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Biobased *de novo* synthesis, upcycling, and recycling — the heartbeat toward a green and sustainable polyethylene terephthalate industry☆

Fabia Weiland, Michael Kohlstedt and Christoph Wittmann

Polyethylene terephthalate (PET) has revolutionized the industrial sector because of its versatility, with its predominant uses in the textiles and packaging materials industries. Despite the various advantages of this polymer, its synthesis is, unfavorably, tightly intertwined with nonrenewable fossil resources. Additionally, given its widespread use, accumulating PET waste poses a significant environmental challenge. As a result, current research in the areas of biological recycling, upcycling, and *de novo* synthesis is intensifying. Biological recycling involves the use of micro-organisms or enzymes to breakdown PET into monomers, offering a sustainable alternative to traditional recycling. Upcycling transforms PET waste into value-added products, expanding its potential application range and promoting a circular economy. Moreover, studies of cascading biological and chemical processes driven by microbial cell factories have explored generating PET using renewable, biobased feedstocks such as lignin. These avenues of research promise to mitigate the environmental footprint of PET, underlining the importance of sustainable innovations in the industry.

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Introduction

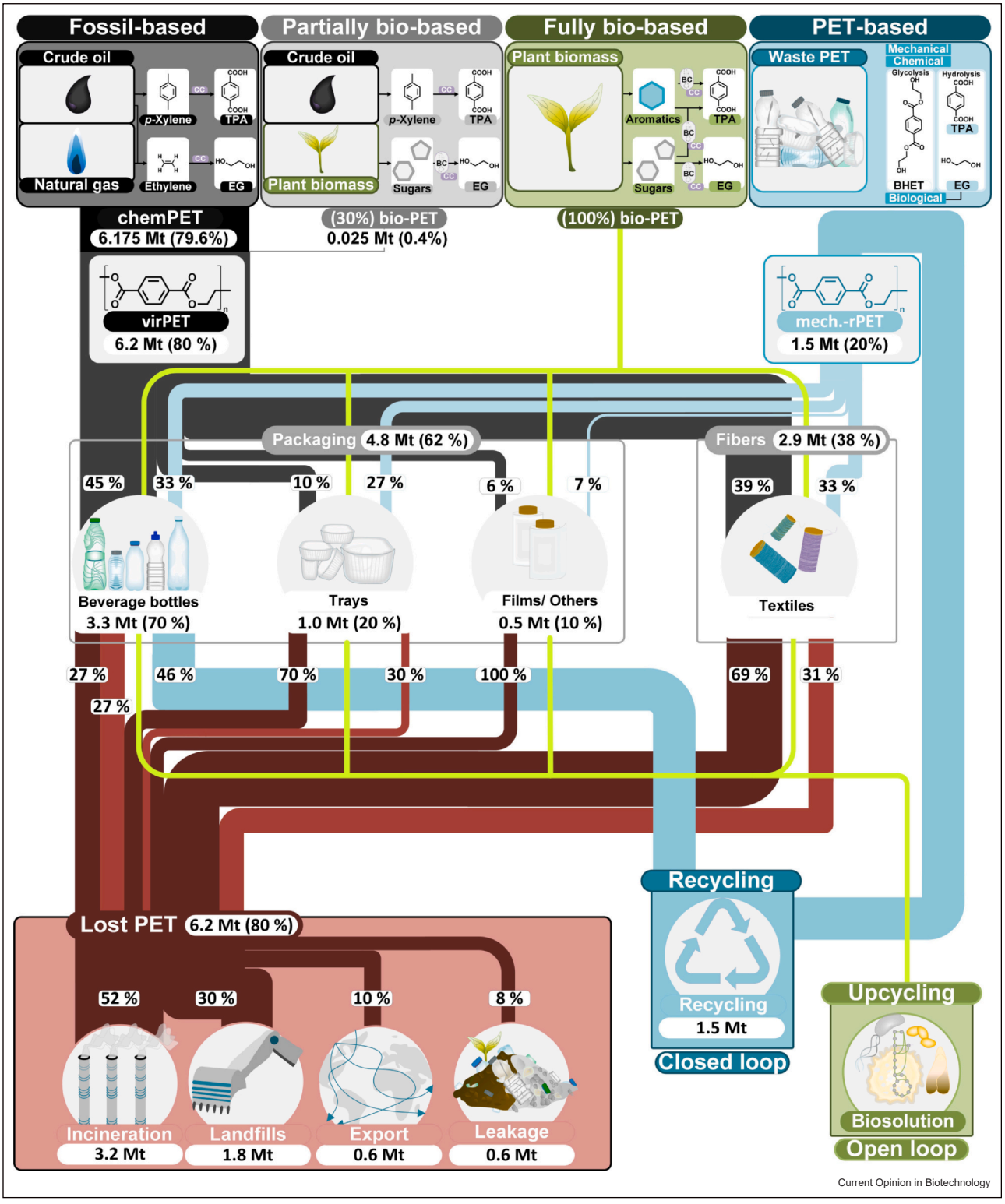
The synthetic polymer polyethylene terephthalate (PET) has a variety of uses in fibers, coatings, and packaging materials and thus plays a major role in our daily lives [1] (Figure 1). Considering its several advantageous properties [2], it is no surprise that the market volume for PET has burgeoned over the years, whereby current global production hovers near approximately 50 million metric tons annually [3].

The process of generating PET begins with two primary raw materials, purified (dimethyl)terephthalic acid (TPA) and ethylene glycol (EG), both of which are derived mainly via petrochemical routes (Figure 1) and undergo polycondensation [4,5]. TPA production starts with petroleum-derived *p*-xylene, which, in the most commonly used American Oil Company (AMOCO) process, undergoes liquid-phase oxidation with molecular oxygen as the oxidant, acetic acid as the solvent, and a catalytic system consisting of cobalt, manganese, and bromide ions 4,5. EG is primarily obtained from ethylene via catalytic oxidation into ethylene oxide and subsequent hydrolysis [4]. Notably, conventional ethylene synthesis by steam cracking naphtha is not only energy intensive but also strongly contributes to global carbon emissions [6], ultimately influencing the environmental footprint of PET production.

However, both petrochemical-based PET-monomer synthesis [5,6] and the fate of postconsumer plastics (Figure 1) are reasons for the manifold environmental concerns [7,8]. An especially unfortunate side effect of the ubiquity of PET is its release into the environment, and the pervasive nature of certain plastics is known to be an urgent, enduring threat to terrestrial and marine ecosystems, as well as human society [8,9]. Additionally, even though the basic framework is ideal from a chemical viewpoint, recycled PET is often considered disadvantageous in terms of production costs, product quality, and energy consumption, limiting its re-entry into a new life cycle [1,2,7,10]. Thus, even in Europe,

☆ Dedicated to Judith Becker (* 2.2. 1981, † 27.4.2021), a gifted metabolic engineer of sustainable cell factories and our cherished colleague and friend at the Institute of Systems Biotechnology of Saarland University.

