



Review

Current biotechnologies on depolymerization of polyethylene terephthalate (PET) and repolymerization of reclaimed monomers from PET for bio-upcycling: A critical review

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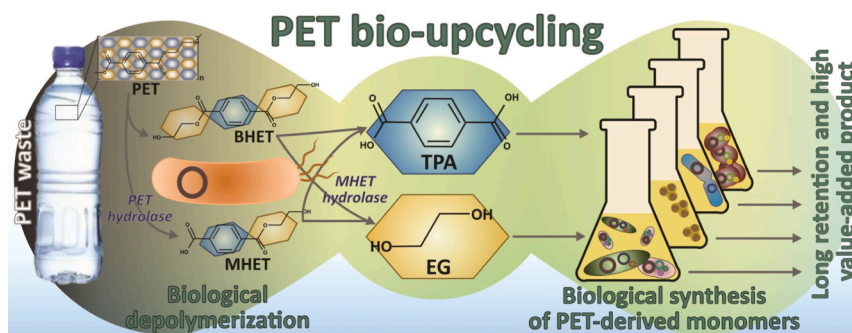
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HIGHLIGHTS

- PET bio-upcycling is a combined process of depolymerization and repolymerization.
- The discovery of *IsPETase* and *IsMHE-Tase* was a milestone in PET hydrolysis.
- New potential PET hydrolases were discovered using Multi-Omics approaches.
- Artificial consortia degrading PET were designed using microbiome engineering.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
 Polyethylene terephthalate
 Bio-upcycling
 Microbial degradation
 Microbiome engineering
 Circular bioeconomy
 PET hydrolase

ABSTRACT

The production of polyethylene terephthalate (PET) has drastically increased in the past half-century, reaching 30 million tons every year. The accumulation of this recalcitrant waste now threatens diverse ecosystems. Despite efforts to recycle PET wastes, its rate of recycling remains limited, as the current PET downcycling is mostly unremunerative. To address this problem, PET bio-upcycling, which integrates microbial depolymerization of PET followed by repolymerization of PET-derived monomers into value-added products, has been suggested. This article critically reviews current understanding of microbial PET hydrolysis, the metabolic mechanisms involved in PET degradation, PET hydrolases, and their genetic improvement. Furthermore, this review includes the use of meta-omics approaches to search PET-degrading microbiomes, microbes, and putative hydrolases. The current development of biosynthetic technologies to convert PET-derived materials into value-added products is also comprehensively discussed. The integration of various depolymerization and repolymerization biotechnologies enhances the prospects of a circular economy using waste PET.

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<https://doi.org/10.1016/j.biortech.2022.127931>

Received 31 July 2022; Received in revised form 4 September 2022; Accepted 6 September 2022

Available online 10 September 2022

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1. Introduction

Plastics are highly versatile, permeating all areas of life, from a kitchen utensil to a construction site. The annual global production of plastics has significantly increased since the early 2000s, reaching 367 million metric tons by 2020 (Plastics-the Facts 2021, Plastics Europe, <https://plasticseurope.org/>) (Tiseo, 2021). The various merits of plastics, including pliability, durability, light weight, and cost-effectiveness, have led to a continuous surge in market demand. However, plastics have had a negative impact on the environment and humans, along with significant mismanagement of post-consumer plastic wastes. Of the plastics estimated to have been produced between 1950 and 2015, 12% was reported to be incinerated, only 9% was recycled, and the rest was discarded in either landfills or other natural environments (Geyer, 2020; Geyer et al., 2017), where the waste can take hundreds of years to break down and return to the natural circulation because of their non-reactive and relatively non-biodegradable properties (Cózar et al., 2014).

Polyethylene terephthalate (PET), as one of the exemplified plastics, is a petroleum-based synthetic polymer produced from the esterification of ethylene glycol (EG) and terephthalic acid (TPA) and polycondensation, with the chemical formula $[C_{10}H_8O_4]_n$. The annual global production of PET exceeded 30 million metric tons in 2019, with an expected 4% market growth rate, contributing 10% to the total primary plastic waste generation (Geyer, 2020; Lebreton and Andradý, 2019). Similar to the plastic waste managements in general, recycling of PET wastes remains limited. PET is the most widely used material in the productions of single-use packaging for foods and beverage bottles, textiles for clothing and fibers, thermoplastic resins, and other consumer products. Millions of tons of plastic debris entering the ocean have created vast collections of plastic litter, such as “the Great Pacific garbage patch,” and have threatened the lives of marine vertebrates (Lamb et al., 2018; Li et al., 2016a). A non-negligible amount of micro- and nano-plastics resulting from physical abrasion and photooxidation further pollutes marine and terrestrial environments, releasing toxic plastic additives and disrupting ecosystems from lower organisms to higher animals along the food chain (Amobonye et al., 2021). These plastics also contaminate the soil environment, adversely affecting soil composition, water potential, nutrients, and consequently terrestrial biodiversity (Wang et al., 2019). Being at the top of the food chain, humans encounter this inevitable plastic pollution, as up to 5 g of microplastics per week was estimated to be ingested (Senathirajah et al., 2021). Considering that nanoparticles of PET internalized by macrophage cell lines induced oxidative stress and altered cell proliferation (Aguilar-Guzmán et al., 2022), despite the lack of direct evidence of toxic effects to humans, various health risks may be potentially caused by inhaled and ingested PET.

The biological degradation of PET, unlike other types of plastic polymers, has been demonstrated; the ester bond of PET between the polymer chains is broken by various hydrolases, including polyethylene

terephthalate hydrolase (PETase), cutinase, lipase, and esterase, and PET is mainly separated into mono-2-hydroxyethyl terephthalic acids (MHET) with minor amounts of bis-2-hydroxyethyl terephthalic acids (BHET), which are cleaved into EG and TPA by mono-2-hydroxyethyl terephthalate hydrolase (MHETase) (Fig. 1) (Hachisuka et al., 2021; Tanasupawat et al., 2016; Yoshida et al., 2016). Microorganisms capable of hydrolyzing PET as carbon and energy sources have been intensively studied to develop solutions for eco-friendly and sustainable waste management of PET (Almeida et al., 2019; Chen et al., 2008; Hachisuka et al., 2021; Herrero Acero et al., 2011; Kleeberg et al., 1998; Zhang et al., 2021b; Zimmermann and Billig, 2011). Although a few species have been discovered to be capable of PET degradation up to date, recent developments in multi-omics-based bioinformatics and data mining have enabled the detection of microbial genomic entities with genes encoding PET hydrolyzing functions and the exploration of various environmental microbiomes, including microbial plastic ecology named “Plastisphere” and extremophile habitats (Danso et al., 2018; Pérez-García et al., 2021; Wright et al., 2021).

