laccase on utilizing and degrading the PE is greatly induced by copper. There was a 13-fold increase when compared the copper treated laccase degradation and the untreated reaction [47]. Laccase is an oxidizing enzyme with four binding sites breaking down non-phenolic aromatic rings, carbon-carbon and other bonds [49]. This enzyme plays a huge role in oxidation of the carbon-carbon backbone on the PE proven by the decrease in molecular weight of the PE and increase in carbonyl index [47].

Manganese peroxidase enzyme has also been confirmed to degrade PE in the presence of malonate buffer and Tween 80 [50]. It was previously assumed by Moen et al. [51] that the degradation is due to manganese peroxidase-lipid peroxidation system generating free radicals that in turn reacts with the carbon-carbon backbone of the PE [51]. Ehara and colleagues [50] concluded that the manganese (III) produced by manganese peroxidase is highly responsible for the PE degradation [50]. On the other hand, an enzyme alkane hydroxylase responsible for PE degradation acts through mineralization of low molecular weight PE into CO₂ [52]. These enzymes modify the PE surface by hydrolysis of the carbon-carbon backbone and most importantly introducing hydrophilic groups thus making the PE surface more hydrophilic and high energy improving adhesion to other material [53]. Some of the enzymes involved in PE degradation are listed in Table 3 [47,48,50,52,54,55].

Table 3 Polyethylene degrading enzymes [47,48,50,52,54,55]

Enzyme	Species	Temperature	References
Laccase	<i>Rhodococcus Ruber</i>	30-70°C	[47]
	<i>Trametes versicolor</i>		[48]
	<i>Aspergillus fumigatus</i>		[54]
Manganese peroxidase	<i>Phanerochaete chrysosporium</i>		[50]
Alkane hydroxylase	<i>Pseudomonas aeruginosa</i>	37°C	[52,58]

5. Enzymatic degradation of polystyrene

An alternative name for polystyrene (PS) is poly-1-phenylethene, a synthetic aromatic hydrocarbon polymer consisting of monomer styrene. It is a high molecular weight hydrocarbon compared to PE which in turn makes it durable and resistant to degradation. Promising enzymatic degradation has been with the help of chemical additives breaking down the higher molecular weight to low molecular weight PS. This phenomenon was confirmed by Nakamiya et al. [56], in 1997 where they used dichloromethane to break PS to small water soluble molecules that were further broken down by hydroquinone peroxidase enzyme isolated from *Azotobacter beijerinckii* (*A. beijerinckii*) [56]. Besides the pre-chemical treatment, a physicochemical process has been observed using mealworms (*Tenebriomolitor* Linnaeus). The mealworms physically chew and ingest PS and with the help of unidentified enzymes produced by the mealworm helps in depolymerization the polymer into CO₂ [57,58]. These enzymes might include the previously mentioned.

6. Enzymatic degradation of polyvinylchloride (PVC)

Like other plastic polymers, polyvinylchloride (PVC) is known for phenomenal resistance to degradation. It is made up of a chain of vinyl chloride. PVC is highly hydrophobic mostly resistant to abrasion by chemical [59] which makes it a good candidate to be used in construction industries. Low-molecular weight PVC has been shown to be viable degraded by some fungi species such as *As. fumigatus*, *Phanerochaete chrysosporium*, *Lentinus tigrinus*, *Aspergillus niger*, and *Aspergillus sydowii* although the mechanism of action is not fully understood [60,61]. In 2016, Sumathi and colleagues [62] finally isolated *Cochliobolus sp.* a fungi species from plastic dump soil [62]. This fungi species uses PVC as the sole carbon source and is capable of producing laccase enzyme which is assumed to

be responsible for the degradation of low-molecular weight PVC. There is no reported enzymatic degradation on high-molecular PVC.