Figure 1


Fossil-based
Partially bio-based
Fully bio-based
PET-based
Crude oil
Natural gas
p-Xylene
TPA
Ethylene
EG
Plant biomass
Sugars
Aromatics
Waste PET
Mechanical
Chemical
Glycolysis
Hydrolysis
BHET
Biological
chemPET
6.175 Mt (79.6%)
(30%) bio-PET
0.025 Mt (0.4%)
(100%) bio-PET
virPET
6.2 Mt (80 %)
mech.-rPET
1.5 Mt (20%)
Packaging 4.8 Mt (62 %)
Fibers 2.9 Mt (38 %)
45 %
33 %
10 %
27 %
6 %
7 %
39 %
33 %
Beverage bottles
3.3 Mt (70 %)
Trays
1.0 Mt (20 %)
Films/ Others
0.5 Mt (10 %)
Textiles
27 %
46 %
70 %
30 %
100 %
69 %
31 %
27 %
Recycling
Recycling
1.5 Mt
Closed loop
Lost PET 6.2 Mt (80 %)
52 %
30 %
10 %
8 %
Incineration
3.2 Mt
Landfills
1.8 Mt
Export
0.6 Mt
Leakage
0.6 Mt
Upcycling
Biosolution
Open loop
Current Opinion in Biotechnology

Sourcing, application, and fate of European PET in numbers. The depicted data are taken from Ref. [11]. Monomers for PET synthesis can be derived from different sources, whereby the most common routes for TPA and EG production rely on fossil resources [4,84]. Synthetic routes for the green production of PET precursors largely rely on separated or integrated chemical catalysis (CC) and biocatalysis (BC) [84]. The available bio-PET contains 30% biomass (EG fraction) [4], whereby its given fraction of the available virgin PET takes into account the fact that 26.5% of bioplastics are produced in Europe and that the share of PET from the total global production of 2.22 million tons of plastics is 4.2% [85]. To date, the major fraction of PET has been lost (red), with light red bars indicating the percentage of PET that enters a sortation process but is not part of a further life cycle 11. Regarding PET recycling (blue), novel developments [7] target an enhanced life cycle from waste PET to recycled PET with virgin-like characteristics (closed loop) [14]. Recent scientific advances in biobased solutions (green) for PET production and the upcycling of PET into various novel products (open loop [14]) could contribute to a sustainable PET industry.

which is generally known for high collection rates [2], the actual PET recycling rate is quite low, as only one-fifth of the available material enters a new life cycle, whereas the rest is ultimately lost without further usage [11] (Figure 1).

In terms of the increase in eco-consciousness worldwide, the magnitude of the PET waste problem underscores the urgent need for sustainable consumption practices, improved recycling technologies, and policies that promote a circular economy for plastics [1,12–15]. In this review, we highlight recent studies demonstrating how biotechnologically engineered cell factories can contribute not only to reviving postconsumer PET waste streams but also to the *de novo* synthesis of PET from green, alternative feedstocks. Based on these impressive achievements, green routes have the potential to drive the entire PET industry for the first time, but further advancements in the future are still needed.

Concepts and strategies for creating a biobased circular polyethylene terephthalate industry

The biobased valorization of postconsumer PET has a comparable start to that of other biotechnological feedstocks [16,17], with depolymerization [10] (Figure 2). Most of the available depolymerization methods are under constant development and result in specific product spectra [7]. Current studies rely either on separate chemical [18] or biobased [19] strategies or a combinatorial (tandem) approach [20] to transform PET polymers into suitable substrates for micro-organisms. However, micro-organisms have not had enough time to adapt to the environmental presence of man-made PET [19], and identifying microorganisms suitable as hosts or gene donors is essential for biobased PET valorization [21,22]. Therefore, the efficient coupling of (1) PET degradation strategies, (2) TPA and EG assimilation, and (3) production often requires other key biotechnological technologies, for example, adaptive laboratory evolution, in addition to genetic engineering [23] (Figure 2). Initiated by pioneering works [21], contemporary contributions (Table 1) have also demonstrated the potential of metabolically engineered cell factories for the open loop recycling or even upcycling of PET (monomers), hence allowing its conversion into new (and often value-added) products [14] (Figure 1). Similarly, the metabolic layout of genetically engineered microbes may also be used for the production of PET precursors from alternative, renewable feedstocks, for example, lignin-based aromatics [24] and (hemi)cellulose-based sugars [25–27], which are chemically converted into biobased PET, thereby closing the circle (Figure 2).

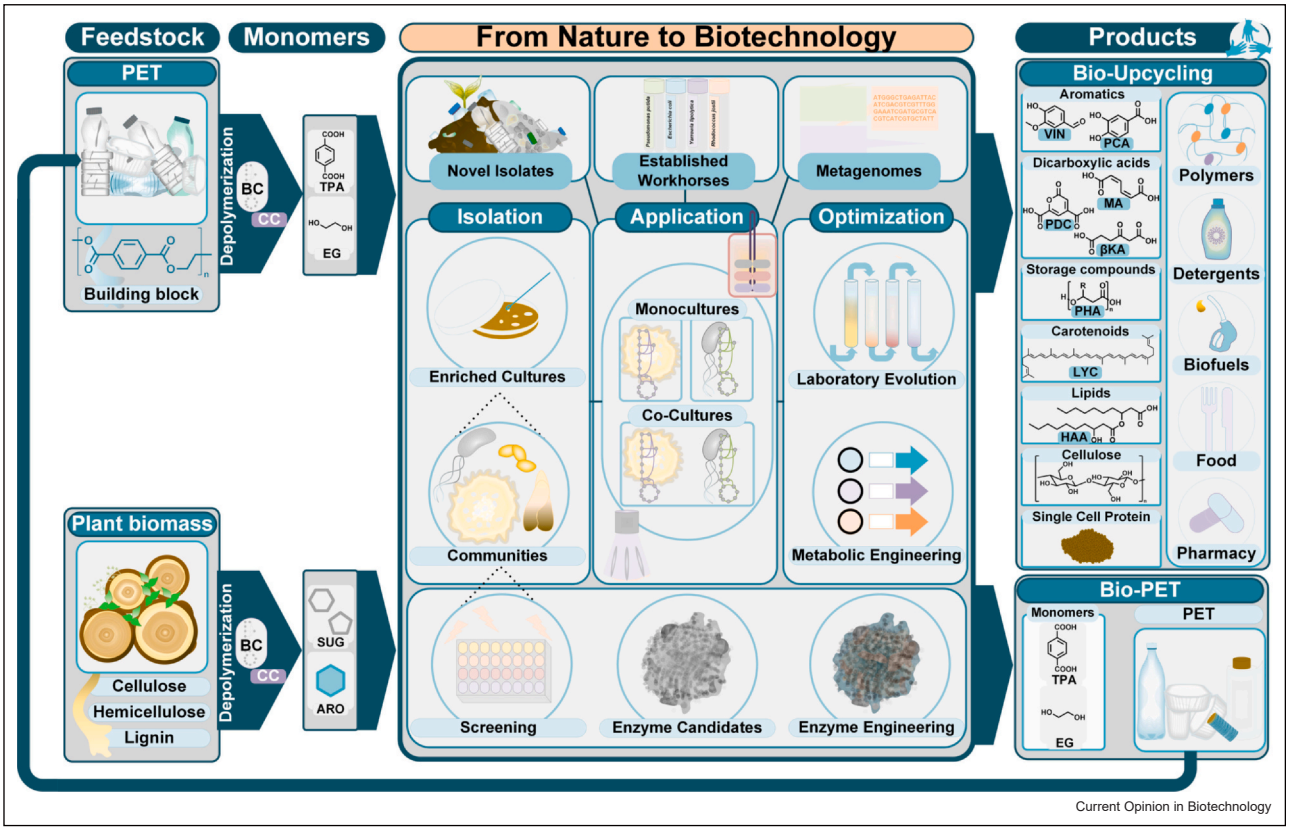
Microbes and hosts relevant to polyethylene terephthalate recycling and upcycling