These novel discoveries not only enlarged the PET hydrolyzing enzyme database but also created the possibility of economically feasible PET upcycling. Traditional PET recycling mostly relies on downcycling; PET wastes are physically reformed or mechanically and chemically depolymerized into TPA and EG, and these monomers are either repolymerized into recycled PET or utilized to produce other products, including carbon fibers, as a blending ingredient (Achilias et al., 2011; Beaucamp et al., 2019; Chaudhary et al., 2013). However, the recycling rate using these methods was limited to only one-fifth of PET waste generation even in Europe and the USA, where recycling of PET waste is relatively well managed, mainly because utilizing recycled PET costs more than utilizing petroleum-based monomers to produce PET of the same quality (García-Manyes and Beedle, 2017; George and Kurian, 2014; López et al., 2014; Vollmer and Jenks, 2020). Alternatively, enzymatic depolymerization of post-consumer PET followed by biological synthesis of monomers and oligomers into value-added polymers and/or aromatic chemicals, including polyhydroxyalkanoate (PHA), hydroxyalkanoxy-alkanoate (HAA), and 2-pyrone-4,6-dicarboxylic acid (PDC), is beginning to enhance rates of upcycling of PET wastes and improving its economic feasibility (Dissanayake and Jayakody, 2021; Kim et al., 2019; Tiso et al., 2021; Tournier et al., 2020).

Given that PET bio-upcycling is a combined process of depolymerization of PET and repolymerization of monomers derived from PET, the technologies in these fields tended to be developed in isolation, and the real-time expansion of the PET hydrolases database resulting from meta-omics analyses of plastisphere microbiomes and data mining was rarely reflected in those studies. It is expected that the integration of advancements in PET depolymerization, repolymerization, meta-multi-omics approaches, and microbiome engineering will open new perspectives in the PET bio-upcycling technology. This review critically discusses 1) our understanding of microbial PET hydrolysis and

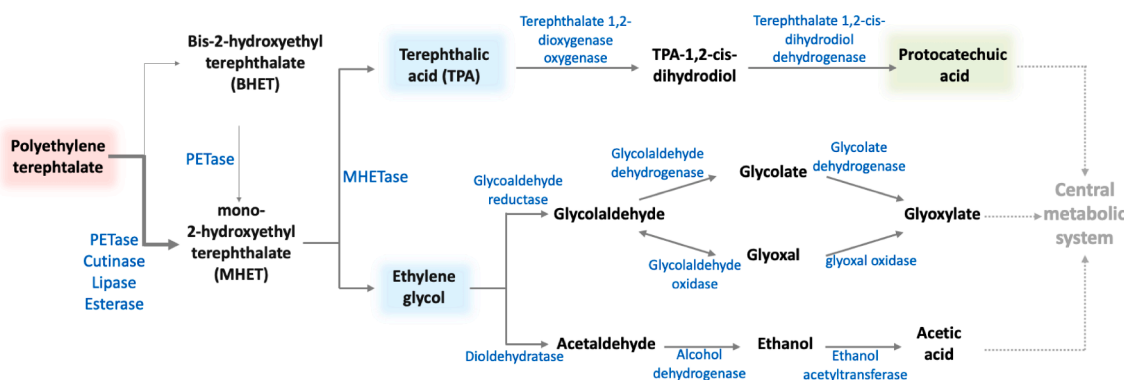


Fig. 1. The metabolic pathway of PET degradation by enzymatic hydrolysis.

metabolic mechanisms; 2) new insights into genomic potentials, diversity of the genomes, and the distribution of putative PET degradation genes revealed by multi-omics technologies; and 3) a comprehensive overview of current biosynthetic technologies utilizing reclaimed PET monomers. These holistic approaches may provide clues for a circular economy by suggesting feasible and sustainable PET bio-upcycling.

2. Enzymatic PET hydrolysis

PET depolymerization is initiated by PET hydrolases, which cleave the ester linkages of PET polymers to generate mainly MHET with trace amounts of BHET, TPA, and EG (Fig. 1) (Yoshida et al., 2016). The hydrolysis of the ester bond in BHET is also activated by the PET hydrolases (Taniguchi et al., 2019; Yoshida et al., 2016). MHET is further converted

to TPA and EG by MHETase. TPA is degraded to TPA-1,2-*cis*-dihydrodiol (DCD) by TPA-1,2-dioxygenase (TphAabc) and subsequently degraded to protocatechuic acid (PCA) by TPA-1,2-*cis*-dihydrodiol dehydrogenase (TphB) (Nomura et al., 1992). EG is sequentially converted to glycoaldehyde, glycolic acid, and glyoxylic acid by glycoaldehyde reductase, glycoaldehyde dehydrogenase, and glycolate dehydrogenase, respectively (Mückschel et al., 2012). PCA and glyoxylic acid are either utilized by anabolic mechanisms or metabolized via the tricarboxylic acid (TCA) cycle (Fig. 1) (Child and Willetts, 1978; Choi et al., 2005; Ru et al., 2020; Sasoh et al., 2006). Microorganisms that produce PET hydrolases tend to form biofilms on the surface of PET and secrete extracellular hydrolases to initiate microbial PET degradation (Vague et al., 2019), which increases the hydrophilicity of PET surfaces where the availability of carboxyl and hydroxyl groups increases (Jaiswal et al., 2020; Kawai

Table 1
Hydrolases that are capable of catalyzing polyethylene terephthalate (PET).