7. Enzymatic degradation of polyvinylchloride polyamide (PA)

The polymer chain of polyvinylchloride polyamide (PA) is joined together by amide bonds (CO-NH). This bond is generally resistant to degradation. Most common synthetic polyamides are nylon 6 (PA 6) and nylon 6,6 (PA 6,6). Proteolytic enzymes such as proteases showed hydrolysis on amide oligomers [63,64]. Recently, a protease from *Bacillus sp.* has been shown to hydrolyze both PA 6 and PA 6,6 [65]. This particular protease functions optimally between 25-30°C at pH 8 under agitation. However, protease and lipase enzymes are normally used to modify and improve PA physical and chemical properties such as hydrophilicity while smoothing the surface [65,66].

Conclusions

Although plastics play a key role in society, their fate has become a major management problem on our environment. The major degradation techniques currently used include thermal and chemical treatments. Enzymatic plastic degradation remains a topic to be explored further with major improvements. Literature shows there has been a lot of research done in PET and PUR compared to other plastic polymers on enzymatic degradation. Occasionally, enzymes are used mainly for modification of plastic such as improving elasticity, hydrophilicity and other important functional physiognomies. Some of the enzymes require a pretreatment either by heat or chemical treatment for maximum degradation. There is very minute research done on the enzyme-substrate interaction, hydrolysis of bonds and enzyme kinetics.

References

- [1] M. Alisch-Mark, A. Herrmann, W. Zimmermann, Increase of the hydrophilicity of polyethylene terephthalate fibres by hydrolases from *Thermomonospora fusca* and *Fusarium solani* f. sp. pisi, *Biotechnol Lett.* 28 (2006) 681–685.
<https://doi.org/10.1007/s10529-006-9041-7>
- [2] Z. Liu, Y. Gosser, P.J. Baker, Y. Ravee, Z. Lu, G. Alemu, H. Li, G.L. Butterfoss, X.-P. Kong, R. Gross, J.K. Montclare, Structural and functional studies of *A. oryzae* Cutinase: enhanced thermostability and hydrolytic activity of synthetic ester and polyester degradation, *J. Am. Chem. Soc.* 131 (2009) 15711–15716.
<https://doi.org/10.1021/ja9046697>
- [3] A.M. Ronkvist, W. Xie, W. Lu, R.A. Gross, Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate), *Macromolecules.* 42 (2009) 5128–5138.
<https://doi.org/10.1021/ma9005318>