PET was long considered nonbiodegradable [7]. However, since the initial discovery of PET depolymerization via the hydrolase TfH from the actinomycete *Thermobifida fusca* in 2005 [28], several enzymes with PET-degrading capabilities have been identified (Table 2) [29–31]. Additionally, the isolation of *Ideonella sakaiensis* 201-F6 from a PET-degrading microbial consortium capable of using PET as a major carbon and energy source [22] made the public aware of the potential for biobased PET degradation. Nevertheless, PET hydrolases are still rare, and identifying them requires extensive screening efforts [32] (Figure 2). Thus, more than 50% of the reported PET degraders have emerged from bacteria, particularly *Bacillus* sp., followed by fungi, including *Aspergillus* sp. [33]. Notably, the search for host strains benefits from the microbial variety in soils [33], whereby widespread PET pollution spurs mining efforts at contaminated sites on land [21] or in aquatic habitats [34].

Despite this clear variety, not all PET degraders are necessarily equally suitable for biobased PET recycling due to slow growth, for example, fungi [33], or the necessity to develop genetic engineering tools to unlock their potential [35]. Thus, so-far natural PET degraders are primarily used as donors for relevant enzymes, which are subsequently introduced into proven hosts, for example, *Escherichia coli* [36] and *Yarrowia lipolytica* [37]. In an attempt to bioremediate polluted seawater, key enzymes have also been introduced into marine microalga [38].

Remarkably, several bacteria associated with lignin valorization [16] also possess interesting properties as hosts for PET valorization. Known to withstand demanding and toxic conditions [10,39,40], *Pseudomonas putida* plays a vital role in the emerging field of PET valorization [20,41–43] (Table 1), and *Comamonas testosteroni* holds

Figure 2



The potential of using micro-organisms in a biobased PET industry**.** The use of PET as a feedstock for biotechnology requires its depolymerization via chemical catalysis (CC) and/or biocatalysis (BC) to afford the monomers TPA and EG [7]. For example, novel strains were isolated from PET-contaminated sites [21,22], and combined with established workhorses [20] and information gained from metagenomes [51], these strains serve as valuable sources for identifying the metabolic pathways and enzymes necessary for PET (monomer) metabolism. Hereby, key biotechnological methods, including adaptive laboratory evolution [23] and metabolic engineering [41], enable enhanced strain performance. Additionally, mining for novel enzymes has benefited from advanced screening strategies [55,103] complemented by biosensors [65] and improved enzyme candidates via protein engineering [49,59]. Monocultures [19] or cocultures [47,72,98] of streamlined cell factories may then be used for the upcycling of PET into various products [18–21,43,72,79–82,101]. Likewise, sugars [25–27] and aromatics [24] from plant biomass serve as substrates for the production of TPA and EG by metabolically engineered microbes, allowing the production of fully biobased PET. Abbreviations: ARO, aromatic compounds; EG, ethylene glycol; HAA, hydroxyalkanoyloxy-alkanoate; βKA, β-ketoadipic acid; LYC, lycopene; MA, *cis, cis*-muconic acid; PCA, protocatechuic acid; PDC, 2-pyrone-4,6-dicarboxylic acid; PHA, polyhydroxyalkanoates; SUG, sugar; VIN, vanillin.

the combined potential for the valorization of lignin- and PET-based aromatic monomers [44].

In addition to single-host strains (Table 1), PET valorization might also benefit from the employment of natural [45] and artificial [46,47] consortia, aiming at synergistic and co-operative PET degradation (Figure 2). This setup matches well with current research focusing on understanding the man-made plastisphere, which refers to the natural microbial communities that colonize plastic waste in terrestrial and aquatic ecosystems [48].

Novel enzymes for polyethylene terephthalate recycling

Compared with other plastics, the presence of ester bonds in PET facilitates the attack and subsequent degradation of PET by microbial enzymes, which have great potential for use in environmental-benign PET recycling [1]. In addition to closed-loop recycling processes [49–51], these enzymes also play vital roles as starting points for microbial upcycling strategies [19,52] (Figure 1). Enzymes with the ability to modify or degrade PET include PETases (EC 3.1.1.101), cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3), and carboxylesterases (EC 3.1.1.1), all of which share the common ability to hydrolyze carboxylic esters (EC 3.1.1) resulting in products with hydroxyl and carboxylic acid residues [7,29]. Additionally, MHETase (EC 3.1.1.102) [22] and the recently reported BHETase (EC number not yet assigned) [53] further breakdown the intermediate products mono-(2-hydroxyethyl) terephthalate (MHET) and bis-(2-hydroxyethyl)-terephthalate (BHET) into TPA and EG, altogether providing the optimal setup for further downstream microbial catabolism (Figure 3).

Table 1

Microbial upcycling of PET and PET-derived monomers. The following examples are categorized based on the strategy used for PET depolymerization, including chemical degradation (○), enzymatic hydrolysis by purified enzymes (•), or hydrolysis by enzymes produced by a cell factory (●). The strains were classified based on their natural genes for TPA (●) and EG (●) metabolism, as well as the application of metabolic engineering strategies for substrate assimilation or product formation (●).