Enzyme	Microorganism	Accession No. ^a	Condition	Substrate ^b	Reference
Fungal enzymes					
FsC	<i>Fusarium solani pisi</i>	1CEX	30–60 °C	PET (7%, 35%)	Ronkvist et al., 2009
HiC	<i>Thermomyces insolens</i>	4OYY	30–85 °C	PET (7%, 35%)	Ronkvist et al., 2009
Lipase	<i>Aspergillus oryzae</i> CCUG 33,812	–	30 °C, pH 5.5–6.0	PET fiber	Wang et al., 2008
Bacterial enzymes					
BhrPETase	<i>Bacillus subtilis</i> HR29	GBD22443	37 °C, pH 7.0	Amorphose PET	Xi et al., 2021
BsEstB	<i>Bacillus subtilis</i>	ADH43200.1	40–45 °C	Bis(benzoyloxyethyl) TPA	Ribitsch et al., 2011
BTA-1BTA-2	<i>Thermobifida fusca</i> DSM43793	AJ810119.1	55 °C pH7.0	Commercial PET bottle	Müller et al., 2005
Cbotu_EstA/Cbotu_EstB	<i>Clostridium botulinum</i> ATCC3502	KP859619/KP859620	50 °C pH7.0	PET film	Biundo et al., 2018
Cut1 Cut2	<i>Thermobifida fusca</i> NRRL B-8184	JN129499.1 JN129500.1	55 °C pH8.0	–	Hegde and Veeranki, 2013
Cut190	<i>Saccharomonospora viridis</i> AHK190	BAO42836.1	60–65 °C pH6.0–8.5	Amorphose PET	Kawai et al., 2014
DuraPETase	<i>Ideonella sakaiensis</i>	–	37 °C, pH 9.0	PET film (30%)	Cui et al., 2019
Est 1	<i>Thermobifida alba</i> AHK119	BAI99230.2	50 °C pH8.0	PET film	Hu et al., 2010
Est119	<i>Thermobifida alba</i> AHK119	BAK48590.1	50 °C pH6.0	PET	Thumarat et al., 2012
Lipase(1JFR)	<i>Streptomyces exfoliatus</i>	AAB51445	30 °C, pH 6.0–7.5	–	Wei et al., 1998
PBS depolymerase	<i>Acidovorax delafieldii</i> BS-3	–	30–37 °C	–	Uchida et al., 2002
PE-H	<i>Pseudomonas aestusnigri</i> VGXO14T	6SBN	30 °C	Amorphose PET film	Bollinger et al., 2020
PETase MHETase	<i>Ideonella sakaiensis</i> 201-F6	GAP38373.1 GAP38911	25–30 °C pH 7.0–9.0	Low-crystallinity PET film (1.9%) Amorphous PET	Yoshida et al., 2016
PmC	<i>Pseudomonas mendocina</i>	–	50 °C pH8.0	Low-crystallinity PET film	Ronkvist et al., 2009
SM14est c	<i>Streptomyces</i> sp. SM14	BK010828	28 °C pH7.0	BHET	Almeida et al., 2019
Tcur0390	<i>Thermomonospora curvata</i> DSM43183	ACY95991.1	50 °C	PET nanoparticle	Wei et al., 2014
Tcur1278	<i>Thermomonospora curvata</i> DSM43183	ACY96861.1	50–60 °C	PET nanoparticle	Wei et al., 2014
TfAXE	<i>Thermobifida fusca</i> NTU22	ADM47605.1	60 °C pH7.5	BHET	Lusty Beech et al., 2022
TfCut1 TfCut2	<i>Thermobifida fusca</i> KW3	CBY05529.1 CBY05530.1	55–65 °C	Low-crystallinity PET film	Herrero Acero et al., 2011
TfH	<i>Thermobifida fusca</i> DSM43793	KR092133.1	55–70 °C pH6.0–7.0	PET (10%)	Müller et al., 2005
Tfu_0882 Tfu_0883	<i>Thermobifida fusca</i> YX	AAZ54920.1 AAZ54921.1	55–65 °C pH8.0	p-nitrophenyl butyrate	Chen et al., 2008
Tha_Cut1	<i>Thermobifida alba</i> DSM43185	ADV92525.1	50 °C	Bis(benzoyloxyethyl) TPA	Ribitsch et al., 2012
Thc_Cut1 Thc_Cut2	<i>Thermobifida cellulositytica</i> DSM44535	ADV92526.1 ADV92527.1	50 °C pH7.0	Bis(benzoyloxyethyl) TPA, PET (37%)	Herrero Acero et al., 2011
Thf42_Cut1	<i>Thermobifida fusca</i> DSM44342	ADV92528.1	50 °C	Bis(benzoyloxyethyl) TPA, PET (37%)	Herrero Acero et al., 2011
Thh_Est	<i>Thermobifida halotolerans</i> DSM44931	AFA45122.1	50 °C	Bis(benzoyloxyethyl) TPA	Ribitsch et al., 2012
Enzymes from metagenome					
LCC	<i>Leaf branch compost</i>	AEV21261.1	50–70 °C pH8.0	Amorphose PET film	Sulaiman et al., 2012
PET12	<i>Schlegelella brevitalea</i>	A0A0G3BI90	50 °C	PET nanoparticle	Danso et al., 2018
PET2	Uncultured bacterium	7ECB_A 7ECB_B 7ECB_A	70 °C pH8.0–9.0	PET nanoparticle	Danso et al., 2018
PET5	<i>Oleispira antarctica</i> RB-8	R4YKL9	50 °C	PET nanoparticle	Danso et al., 2018
PET6	<i>Vibrio gazogenes</i>	UPI0003945E1F	50 °C pH8.0–9.0	PET nanoparticle	Danso et al., 2018
Ple200 Ple201	Marine microbial consortium	MF592405 DQ294206	30 °C pH7.0	Poly butylene adipate-co-terephthalate	Meyer-Cifuentes et al., 2020

a The accession numbers were retrieved from GenBank, NCBI, and PDB.

b Crystallinity of the PET used in each study was indicated in the parenthesis.

et al., 2019; Sohn et al., 2020; Zimmermann and Billig, 2011). Each PET polymer is characterized by its crystallinity (Furukawa et al., 2019; Jog, 1995). The higher the crystallinity of the PET polymer, the more rigid the ester linkages depicted in PET (Zekriardehani et al., 2017). As PET showed the most flexibility around 70–80 °C (the glass transition temperature of PET, T_g) and the flexible structure allowed polyester hydrolases to easily access the target ester linkages (Jog, 1995; Zimmermann and Billig, 2011), hydrolases with thermostable properties are favorable for microbial PET degradation.

2.1. Cutinase hydrolyzing PET

Cutinase is one of the thermophilic hydrolytic enzymes that non-specifically cleave the ester linkages in the PET structure. Cutinase belongs to the α/β -hydrolase superfamily and has been identified as a hydrolytic enzyme for cutin, an insoluble component of plant surface layers (Austin et al., 2018; Zimmermann and Billig, 2011). By catalyzing the ester bonds that interlink monomeric compounds constructing cutin, C_{16} to C_{18} omega-hydroxyfatty acids, cutin is broken down (Gross and Mekala, 2018). Cutinases are widely present in various fungal and bacterial species. Of those, *Fusarium solani* f. sp. *pisii* produces fungal cutinase (FsC) degrading PET (Ronkvist et al., 2009), and *Thermomyces insolens* also has fungal PET hydrolytic cutinase (HiC) (Ronkvist et al., 2009). Cutinase (TfH) produced from *Thermobifida fusca* was the first known bacterial PET hydrolases (Chen et al., 2008; Müller et al., 2005), followed by other cutinases from *T. alba* (Est119) (Thumarat et al., 2012), *T. cellulosilytica* (The_cut1) (Herrero Acero et al., 2011), *Thermomonospora curvata* (Tcur1278) (Wei et al., 2014), *Thermobifida halotolerans* (Thh_est) (Ribitsch et al., 2012), *Saccharomonospora viridis* (Cut190) (Kawai et al., 2014), and leaf-branch compost microbiome (LCC) (Barth et al., 2016; Sulaiman et al., 2012). The available PET-degrading species, their corresponding hydrolases, and their active conditions are listed (Table 1).

2.2. Lipases and esterases hydrolyzing PET

Lipase and esterase are also capable of hydrolyzing PET and modifying surface functional groups. These are available from various microorganisms, including *Aspergillus oryzae* (Liu et al., 2009; Zimmermann and Billig, 2011), *Pseudomonas mendocina* (Boston et al., 1997), *Thermomyces lanuginosus* (Eberl et al., 2009; Fernandez-Lafuente, 2010), and *Candida antarctica* (Carniel et al., 2017) (Table 1). As these enzymes mainly target insoluble triglycerides and soluble esters, their PET-specific hydrolyzing activities are low compared with those of cutinases and PETases (Maurya et al., 2020). However, when one of the carboxylesterases, TfCut2 from *Thermobifida fusca* KW3, was used to degrade PET in combination with LCC, synergetic improvement in PET catalyzing efficiency was observed as the concentration of the released products from hydrolysis of amorphous PET was 2.4 times increased, which resulted from the continuous removal of intermediate products (Barth et al., 2016).