- [4] A.J. Doig, D.H. Williams, Is the hydrophobic effect stabilizing or destabilizing in proteins? The contribution of disulphide bonds to protein stability, *J. Mol. Biol.* 217 (1991) 389–398. [https://doi.org/10.1016/0022-2836\(91\)90551-g](https://doi.org/10.1016/0022-2836(91)90551-g)
- [5] I.S. Chin, A.M.A. Murad, N.M. Mahadi, S. Nathan, F.D.A. Bakar, Thermal stability engineering of *Glomerella cingulata* cutinase, *Protein. Eng. Des. Sel.* 26 (2013) 369–375. <https://doi.org/10.1093/protein/gzt007>
- [6] S. Billig, T. Oeser, C. Birkemeyer, W. Zimmermann, Hydrolysis of cyclic poly(ethylene terephthalate) trimers by a carboxylesterase from *Thermobifida fusca* KW3, *Applied Microbiology and Biotechnology.* 87 (2010) 1753–1764. <https://doi.org/10.1007/s00253-010-2635-y>
- [7] U.T. Bornscheuer, Microbial carboxyl esterases: classification, properties and application in biocatalysis, *FEMS Microbiology Reviews.* 26 (2002) 73–81. [https://doi.org/10.1016/S0168-6445\(01\)00075-4](https://doi.org/10.1016/S0168-6445(01)00075-4)
- [8] S. Amin, M. Amin, Thermoplastic elastomeric (TPE) materials and their use in outdoor electrical insulation, *Rev. Adv. Mater. Sci.* 29 (2011) 15-30.
- [9] B. DemiRel, A. Yara, H. Elç, Crystallization behavior of PET Materials, *BAÜ Fen Bil. Enst. Dergisi Cilt* 13(1) (2011) 26-35.
- [10] R. Wei, W. Zimmermann, Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we?, *Microb Biotechnol.* 10 (2017) 1308–1322. <https://doi.org/10.1111/1751-7915.12710>
- [11] S. Heumann, A. Eberl, H. Pobeheim, S. Liebminger, G. Fischer-Colbrie, E. Almansa, A. Cavaco-Paulo, G. Guebitz, New model substrates for enzymes hydrolysing polyethyleneterephthalate and polyamide fibres, *Journal of Biochemical and Biophysical Methods.* 69 (2006) 89–99. <https://doi.org/10.1016/j.jbbm.2006.02.005>
- [12] E. Herrero Acero, D. Ribitsch, G. Steinkellner, K. Gruber, K. Greimel, I. Eiteljoerg, E. Trotscha, R. Wei, W. Zimmermann, M. Zinn, A. Cavaco-Paulo, G. Freddi, H. Schwab, G. Guebitz, Enzymatic surface hydrolysis of PET: Effect of structural diversity on kinetic properties of cutinases from *Thermobifida*, *Macromolecules.* 44 (2011) 4632–4640. <https://doi.org/10.1021/ma200949p>
- [13] R. Wei, T. Oeser, J. Then, N. Kühn, M. Barth, J. Schmidt, W. Zimmermann, Functional characterization and structural modeling of synthetic polyester-degrading hydrolases from *Thermomonospora curvata*, *AMB Express.* 4 (2014) 44. <https://doi.org/10.1186/s13568-014-0044-9>
- [14] D.L. Ollis, E. Cheah, M. Cygler, B. Dijkstra, F. Frolow, S.M. Franken, M. Harel, S. Jamse-Remington, I. Silman, J. Schrag, J.L. Sussman, K.H.G. Verschueren, A. Goldman, The alpha/beta hydrolase fold. *Protein Eng.* 5 (1992) 197-211. <https://doi.org/10.1093/protein/5.3.197>