Product	Strain designation	Substrate	Relevant carbon source for biosynthesis	Titer (g l^{-1})	Conversion	Method/scale	Reference
Pseudomonas umsongensis GO16							
PHA	Wild type	PET ○	TPA	0.25[a]	27% CDW	Batch, shake flask	[21]
PHA	Wild type	PET ○	TPA	2.61	30% CDW	Fed-batch, reactor	[97]
PHA	KS3	PET ●	TPA_ EG	0.15	7% CDW	Batch, reactor	[19]
HAA	KS3 pSB01	PET ●	TPA	0.04	-	Batch, shake flasks	[19]
Pseudomonas putida GO19							
PHA	Wild type	PET ○	TPA	0.25[a]	23% CDW	Batch, shake flask	[21]
Pseudomona frederiksbergensis GO23							
PHA	Wild type	PET ○	TPA	0.26[a]	24% CDW	Batch, shake flask	[21]
Yarrowia lipolytica and *Pseudomonas stutzeri*							
PHA	$Po1fP_{Ylipo}$	BHET ●	TPA	0.13[a]	3.66% CDW	Batch, shake flask	[98]
	$TPA3P_{Pstutz}$						
PHA	$TPA3P_{Pstutz}$	TPA	TPA	-	11.56% CDW	Batch, shake flask	[98]
Yarrowia lipolytica IMUFRJ 50682							
GLA	Wild type	EG	EG	32.67	74% mol mol^{-1}	Batch, reactor	[83]
Pseudomonas putida KT2440							
β-KA	AW165	PET ○,●	BHET	0.22	45% mol mol $_{BHET}^{-1}$	Fed-batch, shake flask	[20]
β-KA	AW165	BHET ●	TPA_EG	15.1	76% mol mol_{BHET}^{-1}	Fed-batch, reactor	[20]
MA	tacRDL	PET ●	TPA	5.61	100% mol mol_{TPA}^{-1}	Fed-batch, shake flask	[43]
PHA	MFL185	EG	EG	-	32.2% CDW	Batch, shake flask	[75]
Pseudomonas putida EM42							
MA	Pp-T	PET ○	TPA	4.73		Batch, shake flask	[47]
	Pp-E		EG				
PHA	Pp-T	PET ○	TPA	0.64		Fed-batch, shake flask	[47]
	Pp-E		EG				
Ideonella sakaiensis 201-F6							
PHA	Wild type	PET ●	TPA_EG	0.75	48% CDW	Batch, Petri dish	[52]
Rhodococcus jostii PET							
Lycopene	S6	PET ○	TPA_EG	0.001	-	Fed-batch, shake flask	[80]
Escherichia coli							
Vanillin	RARE_pVanX	TPA	TPA	0.12	79% mol mol^{-1}	Batch, shake flask	[81]
Vanillin	RARE_pVanX	PET ●	TPA	0.010	-	Batch, shake flask	[81]
PCA	PCA-1	PET ○	TPA	0.43	81.4% mol mol^{-1}	Batch, shake flask	[72]
GAL	PCA-1	PET ○	TPA	0.46	92.5% mol mol^{-1}	Batch, shake flask	[72]
	HBH-2						
PYG	CTL-1	PET ○	TPA	0.08	-	Batch, shake flask	[72]
	CH-1						
MA	MA-1	PET ○	TPA	0.38	85.4% mol mol^{-1}	Batch, shake flask	[72]
VNA	PCA-1	PET ○	TPA	0.24	41.6% mol mol^{-1}	Batch, shake flask	[72]
	$OMT\text{-}2^{His}$						
PDC		PET ○	TPA	0.57	99% mol mol^{-1}	Batch, shake flask	[18]

Table 1 (*continued*)

Product	Strain designation		Substrate	Relevant carbon source for biosynthesis	Titer (g l^{-1})	Conversion	Method/scale	Reference
	PCA	●						
	PDC_{PCA}	●						
CAT	pKE112TphBaroY + pKM212TphAabc	●	PET ○●	TPA	0.66	99.5% mol mol^{-1}	Batch, shake flask	[99]
Metal-organic frameworks	BL21 (DE3) pET21b-thermoPETase	●	PET ●	TPA	-	-	-	[100]
Gluconobacter oxydans								
GLA	Wild type	●	PET ○	EG	0.80	98.6% mol mol^{-1}	Batch, conical tubes	[72]
Taonella mepensis WT-6								
Bacterial cellulose	Wild type	●,●	PET ○	TPA EG BHET MHET	2.42	-	Batch, shake flask	[101]

GAL, gallic acid; GLA, glycolic acid; HAA, hydroxyalkanoyloxy-alkanoates; MA, *cis,cis*-muconic acid; PCA, protocatechuic acid; PDC, 2-pyrone-4,6-dicarboxylic acid; PYG, pyrogallol; VNA, vanillic acid.

[a] Estimated from the reference.

[b] Improved by adaptive laboratory evolution or deregulating gene expression.

Among many other promising PETases, such as *Is*PETase from *I. sakaiensis* [22], the leaf-branch compost cutinase (LCC) [50] and the metagenomic polyester hydrolase PHL7 [51] are currently undergoing extensive optimization efforts (Table 2). Therefore, rational and structure-guided protein engineering targets, *inter alia*, at an enhanced thermostability over a broad temperature range and increased enzymatic activity [49,54–57]. Another interesting aspect is the broadening of the substrate spectrum of PETases to other polyesters [58]. Overall, protein crystal structures and bioinformatic tools have played pivotal roles in identifying specific amino acid chain mutations that can be used to modulate the desired enzyme features (Table 2). For example, the French company Carbios successfully developed the more efficient variant LCC^{ICCG} from the previously identified LCC cutinase [59]. They patented and industrialized the process under the term C-ZYME, claiming a 30% reduction in CO_2 emissions compared with conventional routes of PET waste management, for example, incineration [60] (Figure 1).

Additionally, other state-of-the-art strategies to enhance enzymatic PET depolymerization include (1) chimeric proteins of PETase and MHETase [61], (2) dual enzyme systems [62], and (3) the surface display of enzymes on microbial surfaces aided by a codisplay of hydrophobin, an adhesion protein facilitating adsorption to hydrophobic surfaces such as PET films [63] or by the addition of rhamnolipids [36]. The current successful application of bioinformatic tools and machine learning [49,57,64] complemented by biosensors [65] has already paved the way for overall accelerated enzyme discovery and improvement, thus facilitating the selection of suitable enzyme candidates via enzyme mining (Figure 2).