2.3. Petase and MHETase from *Ideonella sakaiensis*

In 2016, Yoshida and colleagues discovered two enzymes that specifically degraded PET films with 1.9% of crystallinity under mesophilic conditions, named IsPETase and IsMHETase, from *Ideonella sakaiensis* 201-F6 strain, a new bacterium isolated from environmental samples collected from a PET-bottle recycling site (Tanasupawat et al., 2016; Yoshida et al., 2016). Consequently, microbial PET degradation was spotlighted as a promising solution for the PET waste managements. The *I. sakaiensis* 201-F6 strain synergistically using IsPETase and IsMHETase was capable of complete degradation of the PET film in 6 weeks under mesophilic conditions (30 °C) at a rate of 0.13 mg cm⁻² day⁻¹ by utilizing PET as the sole carbon and energy source (Hachisuka et al., 2021; Yoshida et al., 2016). IsPETase showed high activity even on high-

crystallinity PET and wide active sites compared to other PET hydrolases (Chen et al., 2018; Yoshida et al., 2016). IsMHETase showed exclusive substrate specificity for MHET and did not hydrolyze PET, BHET, and other aromatic and aliphatic esters (Palm et al., 2019).

2.4. Genetic improvement by engineering PET hydrolases

Given the currently available PET hydrolases, many studies have been conducted to improve the efficiency of degradation by optimizing the rate of enzymatic production and modifying the functionality, availability, and accessibility of enzymes using genetic and protein engineering techniques. Cutinase from *T. fusca*, Tfu_0883, was genetically mutated to enhance substrate adjustment by substituting the amino acids at its active sites (Silva et al., 2011). In *S. viridis*, the active state and structural stability of cutinase, Cut190, required binding of calcium ions (Ca²⁺). Consequently, an enzymatic mutant was engineered by replacing amino acid sequences and introducing disulfide bonds imitating Ca²⁺ binding sites, which resulted in increased thermal stabilization and degradation of PET microfibers with increasing melting temperature (Oda et al., 2018). Recently, the fastest degradation rate of amorphous PET, 200 g kg⁻¹ PET hour⁻¹ at 72 °C, was reported by mutagenesis of LCC in the enzymatic active sites, which enabled 90% depolymerization of PET in 10 h (Tournier et al., 2020). Furukawa et al. applied IsPETase and cutinase from *T. fusca* (TfCut2), which were mutated to have enriched ionic regions, to PET surfaces pretreated with anionic and cationic surfactants to accelerate the hydrolysis of the low-crystalline PET films (Furukawa et al., 2018; Furukawa et al., 2019).

Because extracellularly secreted enzymes might compete by binding to active sites, which may reduce their activity, to prevent enzymatic aggregation, IsPETase was immobilized on the surface of *Escherichia coli* (*E. coli*) UT5600 to suppress the outer membrane protease and conserve surface-displayed proteins. In this manner, enzymatic production was increased, aggregation was prevented, and consequently the efficiency of BHET degradation was enhanced (Gercke et al., 2021). Because the primary materials normally used in the PET manufacturing industry are characterized by high crystallinity, in the process of PET degradation, temperature around 70 °C, T_g of PET, allowed PET hydrolases to easily access to the flexible regions of ester linkages. To overcome thermal instability, IsPETase^{S121E/D186H/R280A} variants with enhanced enzymatic activity and stable hydrolysis at 40 °C have been designed (Son et al., 2019). IsPETase variants with functional modification of substrate-binding affinity exhibited improved degradability for highly crystalline PET structures (Austin et al., 2018). In addition, various attempts have been made to improve the activity of IsPETase by mutagenesis using *E. coli* as a host strain (Liu et al., 2018; Ma et al., 2018; Singh Jadaun et al., 2022). The expression level of IsPETase mutated in *Bacillus subtilis* was increased by optimizing signal peptides and promoters in the expressional secretory gene cascade (Kim et al., 2020b; Wang et al., 2020; Xi et al., 2021). Signal peptides in *E. coli* have also been modified to improve secretion (Seo et al., 2019; Wang et al., 2020). The supply of synthetic or biological surfactants has been demonstrated to amplify PET hydrolyzing activity (Furukawa et al., 2018; Gercke et al., 2021). IsPETase engineered in photosynthetic marine microalgae, *Phaeodactylum tricoratum* and *Chlamydomonas reinhardtii*, also presented a solution for the remediation of the imperative ocean plastic contamination (Kim et al., 2020a; Moog et al., 2019). Structural modification of IsMHETase variants led to increased MHET hydrolyzing activity by altering the active site conformation to broaden substrate specificity towards BHET (Palm et al., 2019; Sagong et al., 2020). IsPETase and IsMHETase consecutively depolymerized PET to TPA and EG via MHET, and a synergistic efficiency of PET degradation was observed when a mixture of these two enzymes was applied. Given this, amorphous PET degradation efficiency by proteins modified to physically link IsPETase and IsMHETase at different proximities was determined based on TPA production (Knott et al., 2020). The released monomer concentration was more than sixfold higher with the modified proteins and was even more

than three times greater than that released by an equimolar mixture of the two enzymes.

3. Biodegradation of PET in microbiomes examined by multi-omics approaches

Studies on biological PET degradation have tended to focus on culture-based isolation of microorganisms that produced PET hydrolyzing enzymes and mutagenesis of the PET hydrolases to enhance enzymatic activity, thermal stability, and surface affinity. Barth et al. (2016) further demonstrated that PET degradation efficiency could be boosted by the combination of multiple PET hydrolases from different microorganisms, which implies that the application of co-cultures with a cocktail of microbes synergistically working on PET depolymerization and even well-designed microbial consortia might produce significant improvements (Skariyachan et al., 2022). In addition, the recent developments in multi-omics (e.g., metagenomics, metatranscriptomics, proteogenomics, and metabolomics), together with bioinformatics, have accelerated the investigation of cooperative PET degradation at the uncultured microbial community level, diversified potential PET metabolic pathways and mechanisms, and enabled the exploration of extremophilic microbiomes (Fig. 2) (Bhatt et al., 2021; Skariyachan et al., 2022). Like *I. sakaiensis* isolated from an environmental microbiome collected from a PET bottle recycling site (Yoshida et al., 2016), diverse marine and terrestrial plastispheres have been comprised of plastic-biodegrading microorganisms that resulted from selective enrichment, going through multifarious stages of community succession in a long term (Datta et al., 2016; Kirstein et al., 2019; Oberbeckmann et al., 2018). Considering that PET, BHET, and MHET hydrolases are secreted exoenzymes and hydrolysis of PET to unit monomers occurs extracellularly (Taniguchi et al., 2019), interactions among the commensal microbes in the biofilm plastisphere communities might affect PET degradation, such as the metabolisms of intermediates during PET degradation, although little information is available.