- [15] S. Yoshida, K. Hiraga, T. Takehana, I. Taniguchi, H. Yamaji, Y. Maeda, K. Toyohara, K. Miyamoto, Y. Kimura, K. Oda, A bacterium that degrades and assimilates poly(ethylene terephthalate), *Science*. 351 (2016) 1196–1199.
<https://doi.org/10.1126/science.aad6359>
- [16] J.T. Ranz-Korpecka, S. Heumann, G. Gübitz, S. Billig, W. Zimmermann, M. Zinn, J. Ihssen, A. Cavaco-Paulo, Cutinase activity of PET-hydrolases., *Macromolecular Symposia*. 296 (2010) 342–346.
- [17] T. Brueckner, A. Eberl, S. Heumann, M. Rabe, G.M. Guebitz, Enzymatic and chemical hydrolysis of poly(ethylene terephthalate) fabrics, *Journal of Polymer Science Part A: Polymer Chemistry*. 46 (2008) 6435–6443.
<https://doi.org/10.1002/pola.22952>
- [18] I. Kleeberg, C. Hetz, R.M. Kroppenstedt, R.-J. Müller, W.D. Deckwer, Biodegradation of aliphatic-aromatic copolyesters by *Thermomonospora fusca* and other thermophilic compost isolates, *Appl Environ Microbiol*. 64 (1998) 1731–1735.
- [19] A. Eberl, S. Heumann, T. Brückner, R. Araujo, A. Cavaco-Paulo, F. Kaufmann, W. Kroutil, G.M. Guebitz, Enzymatic surface hydrolysis of poly(ethylene terephthalate) and bis(benzoyloxyethyl) terephthalate by lipase and cutinase in the presence of surface active molecules, *J. Biotechnol*. 143 (2009) 207–212.
<https://doi.org/10.1016/j.jbiotec.2009.07.008>
- [20] S. Liebming, A. Eberl, F. Sousa, S. Heumann, G. Fischer-Colbrie, A. Cavaco-Paulo, G.M. Guebitz, Hydrolysis of PET and bis-(benzoyloxyethyl) terephthalate with a new polyesterase from *Penicillium citrinum*, *Biocatalysis and Biotransformation*. 25 (2007) 171–177. <https://doi.org/10.1080/10242420701379734>
- [21] D. Ribitsch, E.H. Acero, K. Greimel, I. Eiteljoerg, E. Trotscha, G. Freddi, H. Schwab, G.M. Guebitz, Characterization of a new cutinase from *Thermobifida alba* for PET-surface hydrolysis, *Biocatalysis and Biotransformation*. 30 (2012) 2–9.
<https://doi.org/10.3109/10242422.2012.644435>
- [22] M. Alisch, A. Feuerhack, H. Müller, B. Mensak, J. Andreaus, W. Zimmermann, Biocatalytic modification of polyethylene terephthalate fibres by esterases from actinomycete isolates, *Biocatalysis and Biotransformation*. 22 (2004) 347–351.
<https://doi.org/10.1080/10242420400025877>
- [23] A. O'Neill, R. Araújo, M. Casal, G. Guebitz, A. Cavaco-Paulo, Effect of the agitation on the adsorption and hydrolytic efficiency of cutinases on polyethylene terephthalate fibres, *Enzyme and Microbial Technology*. 40 (2007) 1801–1805.
<https://doi.org/10.1016/j.enzmictec.2007.02.012>
- [24] F. Kawai, T. Kawase, T. Shiono, H. Urakawa, S. Sukigara, C. Tu, M. Yamamoto, Enzymatic hydrophilization of polyester fabrics using a recombinant cutinase Cut 190 and their surface characterization, *Journal of Fiber Science and Technology*. 73 (2017) 8–18. <https://doi.org/10.2115/fiberst.fiberst.2017-0002>

- [25] J. Hooker, D. Hinks, G. Montero, M. Icherenska, Enzyme-catalyzed hydrolysis of poly(ethylene terephthalate) cyclic trimer, *J. App. Polymer Sci.* 89 (2003) 2545–2552. <https://doi.org/10.1002/app.11963>
- [26] F. Kawai, M. Oda, T. Tamashiro, T. Waku, N. Tanaka, M. Yamamoto, H. Mizushima, T. Miyakawa, M. Tanokura, A novel Ca^{2+} -activated, thermostabilized polyesterase capable of hydrolyzing polyethylene terephthalate from *Saccharomonospora viridis* AHK190, *Applied Microbiology and Biotechnology*. 98 (2014) 10053–10064. <https://doi.org/10.1007/s00253-014-5860-y>
- [27] M.A.M.E. Vertommen, V.A. Nierstrasz, M. van der Veer, M.M.C.G. Warmoeskerken, Enzymatic surface modification of poly(ethylene terephthalate), *Journal of Biotechnology*. 120 (2005) 376–386. <https://doi.org/10.1016/j.jbiotec.2005.06.015>
- [28] C.W. Lee, J.D. Chung, Synthesis and Biodegradation behavior of poly(ethylene terephthalate) oligomers, *Polymer Korea*. 33 (2009) 198-202.
- [29] A. Nechwatal, A. Blokesch, M. Nicolai, M. Krieg, A. Kolbe, M. Wolf, M. Gerhardt, A contribution to the investigation of enzyme-catalysed hydrolysis of poly(ethylene terephthalate) oligomers, *Macromol. Mater. Eng.* 291 (2006) 1486–1494. <https://doi.org/10.1002/mame.200600204>
- [30] D. Ribitsch, E. Herrero Acero, K. Greimel, A. Dellacher, S. Zitzenbacher, A. Marold, R.D. Rodriguez, G. Steinkellner, K. Gruber, H. Schwab, G.M. Guebitz, A new esterase from thermobifida halotolerans hydrolyses polyethylene terephthalate (PET) and polylactic acid (PLA), *Polymers*. 4 (2012) 617–629. <https://doi.org/10.3390/polym4010617>
- [31] H.R. Kim, W.S. Song, Optimization of papain treatment for improving the hydrophilicity of polyester fabrics, *Fibers and Polymers*. 11 (2010) 67–71. <https://doi.org/10.1007/s12221-010-0067-z>
- [32] J. Schmidt, R. Wei, T. Oeser, L.A. Dedavid e Silva, D. Breite, A. Schulze, W. Zimmermann, degradation of polyester polyurethane by bacterial polyester hydrolases, *Polymers*. 9 (2) (2017) 65. <https://doi.org/10.3390/polym9020065>
- [33] J.P. Santerre, R.S. Labow, D.G. Duguay, D. Erfle, G.A. Adams, Biodegradation evaluation of polyether and polyester-urethanes with oxidative and hydrolytic enzymes, *J. Biomed. Mater. Res.* 28 (1994) 1187–1199. <https://doi.org/10.1002/jbm.820281009>
- [34] RE Vega, T. Main, G.T. Howard, Cloning and expression in *Escherichia coli* of a polyurethane-degrading enzyme from *Pseudomonas ~uorescens*, *International Biodeterioration & Biodegradation*. 43 (1999) 49–55.
- [35] R.V. Stern, G.T. Howard, The polyester polyurethanase gene (pueA) from *Pseudomonas chlororaphis* encodes a lipase, *FEMS Microbiol. Lett.* 185 (2000) 163–168. <https://doi.org/10.1111/j.1574-6968.2000.tb09056.x>