Metabolic pathways for the degradation of polyethylene terephthalate-based monomers

Notably, it has been known for several decades that different micro-organisms can metabolize the PET monomers TPA and EG; readers interested in these micro-organisms are referred to [15,66] for a more detailed overview of the underlying pathways, which in total provides a powerful basis for metabolic engineering (Figure 2). The aerobic TPA degradation pathway has been identified in different *Proteobacteria* [67–69] and the *Rhodococcus* genus [70,71] and generally follows the same pattern (Figure 3). Despite many similarities, including the genetic organization of the *tph* operon [15], the different cofactor preferences indicate the potential of certain adjustments for metabolic engineering [72]. Additionally, the downstream pathways involved in the degradation of protocatechuate, a key intermediate in aromatic degradation pathways, are quite diverse among host strains (Figure 3) [16], suggesting that the metabolic engineering of TPA

Table 2

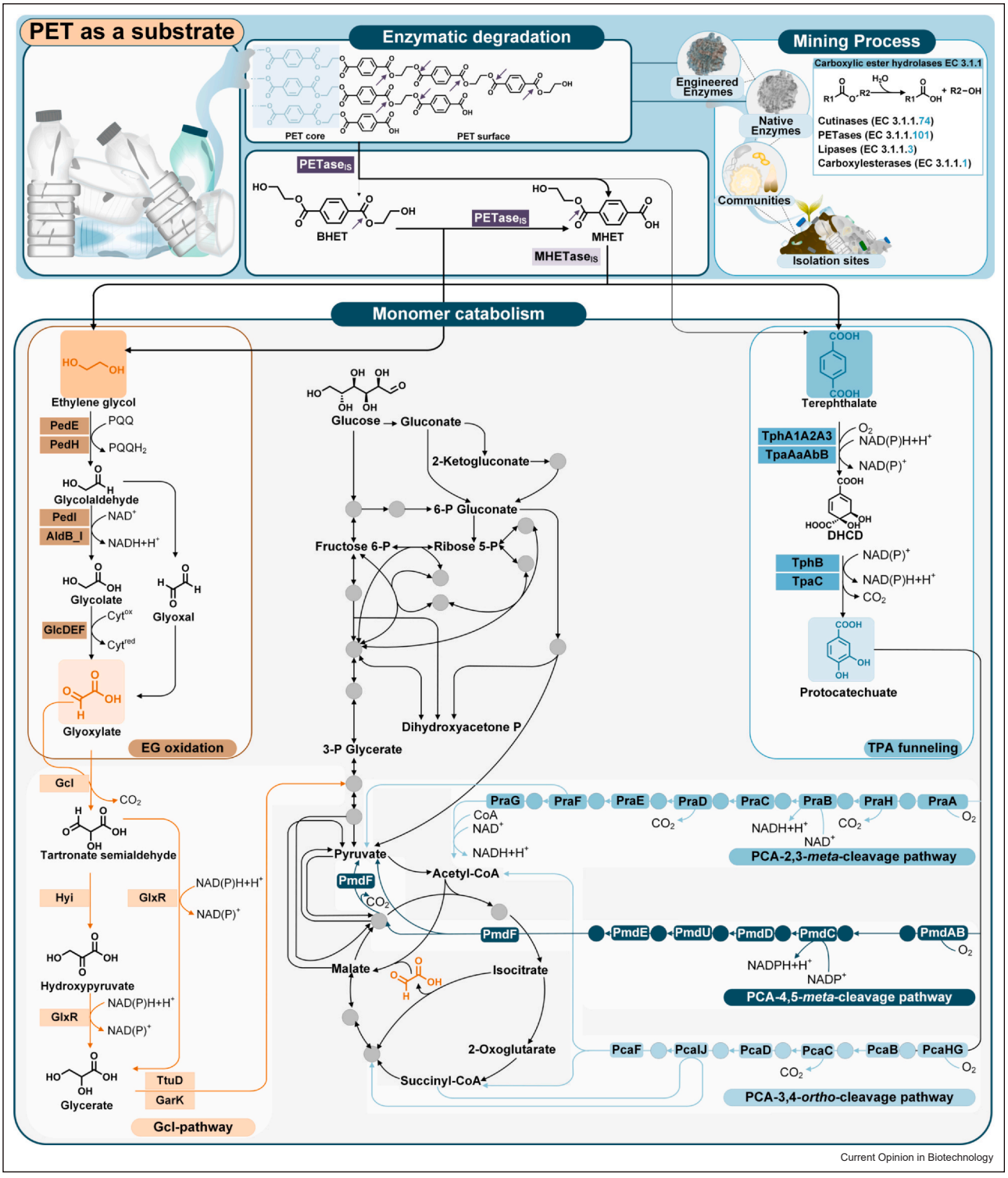
Blockbuster enzymes for biological PET depolymerization.

Source	Name	Engineered variant	Substrate	Comment	Reference (s)
Ideonella sakaiensis 210 F6	IsPETase		PET		[22,102]
		IsPETase$^{S238F/W159H}$	PET	Evolved from IsPETase	[58]
		ThermoPETase/IsPETaseTM/ IsPETase$^{S121E/D186H/R280A}$	PET	Evolved from IsPETase	[54]
		IsPETase$^{S121E/D186H/S242T/N246D}$	PET	Evolved from IsPETase	[56]
		FAST-PETase	PET	Evolved from IsPETase	[49]
		HotPETase	PET	Evolved from IsPETase	[55]
		DuraPETase	PET	Evolved from IsPETase	[57]
		TS-PETase/IsPETase$^{R280A/S121E/D186H/N233C/S282C}$	PET	Evolved from IsPETase	[103]
		Z1-PETase	PET	Evolved from IsPETase	[104]
Ideonella sakaiensis 210 F6	IsMHETase		MHET		[22,102]
Thermobifida fusca DSM43793	TfH		PET		[28,105]
Thermobifida fusca KW3	TfC (TfCut1, TfCut2)		PET		[106,107]
Thermobifida fusca KW3	TfCa		MHET, BHET	Carboxyl esterase	[62]
		TfCa WA$^{I69W/V376A}$	MHET, BHET	Evolved from TfCa	[62]
Rhizobacter gummiphilus	RgPETase		PET		[108]
Brachybacterium ginsengisoli	BgP		PET	Marine bacterium (deep sea)	[109]
Streptomyces sp. SM14	SM14est		PET	Marine bacterium	[110]
Pseudomonas aestusnigri	PE-H		PET	Marine bacterium	[111]
Burkholderiales bacterium	BbPETase		PET		[112]
		BbPETase$^{S335N/T338I/M363I/N365G}$	PET		[112]
Thermobifida cellulosilytica	Thc (Thc_Cut1, Thc_Cut2)		PET		[106,113]
Humicola/Humilica/ Thermomyces insolens	HiC		PET	Fungal enzyme	[114]
Fusarium solani f. sp. *pisi*	FsC		PET	Fungal enzyme	[114]
Pseudomonas mendocina	PmC		PET		[114]
Bacillus subtilis	Bs2Est		MHET, BHET	Carboxyl esterase	[99]
HR29	BhrPETase		PET		[115]
Leaf-branch compost metagenome	LCC		PET		[50,116]
		LCC^{ICCG}	PET		[50,59]
Compost metagenome	PHL7		PET		[51]
		PHL7^{L210T}	PET		[117]
Metagenome	PES-H1/PES-H2		PET	Polyester hydrolase	[118]
		PES-H1$^{L92F/Q94Y}$	PET		[118]
Metagenome	ChryBHETase		BHET	Carboxyl esterase	[53]
	BsEst		BHET	Carboxyl esterase	[53]