3.1. Discovery of PET hydrolyzing microorganisms in plastisphere community

Marine plastisphere community compositions that were post-enriched with PET and its derivatives for six weeks have been investigated (Wright et al., 2021). In their study, abundant potential enzymes involved in PET degradation were analyzed using metagenomics, which supported the physical oxidation of PET polymers detected by Fourier transform infrared (FTIR) spectroscopy. Furthermore, among the two strains isolated from the microbiomes, *Bacillus* sp. was found, using proteogenomics and metabolomics, to use a novel PET metabolic pathway with an unprecedented PET hydrolase. Abundance analysis of the isolates against publicly available Tara Ocean datasets (Logares et al., 2014; Sunagawa et al., 2015; Tully et al., 2018) confirmed their global presence in marine environments. This type of interactive research using cultivation, isolation, enzymatic verification, and metagenomics have also been conducted in several studies targeting various environmental samples, including marine plastispheres (Gao and Sun, 2021; Meyer-Cifuentes et al., 2020a), petroleum-contaminated sites (Roberts et al., 2020), and landfill soil (Kumar et al., 2021).

3.2. PET hydrolases identified by public metagenome data mining

Public data mining has emerged as a tool for identifying novel PETases. An artificially engineered PET hydrolase, named FAST-PETase, was designed by analyzing tens of thousands protein structures from the Worldwide Protein Data Bank (PDB) (Berman et al., 2003) using Mut-Compute (<https://mutcompute.com>), a deep learning guided prediction algorithm using Convolutional Neural Network (Lu et al., 2022). FAST-PETase, showing superior activity at a broad range of temperatures and pH levels, was verified for its ability to be directly applicable to the treatment of post-consumer PET with relatively high crystallinity. A workflow of intensive database search using a Hidden Markov Model (HMM) followed by an expression and functional activity test was established to identify novel PET hydrolases (Danso et al., 2019; Danso et al., 2018). The HMM search algorithm was constructed based on the

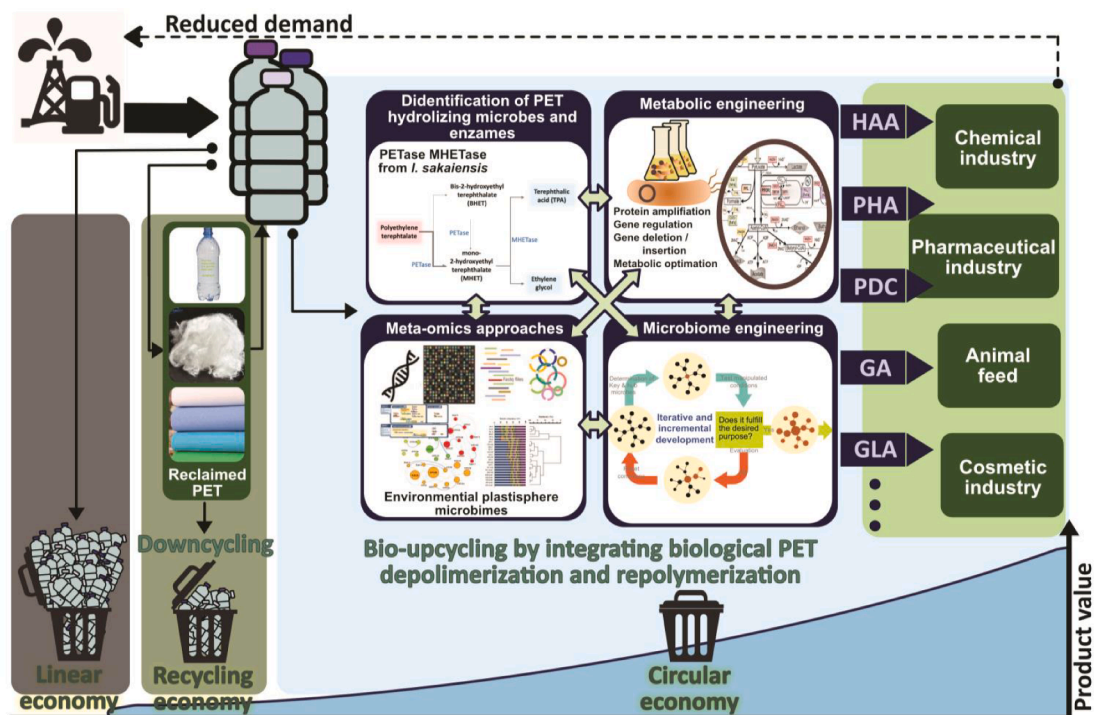


Fig. 2. Advancement of PET bio-upcycling technologies in the circular economy. HAA, hydroxyalkanoyloxy-alkanoate; PHA, polyhydroxyalkanoate; PDC, 2-pyrone-4,6-dicarboxylic acid; GA, gallic acid; and GLA, glycolic acid.

amino acid sequences of nine representative IsPETase and cutinases and used to detect more than 500 PET hydrolases from the UniProtKB database (Consortium, 2020), the NCBI database (Sayers et al., 2022), and diverse environmental metagenomes retrieved from the Integrated Microbial Genome (IMG) server (Chen et al., 2020). The viability of the HMM algorithm for PET hydrolase search was confirmed in the following expression and functionality tests of the candidate enzymes selected. This work has spurred the discovery of an abundant pool of potential PET hydrolases. Using the HMM search algorithm (Danso et al., 2018), an esterase from *Chryseobacterium jeonii* (PET30) was identified to be capable of catalyzing PET over a broad temperature range from 4 °C to 30 °C with a degradation rate similar to that of IsPETase (Zhang et al., 2021a). A further metagenomic search of the esterase indicated that the enzyme was universally distributed in marine environments at various latitudes. Another database allowing searching for new PET hydrolases is the Plastic Microbial Biodegradation Database (Gan and Zhang, 2019). More than two hundreds genes involved in plastic degradation were identified, of which the activity of PET-degrading genes in contaminated site consortia was confirmed using RNAseq differential expression (Edwards et al., 2022).

3.3. Microbiome engineering for PET degradation

Along with emerging meta-omics approaches, various attempts to harness naturally evolved microbiomes have been made to suggest solutions for urgent environmental challenges, including the degradation of recalcitrant contaminants, climate management, and soil remediation. However, the applications of microbiomes might rarely result in what the researchers expect. To address this limitation, microbiome engineering is emerging; the functions of harnessed microbiomes are improved by integrating newly introduced and/or augmented microbes depending on the desired purposes (Lawson et al., 2019). As one example of simplified microbiome engineering, an artificial consortium composed of four selected species was designed to accelerate the degradation of an amorphous PET film: two *Bacillus subtilis* 168 mutated to produce PETase and MHETase, respectively, *Rhodococcus jostii* for utilization of TPA, and *Pseudomonas putida* for uptake of EG (Qi et al., 2021). Among the various combinations of the consortia, the combination of *R. jostii* and *P. putida* showed the most efficient PET degradation by preventing intermediate inhibition effects. In the study of Gao and Sun (2021), three key PET degrading microorganisms, *Exiguobacterium* sp., *Halomonas* sp., and *Ochrobactrum* sp., were isolated from a marine consortium (CAS6), which was selected among various environmental plastisphere samples for its distinct PET degradation. In a following PET degradation test using co-cultures of an artificial microbial community consisting of those three isolates, significantly deconstructed PET films were observed in 2 weeks through FTIR analysis (Gao and Sun, 2021). Additionally, a differential expression analysis based on macro transcriptome of the artificial microbiome indicated common upregulations in putative lipases, esterases, cutinases and hydrolases, which was an indirect evidence of PET hydrolysis by the community. Together with a deep understanding of PET plastisphere community assemblages (Dey et al., 2022), microbiome engineering can provide solutions derived from the functionality of the entire microbial ecology and suggest additional areas of research.