- [36] Y. Akutsu, T. Nakajima-Kambe, N. Nomura, T. Nakahara, Purification and properties of a polyester polyurethane-degrading enzyme from *Comamonas acidovorans* TB-35, *Appl Environ Microbiol.* 64 (1998) 62–67.
- [37] T. Nakajima-Kambe, F. Onuma, N. Kimpara, T. Nakahara, Isolation and characterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source, *FEMS Microbiol. Lett.* 129 (1995) 39–42.
[https://doi.org/10.1016/0378-1097\(95\)00131-N](https://doi.org/10.1016/0378-1097(95)00131-N)
- [38] J.R. Russell, J. Huang, P. Anand, K. Kucera, A.G. Sandoval, K.W. Dantzler, D. Hickman, J. Jee, F.M. Kimovec, D. Koppstein, D.H. Marks, P.A. Mittermiller, S.J. Núñez, M. Santiago, M.A. Townes, M. Vishnevetsky, N.E. Williams, M.P.N. Vargas, L.-A. Boulanger, C. Bascom-Slack, S.A. Strobel, Biodegradation of polyester polyurethane by endophytic fungi ∇ , *Appl. Environ. Microbiol.* 77 (2011) 6076–6084.
<https://doi.org/10.1128/AEM.00521-11>
- [39] J. Knowles, P. Lehtovaara, T. Teeri, Cellulase families and their genes, *Trends Biotechnol.* 5 (1987) 255–261. [https://doi.org/10.1016/0167-7799\(87\)90102-8](https://doi.org/10.1016/0167-7799(87)90102-8)
- [40] C. Ruiz, T. Main, N.P. Hilliard, G.T. Howard, Purification and characterization of twopolyurethanase enzymes from *Pseudomonas chlororaphis*, *International Biodeterioration & Biodegradation.* 43 (1999) 43–47. [https://doi.org/10.1016/S0964-8305\(98\)00067-5](https://doi.org/10.1016/S0964-8305(98)00067-5)
- [41] G.T. Howard, B. Crother, J. Vicknair, Cloning, nucleotide sequencing and characterization of a polyurethanase gene (pueB) from *Pseudomonas chlororaphis*, *International Biodeterioration & Biodegradation.* 47 (2001) 141–149.
[https://doi.org/10.1016/S0964-8305\(01\)00042-7](https://doi.org/10.1016/S0964-8305(01)00042-7)
- [42] J.R. Crabbe, J.R. Campbell, L. Thompson, S.L. Walz, W.W. Schultz, Biodegradation of a colloidal ester-based polyurethane by soil fungi, *International Biodeterioration & Biodegradation.* 33 (1994) 103–113. [https://doi.org/10.1016/0964-8305\(94\)90030-2](https://doi.org/10.1016/0964-8305(94)90030-2)
- [43] G.T. Howard, R.C. Blake, Growth of *Pseudomonas fluorescens* on a polyester–polyurethane and the purification and characterization of a polyurethanase–protease enzyme, *International Biodeterioration & Biodegradation.* 42 (1998) 213–220.
[https://doi.org/10.1016/S0964-8305\(98\)00051-1](https://doi.org/10.1016/S0964-8305(98)00051-1)
- [44] Suhas, P.J.M. Carrott, M.M.L. Ribeiro Carrott, Lignin – from natural adsorbent to activated carbon: A review, *Bioresource Technology.* 98 (2007) 2301–2312.
<https://doi.org/10.1016/j.biortech.2006.08.008>
- [45] J.-M. Restrepo-Flórez, A. Bassi, M.R. Thompson, Microbial degradation and deterioration of polyethylene – A review, *International Biodeterioration & Biodegradation.* 88 (2014) 83–90. <https://doi.org/10.1016/j.ibiod.2013.12.014>