assimilates will largely benefit from recent developments in bacterial lignin upgrading [73].

Recent efforts have specifically focused on characterizing *P. putida* KT2440 in relation to EG metabolism. Starting from the initial insights from comparative proteomics with *Pseudomonas* strain JM37 [74], strain KT2440 was found to possess a *gcl* operon, providing the genomic setup for the glyoxylate carboligase (Gcl) pathway [75] (Figure 3). This pathway is naturally repressed and must first be activated either by deletion of the specific transcriptional repressor GclR [23] or by constitutive overexpression [75], after which it could be allowed to grow on EG as the sole carbon and energy source. This pathway has also been identified in *Pseudomonas umsongensis* GO16 [69] and suggested for *I. sakaiensis* [76]; however, based on CO_2 release and the input of reducing equivalents metabolically, the use of this pathway is quite costly [77]. Establishing the β-hydroxyaspartate cycle from *Paracoccus denitrificans* as an

Figure 3


PET as a substrate
Enzymatic degradation
PET core
PET surface
Engineered Enzymes
Native Enzymes
Mining Process
Carboxylic ester hydrolases EC 3.1.1
Cutinases (EC 3.1.1.74)
PETases (EC 3.1.1.101)
Lipases (EC 3.1.1.3)
Carboxylesterases (EC 3.1.1.1)
Communities
Isolation sites
PETase$_{IS}$
BHET
MHET
MHETase$_{IS}$
Monomer catabolism
Ethylene glycol
PedE
PedH
PQQ
PQQH$_2$
Glycolaldehyde
PedI
AldB_I
NAD$^+$
NADH+H$^+$
Glycolate
GlcDEF
Cyt^{ox}
Cyt^{red}
Glyoxal
Glyoxylate
EG oxidation
Glucose
Gluconate
2-Ketogluconate
6-P Gluconate
Fructose 6-P
Ribose 5-P
Dihydroxyacetone P
3-P Glycerate
Terephthalate
TphA1A2A3
TpaAaAbB
O$_2$
NAD(P)H+H$^+$
NAD(P)$^+$
DHCD
TphB
TpaC
CO$_2$
Protocatechuate
TPA funneling
Gcl
Tartronate semialdehyde
Hyi
GlxR
Hydroxypyruvate
Glycerate
TtuD
GarK
Gcl-pathway
Pyruvate
Acetyl-CoA
CoA
PmdF
Malate
Isocitrate
2-Oxoglutarate
Succinyl-CoA
PraG
PraF
PraE
PraD
PraC
PraB
PraH
PraA
PCA-2,3-*meta*-cleavage pathway
PmdE
PmdU
PmdD
PmdC
PmdAB
NADPH+H$^+$
NADP$^+$
PCA-4,5-*meta*-cleavage pathway
PcaF
PcaIJ
PcaD
PcaC
PcaB
PcaHG
PCA-3,4-*ortho*-cleavage pathway
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PET as a feedstock for microbial upgrading of postconsumer waste streams. Microbial enzymes are considered attractive tools for PET degradation, spurring recent efforts to identify novel and superior enzymes (enzyme mining) [1,7]. Relevant enzymes belong to the group of carboxylic ester hydrolases (EC 3.1.1) [7,29], whereby only a small fraction of these are suitable for both surface modification and the degradation of the inner PET core [29]. One interesting PET depolymerizing enzyme donor is the bacterium *I. sakaiensis*, which supplies PETase and MHETase [22]. A recent study specifically focused on developing a BHETase mining process [53]. Once depolymerized, EG and TPA are metabolized via different pathways [15,66]. Using *P. putida* as a case study, in addition to the stepwise oxidation of EG [74], the Gcl pathway enables growth on EG as the sole carbon and energy source [23,75] and was also identified in *P. umsongensis* [69]. TPA degradation involves oxygen-dependent dihydroxylation catalyzed by TPA dioxygenase, followed by decarboxylation of the intermediate 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate (DHCD) [68,71]. Depending on the host, PCA is further metabolized via different pathways [44,68,70], which are initiated by oxygen-dependent ring cleavage at different positions [16]. Enzymes: AldB_I: aldehyde dehydrogenase; GarK: glycerate kinase; GlcDEF: glycolate oxidase; Hyi: hydroxypyruvate isomerase; GlxR: tartronate semialdehyde reductase; PcaB: 3-carboxy-*cis,cis*-muconate cycloisomerase; PcaC: 4-carboxymuconolactone decarboxylase; PcaD: β-ketoadipate enol-lactonase; PcaHG: protocatechuate-3,4-dioxygenase; PcaIJ: β-ketoadipate CoA-transferase; PcaF: β-ketoadipyl-CoA thiolase; PedI: aldehyde reductase; PedE: PQQ-dependent (m)ethanol family dehydrogenase; PmdAB: protocatechuate-4,5-dioxygenase; PmdC: 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase; PmdD: 2-pyrone-4,6-dicarboxylate hydrolase; PedH: quinoprotein (m)ethanol family dehydrogenase; PmdF: 4-carboxy-4-hydroxy-2-oxoadipate aldolase; PmdU: 4-oxalomesaconate tautomerase; PmdE: 4-oxalomesaconate hydratase; PraA: protocatechuate-2,3-dioxygenase; PraB: 2-hydroxymuconate-6-semialdehyde dehydrogenase; PraC: 2-hydroxymuconate tautomerase; PraD: 4-oxalocrotonate decarboxylase; PraE: 2-hydroxypenta-2,4-dienoate hydratase; PraF: 4-hydroxy-2-oxovalerate aldolase; PraG: acetaldehyde dehydrogenase; PraH: 5-carboxy-2-hydroxymuconate-6-semialdehyde decarboxylase; TphA1A2A3/TpaAaAbB: terephthalate 1,2-dioxygenase; TphB/TpaC: 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate dehydrogenase; TtuD: hydroxypyruvate reductase.

alternative route for glyoxylate metabolism in *P. putida* KT2440 combined with adaptive laboratory evolution markedly enhanced growth on EG [77].