4. Recent upcycling biotechnologies and valorization of PET

The fundamental goals of upcycling are environmental protection by minimizing end-of-life waste and recovering resources by converting waste into value-added products. In PET bio-upcycling, the goals, like a bowtie structure, are achieved by integrating the depolymerization of PET to intermediary platform chemicals, that is, TPA and EG as monomers and BHET and MHET as oligomers, and repolymerization of the oligomers and monomers to higher value-added materials than currently recycled PET (Wei et al., 2020). As the enzymatic and microbial

depolymerization of PET has thus far been reviewed, the development of biotechnology in repolymerization, the other side of the bowtie, must be critically reviewed. Although enzymatically and biologically reclaimed TPA and EG from PET can be funneled into chemical repolymerization (Rorrer et al., 2019; Vollmer et al., 2020), their bioconversion is discussed in the following section (Fig. 2).

4.1. EG utilization in synthesis of value-added products

In bio-upcycling of PET, since TPA and EG are the most commonly used platform chemicals, identification of microorganisms and metabolic pathways utilizing these chemicals is the most crucial first step. Many *Pseudomonas* species are equipped with C2 metabolic pathways that utilize EG via glycolaldehyde and glycolate, which are toxic compounds. *P. putida* KT2440 was engineered to produce medium-chain-length PHAs (mcl-PHA, C₆–C₁₄) (Li et al., 2016b), by overexpressing the oxidase operon (glcDEF) to prevent accumulation of a toxic glycolaldehyde and suppressing the PHA depolymerase and genes involved in β -oxidation, which led to an increase in overall PHA production (Frandsen et al., 2018; Rodriguez-Contreras, 2019). PHAs, biodegradable polyesters stored in microorganisms, were utilized to produce biomedical devices, pharmaceutical products, and biodegradable plastics (Kenny et al., 2008). *Gluconobacter oxydans* KCCM 40,109 produced glycolate, which was widely used as an ingredient in cosmetic exfoliants (Kim et al., 2019; Kraeling and Bronaugh, 1997; Wei et al., 2009). Engineered *P. putida* also converted EG into glycolate (Mückschel et al., 2012). Engineered *Klebsiella pneumoniae* channeled EG into 3-hydroxypropionic acid via glycerol, the products of which were used in the synthesis of acrylic acid to produce adhesives and elastomers (Matsakas et al., 2018). This application may reduce costs by half compared to petroleum-based production (Jung et al., 2014).

4.2. TPA utilization in synthesis of value-added products

The most widely used reclaimed platform chemical from the PET depolymerization is TPA in bio-upcycling. Whereas EG can diffuse into cellular membranes, microorganisms should be equipped with a transport protein family (TphABC) to bring and metabolize TPA intracellularly (Hara et al., 2007; Maruyama et al., 2004; Yoshida et al., 2016). PCA is a precursor chemical funneling into the synthesis of diverse value-added products; PCA was converted from the transported TPA via DCD by a Tph protein family (TphAabc and TphB) (Kim et al., 2019; Mückschel et al., 2012; Nomura et al., 1992). Since only a few genera, that is, *Comamonas* (Fukuhara et al., 2008; Sasoh et al., 2006), *Delftia* (Shigematsu et al., 2003), *Ideonella* (Yoshida et al., 2016), *Pseudomonas* (Kenny et al., 2008), and *Rhodococcus* (Choi et al., 2005), encode the genes that express the Tph protein family to produce PCA from TPA, species in these genera or engineered microorganisms were often employed to express the Tph enzymes (Kim et al., 2019; Tiso et al., 2021; Wei et al., 2020). Engineered *E. coli* hosts with the TphAabc and TphB from *Comamonas* sp. E6 were used to convert PCA into various molecules, such as pyrogallol, gallic acid, muconic acid, catechol, and vanillic acid (Kim et al., 2019). The microbial conversion of TPA generated by pyrolysis into PHA using various strains of *P. putida* (sp. GO16, GO19, and GO23) has been conducted by many research groups (Goff et al., 2007; Guzik et al., 2014; Kenny et al., 2008; Kenny et al., 2012), offering great promise for PET bio-upcycling when combined with enzymatic and microbial PET hydrolysis. Extracellular conversion of TPA and EG to HAA using an engineered *P. putida* sp. GO16 was successfully demonstrated, which may ease the product recovery process (Tiso et al., 2021; Wei et al., 2020). An improved kinetic model for the β -ketoacid pathway in *P. putida* KT2440 was developed by repressing a transporter protein for β -ketoacid (pcaT) (Sudarsan et al., 2016). Co-cultures of two engineered *E. coli* variants, one with the Tph gene family from *Comamonas* sp. E6 and the other with ligABC genes from *Sphingobium* sp. SYK-6, were used to convert TPA chemo-physically hydrolyzed from

PET waste to 2-pyrone-4,6-dicarboxylic acid via 4-carboxy-2-hydroxy-muconate semialdehyde with high efficiency (Kang et al., 2020). A complete bio-upcycling of PET depolymerization and polyhydroxybutyrate (PHB) biosynthesis was demonstrated in co-cultures of *Yarrowia lipolytica* Po1f, a yeast strain engineered with PETase from *Idenella sakaiensis*, and *Pseudomonas stutzeri* TPA3P engineered with a plasmid containing phbCAB operons from *Ralstonia eutropha* for PHB production (Liu et al., 2021). *Y. lipolytica* Po1f hydrolyzed BHET and PET and produced TPA and EG using the PETase engineered to be efficiently secreted into extracellular media by a signaling peptide from lipase. Subsequently, the TPA was metabolized by *P. stutzeri* TPA3P using Tph protein family and converted into PHB using the engineered phbCAB. This demonstration suggests that artificial model consortia with functionally designed strains may realize theoretical completion of biological recycling of PET wastes with further research on microbiome engineering (Christova et al., 2004; Wierckx et al., 2015), may lead to the fundamental goal of achieving in a circular economy. Lastly, upcycling of BHET, the oligomer form derivatives from PET degradation, was undertaken to produce biologically degradable fiber-reinforced plastics, blended with muconic acid and acrylic acid (Rorrer et al., 2019).