- [46] M.C. Krueger, H. Harms, D. Schlosser, Prospects for microbiological solutions to environmental pollution with plastics, *Appl. Microbiol. Biotechnol.* 99 (2015) 8857–8874. <https://doi.org/10.1007/s00253-015-6879-4>
- [47] M. Santo, R. Weitsman, A. Sivan, The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*, *International Biodeterioration & Biodegradation.* 84 (2013) 204–210. <https://doi.org/10.1016/j.ibiod.2012.03.001>
- [48] M. Fujisawa, H. Hirai, T. Nishida, Degradation of polyethylene and Nylon-66 by the Laccase-Mediator System, 9 (2001) 103-108.
- [49] S. Kim, S.C. Chmely, M.R. Nimlos, Y.J. Bomble, T.D. Foust, R.S. Paton, G.T. Beckham, Computational Study of Bond Dissociation Enthalpies for a Large Range of Native and Modified Lignins, *J. Phys. Chem. Lett.* 2 (2011) 2846–2852. <https://doi.org/10.1021/jz201182w>
- [50] K. Ehara, Y. Iiyoshi, Y. Tsutsumi, T. Nishida, Polyethylene degradation by manganese peroxidase in the absence of hydrogen peroxide, *J. Wood Sci.* 46 (2000) 180–183. <https://doi.org/10.1007/BF00777369>
- [51] M.A. Moen, K.E. Hammel, Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the Intact Fungus, *Appl. Environ. Microbiol.* 60 (1994) 1956–1961. <https://doi.org/10.1128/AEM.60.6.1956-1961.1994>
- [52] M.G. Yoon, H.J. Jeon, M.N. Kim, Biodegradation of Polyethylene by a soil bacterium and AlkB Cloned Recombinant Cell, *Journal of Bioremediation & Biodegradation.* 03 (2012) undefined-undefined.
- [53] J. Zhao, Z. Guo, X. Ma, G. Liang, J. Wang, Novel surface modification of high-density polyethylene films by using enzymatic catalysis, *Journal of Applied Polymer Science.* 91 (2004) 3673–3678. <https://doi.org/10.1002/app.13619>
- [54] C. Ndahebwa Muhonja, G. Magoma, M. Imbuga, H.M. Makonde, Molecular Characterization of low-density polyethene (LDPE) degrading bacteria and fungi from Dandora Dumpsite, Nairobi, Kenya, *Int. J. Microbiol.* 2018 (2018) 1-10. <https://doi.org/10.1155/2018/4167845>
- [55] A. Belhaj, N. Desnoues, C. Elmerich, Alkane biodegradation in *Pseudomonas aeruginosa* strains isolated from a polluted zone: identification of *alkB* and *alkB*-related genes, *Res. Microbiol.* 153 (2002) 339–344. [https://doi.org/10.1016/S0923-2508\(02\)01333-5](https://doi.org/10.1016/S0923-2508(02)01333-5)
- [56] K. Nakamiya, G. Sakasita, T. Ooi, S. Kinoshita, Enzymatic degradation of polystyrene by hydroquinone peroxidase of *Azotobacter beijerinckii* HM121, *J. Ferment. Bioeng.* 84 (1997) 480–482. [https://doi.org/10.1016/S0922-338X\(97\)82013-2](https://doi.org/10.1016/S0922-338X(97)82013-2)