From polyethylene terephthalate to novel products via microbial pathways

In recent years, several researchers have made great contributions to microbial PET valorization, giving rise to a versatile spectrum of products (Table 1; Figure 2). Native EG metabolism harbors interesting intermediates, for example, glycolate [72] and ethanol [78], and is often used as a complementary carbon source for holistic PET valorization concepts [20,43]. Aromatic TPA metabolism via protocatechuate (PCA; Figure 2) allows the adaptable production of other aromatic compounds (AROs) [72] or plastic precursors [18,43] (Table 2). For example, Werner et al. extensively engineered *P. putida* KT2440 for the production of β-ketoadipate, a building block for a nylon-6,6 analog (Figure 4a) [20]. In addition to enhancing EG utilization, efficient TPA catabolism required the screening of a combinatorial gene library. The best strain for TPA catabolism contained the TPA transporter *tpaK* from *R. jostii* and the *tph* operon from *Comamonas* sp. E6. Moreover, PETase and MHETase from *I. sakaiensis* enabled the conversion of BHET, derived from PET glycolysis, into TPA and EG. Strain AW165, with deletion of *pcaIJ* to allow β-ketoadipate accumulation, produced 1.39 mM product from depolymerized PET under supplementation with additional glucose and 15.1 g l^{-1} product from commercial BHET powder [20]. In addition to biotransformation strategies, the *de novo* synthesis of polyhydroxyalkanoates (PHA) [21,52] or hydroxyalkanoyloxy alkanoates [19,79] represents an alternative approach for converting waste PET into novel plastics with different properties. Next to high-value products, for example, lycopene [80], recent efforts have suggested potential applications of microbial PET valorization in the food sector, for example, via the production of vanillin [81] or single-cell proteins [82] (Figure 2).

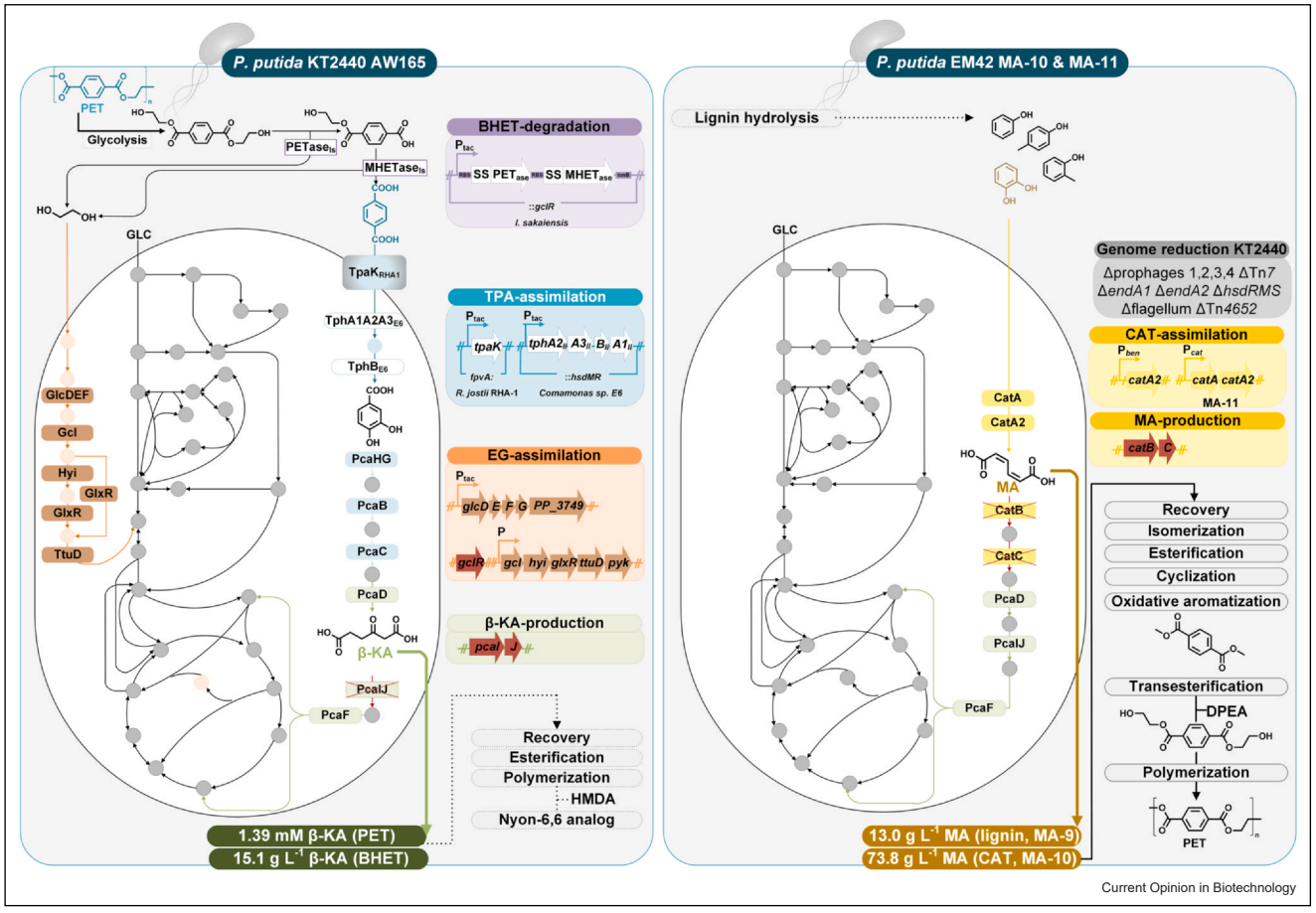
Overall, PET valorization is likewise believed to be easier than the valorization of other biotechnological feedstocks because of its comparatively simple composition [10]. Nevertheless, according to recent studies, some aspects have been proven to be crucial when fine-tuning cell factories to metabolize TPA and EG, particularly in terms of tolerance to (1) high concentrations of substrates [80,83], (2) toxic metabolic intermediates [75], and (3) accompanying stressors from certain chemical depolymerization methods [80]. Moreover, for non-native degraders, TPA import [20], as well as sufficient coconsumption of both TPA and EG, seem to be relevant targets [20,43]. As noted in Ref. [20], an issue that has not been widely considered thus far is that in an authentic postconsumer PET scenario, the presence of various additives [30] will also pose additional challenges for host strains.