5. Future research prospects

Advancements in bio-upcycling technologies for alternative PET waste management exhibit great promise for the development of eco-friendly approaches. Compared to the current mechanical and chemical PET recycling, microbial recycling of PET can reduce CO₂ emissions derived from PET waste treatments and achieve 30–40% reduction in CO₂ emissions from landfills and incineration (Blank et al., 2020; Rorrer et al., 2019; Vollmer et al., 2020).

Considering the increasing demand for TPA production annually (Lee et al., 2021), the depolymerization of PET to reclaim TPA could partially meet the demand and further reduce petroleum consumption to produce virgin TPA. Furthermore, compared to conventional mechanical and chemical processes, a biocatalytic treatment of waste PET using microbial hydrolases depolymerized PET under milder reaction conditions (Barth et al., 2016) and also offered possible treatment of complex mixtures of PET with additives, requiring less feedstock specificity (Singh et al., 2021). Modeling life cycle impacts for TPA production by enzymatic depolymerization of waste PET compared to petroleum-based production assessed that, in spite of improvement required in various aspects for a feasible application, the enzymatic method gave benefits in energy requirements and greenhouse gas emission from supply chain processes (Uekert et al., 2022).

Much effort has been made to improve the microbial depolymerization of PET by increasing hydrolysis efficiency, enzymatic activity, thermal and pH level stability, and kinetics. However, considerable room for improvement remains in the identification of new microorganisms capable of PET degradation with diverse pathways, expansion of the hydrolase database, and simultaneous characterization of these entities, together with genetic engineering. Meanwhile, it is necessary to develop computational and machine learning algorithms by iteratively searching metagenomes of environmental plastispheres, reflecting the expanded PET hydrolase database, and characterizing the newly introduced hydrolases by analyzing protein structures.

The circular PET economy aims to prolong the retention of value and utility of products in the economic material cycle. Since the integration of biosynthesis of constituent monomers reclaimed from PET depolymerization completes bio-upcycling of PET, improvement of the biosynthetic technology and intensive metabolic engineering are required to produce diverse materials with long retention times and high value, beyond current capacities. Diversifying the biosynthesis of value-added products, not limited to bio-based PET production, is necessary for a sustainable circular PET economy. Finally, systematic augmentation to develop artificial model consortia should be undertaken using the iterative design–build–test–learn cycle (Lawson et al., 2019), which will

bring PET bio-upcycling technologies closer to practical application.

6. Conclusions

Bio-upcycling of PET is based on integrated technologies of microbial depolymerization and the subsequent biosynthesis of reclaimed monomers to value-added products, which presents alternative plastic waste management and allows achieving a circular economy. Microbes capable of hydrolyzing PET and novel PET hydrolases have been intensively investigated, together with their functional improvement using genetic engineering to achieve efficient PET hydrolysis. Identification of novel microorganisms and PET hydrolases can be expedited using meta-omics approaches and computational biology. Scrutinizing metabolic pathways and metabolic engineering for biosynthesis of PET-derived monomers to value-added products enables establishment of a circular economy.

Funding

This work was supported by the National Research Foundation of Korea (NRF-2021R1A2C2007319) and a Korea University Grant.

CRediT authorship contribution statement

Na-Kyung Kim: Conceptualization, Resources, Formal analysis, Visualization, Data curation, Writing – original draft, Writing – review & editing. **Sang-Hoon Lee:** Resources, Data curation, Formal analysis, Writing – original draft. **Hee-Deung Park:** Conceptualization, Resources, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- Achillas, D.S., Tsintzou, G.P., Nikolaidis, A.K., Bikiaris, D.N., Karayannidis, G.P., 2011. Aminolytic depolymerization of poly (ethylene terephthalate) waste in a microwave reactor. *Polym. Int.* 60 (3), 500–506.
- Aguilar-Guzmán, J.C., Bejtka, K., Fontana, M., Valsami-Jones, E., Villezas, A.M., Vazquez-Duhalt, R., Rodríguez-Hernández, A.G., 2022. Polyethylene terephthalate nanoparticles effect on RAW 264.7 macrophage cells. *Microplast. Nanoplast.* 2 (1), 9.
- Almeida, E.L., Carrillo Rincón, A.F., Jackson, S.A., Dobson, A.D.J.F.i.m., 2019. In silico Screening and heterologous expression of a polyethylene terephthalate hydrolase (PETase)-Like Enzyme (SM14est) With Polycaprolactone (PCL)-degrading activity, from the marine sponge-derived strain *Streptomyces* sp. SM14 10, 2187.
- Amobonye, A., Bhagwat, P., Raveendran, S., Singh, S., Pillai, S., 2021. environmental impacts of microplastics and nanoplastics: A current overview. *Front. Microbiol.* 12.
- Austin, H.P., Allen, M.D., Donohoe, B.S., Rorrer, N.A., Kearns, F.L., Silveira, R.L., Pollard, B.C., Dominick, G., Duman, R., El Omari, K., 2018. Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proc. Natl. Acad. Sci.* 115 (19), E4350–E4357.
- Barth, M., Honak, A., Oeser, T., Wei, R., Belisário-Ferrari, M.R., Then, J., Schmidt, J., Zimmermann, W., 2016. A dual enzyme system composed of a polyester hydrolase and a carboxylesterase enhances the biocatalytic degradation of polyethylene terephthalate films. *Biotechnol. J.* 11 (8), 1082–1087.
- Beaucamp, A., Wang, Y., Culebras, M., Collins, M.N., 2019. Carbon fibres from renewable resources: the role of the lignin molecular structure in its blendability with biobased poly (ethylene terephthalate). *Green Chem.* 21 (18), 5063–5072.
- Berman, H., Henrick, K., Nakamura, H., 2003. Announcing the worldwide protein data bank. *Nat. Struct. Mol. Biol.* 10 (12), 980.
- Bhatt, P., Zhou, X., Huang, Y., Zhang, W., Chen, S., 2021. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. *J. Hazard. Mater.* 411, 125026.