- [57] Y. Yang, J. Yang, W.M. Wu, J. Zhao, Y. Song, L. Gao, R. Yang, L. Jiang, Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. chemical and physical characterization and isotopic tests, *Environ. Sci. Technol.* 49 (2015) 12080–12086. <https://doi.org/10.1021/acs.est.5b02661>
- [58] Y. Yang, J. Yang, W.-M. Wu, J. Zhao, Y. Song, L. Gao, R. Yang, L. Jiang, Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. role of gut microorganisms, *Environ. Sci. Technol.* 49 (2015) 12087–12093. <https://doi.org/10.1021/acs.est.5b02663>
- [59] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: A comprehensive review, *Biotechnol. Adv.* 26 (2008) 246–265. <https://doi.org/10.1016/j.biotechadv.2007.12.005>
- [60] M.I. Ali, S. Ahmed, I. Javed, N. Ali, N. Atiq, A. Hameed, G. Robson, Biodegradation of starch blended polyvinyl chloride films by isolated *Phanerochaete chrysosporium* PV1, *Int. J. Environ. Sci. Technol.* 11 (2014) 339–348. <https://doi.org/10.1007/s13762-013-0220-5>
- [61] M.I. Ali, S. Ahmed, G. Robson, I. Javed, N. Ali, N. Atiq, A. Hameed, Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates, *J. Basic Microbiol.* 54 (2014) 18–27. <https://doi.org/10.1002/jobm.201200496>
- [62] T. Sumathi, B. Viswanath, A. Sri Lakshmi, D.V.R. SaiGopal, Production of Laccase by *Cochliobolus* sp. isolated from plastic dumped soils and their ability to degrade low molecular weight PVC, *Biochem Res Int.* 2016 (2016). <https://doi.org/10.1155/2016/9519527>
- [63] I.D. Prijambada, S. Negoro, T. Yomo, I. Urabe, Emergence of nylon oligomer degradation enzymes in *Pseudomonas aeruginosa* PAO through experimental evolution., *Appl. Environ. Microbiol.* 61 (1995) 2020–2022.
- [64] T. Deguchi, M. Kakezawa, T. Nishida, Nylon biodegradation by lignin-degrading fungi, *Appl. Environ. Microbiol.* 63 (1997) 329–331.
- [65] I. Jordanov, D.L. Stevens, A. Tarbuk, E. Magovac, S. Bischof, J.C. Grunlan, Enzymatic Modification of Polyamide for Improving the Conductivity of water-based multilayer nanocoatings, *ACS Omega.* 4 (2019) 12028–12035. <https://doi.org/10.1021/acsomega.9b01052>
- [66] A. Kiumarsi, M. Parvinzadeh, Enzymatic hydrolysis of nylon 6 fiber using lipolytic enzyme, *J. Appl. Polym. Sci.* 116 (2010) 3140–3147. <https://doi.org/10.1002/app.31756>