- Tanasupawat, S., Takehana, T., Yoshida, S., Hiraga, K., Oda, K., 2016. *Ideonella sakaiensis* sp. nov., isolated from a microbial consortium that degrades poly (ethylene terephthalate). *Int. J. Syst. Evol. Microbiol.* 66 (8), 2813–2818.
- Taniguchi, I., Yoshida, S., Hiraga, K., Miyamoto, K., Kimura, Y., Oda, K., 2019. Biodegradation of PET: current status and application aspects. *ACS Catal.* 9 (5), 4089–4105.
- Thumarat, U., Nakamura, R., Kawabata, T., Suzuki, H., Kawai, F., 2012. Biochemical and genetic analysis of a cutinase-type polyesterase from a thermophilic *Thermobifida alba* AHK119. *Appl. Microbiol. Biotechnol.* 95 (2), 419–430.
- Tiseo, I. 2021. Global plastic production 1950-2020, Statista.
- Tiso, T., Narancic, T., Wei, R., Pollet, E., Beagan, N., Schröder, K., Honak, A., Jiang, M., Kenny, S.T., Wierckx, N., 2021. Towards bio-upcycling of polyethylene terephthalate. *Metab. Eng.* 66, 167–178.
- Tournier, V., Topham, C.M., Gilles, A., David, B., Folgoas, C., Moya-Leclair, E., Kamionka, E., Desrousseaux, M.L., Texier, H., Gavalda, S., Cot, M., Guémard, E., Dalibey, M., Nomme, J., Cioci, G., Barbe, S., Chateau, M., André, I., Duquesne, S., Marty, A., 2020. An engineered PET depolymerase to break down and recycle plastic bottles. *Nature* 580 (7802), 216–219.
- Tully, B.J., Graham, E.D., Heidelberg, J.F., 2018. The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. *Sci. Data* 5 (1), 1–8.
- Uchida, H., Shigeno-Akutsu, Y., Nomura, N., Nakahara, T., Nakajima-Kambe, T., 2002. Cloning and sequence analysis of poly (tetramethylene succinate) depolymerase from *Acidovorax delafieldii* strain BS-3. *J. Biosci. Bioeng.* 93 (2), 245–247.
- Uekert, T., DesVeaux, J.S., Singh, A., Nicholson, S.R., Lamers, P., Ghosh, T., McGeehan, J.E., Carpenter, A.C., Beckham, G.T., 2022. Life cycle assessment of enzymatic poly(ethylene terephthalate) recycling. *Green Chem.* 24 (17), 6531–6543.
- Vague, M., Chan, G., Roberts, C., Swartz, N.A., Mellies, J.L., 2019. *Pseudomonas* isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic. [bioRxiv](https://doi.org/10.1101/2019.06.11.247321), 647321.
- Vollmer, I., Jenks, M. 2020. JF, Roelands, M. CP, White, RJ, Harmelen, T. van, de Wild, P., van der Laan, GP, Meirer, F., Keurentjes, J. TF & Weckhuysen, BM Beyond mechanical recycling: giving new life to plastic waste. *Angew. Chem. Int. Ed.* 59, 15402-15423.
- Vollmer, I., Jenks, M.J.F., Roelands, M.C.P., White, R.J., van Harmelen, T., de Wild, P., van der Laan, G.P., Meirer, F., Keurentjes, J.T.F., Weckhuysen, B.M., 2020. Beyond mechanical recycling: giving new life to plastic waste. *Angew. Chem. Int. Ed.* 59 (36), 15402–15423.
- Wang, N., Guan, F., Lv, X., Han, D., Zhang, Y., Wu, N., Xia, X., Tian, J., 2020. Enhancing secretion of polyethylene terephthalate hydrolase PETase in *Bacillus subtilis* WB600 mediated by the SPamy signal peptide. *Lett. Appl. Microbiol.* 71 (3), 235–241.
- Wang, J., Liu, X., Li, Y., Powell, T., Wang, X., Wang, G., Zhang, P., 2019. Microplastics as contaminants in the soil environment: A mini-review. *Sci. Total Environ.* 691, 848–857.
- Wang, X., Lu, D., Jönsson, L., Hong, F., 2008. Preparation of a PET-hydrolyzing lipase from *Aspergillus oryzae* by the addition of bis (2-hydroxyethyl) terephthalate to the culture medium and enzymatic modification of PET fabrics. *Eng. Life Sci.* 8 (3), 268–276.
- Wei, R., Oeser, T., Zimmermann, W., 2014. Chapter seven - synthetic polyester-hydrolyzing enzymes from thermophilic actinomycetes. In: Sariaslani, S., Gadd, G.M. (Eds.), *Advances in Applied Microbiology*, vol. 89. Academic Press, pp. 267–305.
- Wei, Y., Swenson, L., Castro, C., Derewenda, U., Minor, W., Arai, H., Aoki, J., Inoue, K., Servin-Gonzalez, L., Derewenda, Z.S., 1998. Structure of a microbial homologue of mammalian platelet-activating factor acetylhydrolases: *Streptomyces exfoliatus* lipase at 1.9 Å resolution. *Structure* 6 (4), 511–519.
- Wei, R., Tiso, T., Bertling, J., O'Connor, K., Blank, L.M., Bornscheuer, U.T., 2020. Possibilities and limitations of biotechnological plastic degradation and recycling. *Nat. Catal.* 3 (11), 867–871.
- Wei, G., Yang, X., Gan, T., Zhou, W., Lin, J., Wei, D., 2009. High cell density fermentation of *Gluconobacter oxydans* DSM 2003 for glycolic acid production. *J. Ind. Microbiol. Biotechnol.* 36 (8), 1029–1034.
- Wierckx, N., Prieto, M.A., Pomposiello, P., de Lorenzo, V., O'Connor, K., Blank, L.M., 2015. Plastic waste as a novel substrate for industrial biotechnology. *Microb. Biotechnol.* 8 (6), 900–903.
- Wright, R.J., Bosch, R., Langille, M.G.I., Gibson, M.I., Christie-Oleza, J.A., 2021. A multi-OMIC characterisation of biodegradation and microbial community succession within the PET plastisphere. *Microbiome* 9 (1), 1–22.
- Xi, X., Ni, K., Hao, H., Shang, Y., Zhao, B., Qian, Z., 2021. Secretory expression in *Bacillus subtilis* and biochemical characterization of a highly thermostable polyethylene terephthalate hydrolase from bacterium HR29. *Enzyme Microb. Technol.* 143, 109715.
- Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K., 2016. A bacterium that degrades and assimilates poly (ethylene terephthalate). *Science* 351 (6278), 1196–1199.
- Zekriardehani, S., Jabarin, S., Gidley, D., Coleman, M., 2017. Effect of chain dynamics, crystallinity, and free volume on the barrier properties of poly (ethylene terephthalate) biaxially oriented films. *Macromolecules* 50 (7), 2845–2855.
- Zhang, H., Dierkes, R., Pérez-García, P., Weigert, S., Sternagel, S., Hallam, S., Schott, T., Juergens, K., Vollstedt, C., Chibani, C. 2021a. The abundance of mRNA transcripts of bacteroidetal polyethylene terephthalate (PET) esterase genes may indicate a role in marine plastic degradation.
- Zhang, H., Perez-Garcia, P., Dierkes, R.F., Applegate, V., Schumacher, J., Chibani, C.M., Sternagel, S., Preuss, L., Weigert, S., Schmeisser, C., Danso, D., Pleiss, J., Almeida, A., Höcker, B., Hallam, S.J., Schmitz, R.A., Smits, S.H.J., Chow, J., Streit, W.R., 2021. The Bacteroidetes *Aequorivita* sp. and *Kaistella jeonii* Produce Promiscuous Esterases With PET-Hydrolyzing Activity. *Front. Microbiol.* 12, 803896.
- Zimmermann, W., Billig, S., 2011. Enzymes for the Biofunctionalization of Poly(Ethylene Terephthalate). In: Nyanhongo, G.S., Steiner, W., Gübitz, G. (Eds.), *Biofunctionalization of Polymers and Their Applications*. Springer, Berlin Heidelberg. Berlin, Heidelberg, pp. 97–120.