Toward alternative routes for polyethylene terephthalate production — *de novo* synthesis of polyethylene terephthalate from alternative feedstocks

Regarding the far-reaching consequences of conventional PET synthesis, several attempts have been made to establish alternative and sustainable production routes for TPA and EG by the chemical conversion of biomass [4,84]. However, most of these processes are not accessible on a commercial scale, and the available bio-PET consists of sugarcane-derived EG and fossil-based TPA [4] (Figure 1). According to European Bioplastics, only 4.2% of the PET produced in 2022 worldwide was bio-based [85].

The production of PET monomers from different feedstocks is also an emerging field of biotechnology [15,86]. In particular, for EG, several pathways have

Figure 4

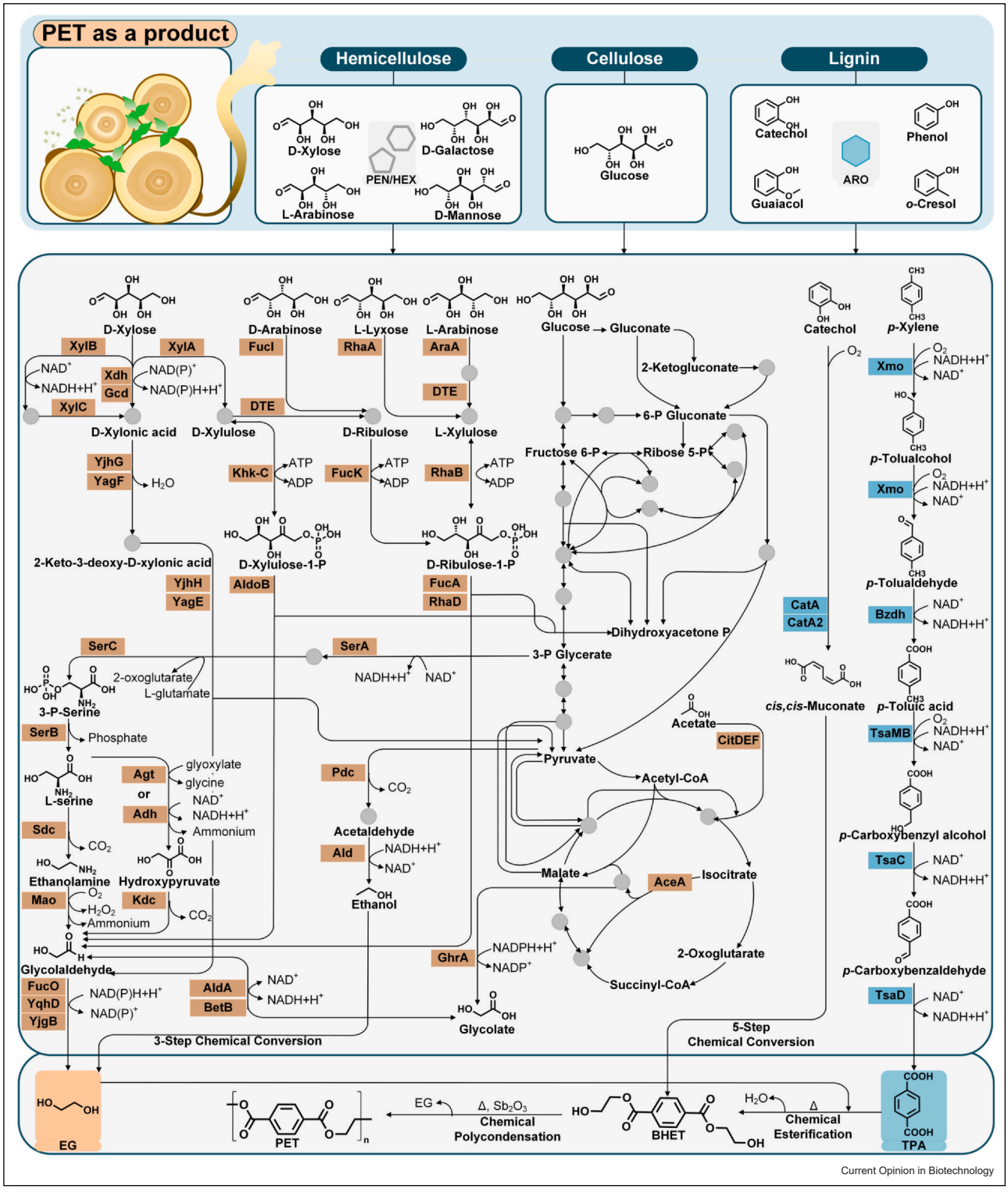


P. putida KT2440 as a case study for a biobased PET industry. **(a)** From PET to alternative polymers. *P. putida* KT2440 was streamlined for β-KA production from PET depolymerized by glycolysis. Strain AW165 is characterized by the following: (1) constitutive overexpression of genes involved in EG oxidation, (2) deletion of *gclR* for activation of the Gcl pathway, (3) expression of the *tphA2$_{II}$A3$_{II}$B$_{II}$A1$_{II}$* operon from *Comamonas* sp. E6 and *tpaK* from *R. jostii* for TPA catabolism and import, respectively, (4) expression of PETase and MHETase from *I. sakaiensis* for BHET hydrolysis, and (5) deletion of *pcaIJ* for β-KA accumulation. The strain produced β-KA from PET, depolymerized using a combination of chemical and biological methods, which subsequently can be used to produce a nylon-6,6 analog [20]. **(b)** From lignin to PET. The genome-reduced *P. putida* KT2440 derivative EM42 was metabolically engineered for MA production by deleting the *catBC* genes, thereby generating the MA-10 strain. Additionally, strain MA-11 harbors a second copy of the native *catA2* gene, which is introduced downstream of *catA,* the major catechol-1,2-dioxygenase. The genetic setup of both strains enabled enhanced *cis,cis*-muconate production from the toxic lignin model compound CAT in comparison to the KT2440 strains without genome reduction in terms of productivity, titer, and yield. MA was recovered from the culture broth, followed by stepwise chemical conversion into BHET and final polymerization into PET [24]. Considering the results of previous works, a full value chain from lignin to PET is possible [40]. Color coding: deletions are highlighted in red, and native genes and enzymes are depicted in full color, whereas heterologous genes are shown in white with a colored frame. HMDA, hexamethylenediamine; DPEA, *N,N*-diisopropylethylamine.

been identified, allowing production from D-xylose [25,87–90], the downstream intermediate xylonic acid [91,92] or other pentose sugars [26] via the formation of glycolaldehyde, which is subsequently enzymatically reduced to EG (Figure 5). Alternative pathways start with glucose via L-serine as an intermediate [27,93], and, additionally, a novel biosynthetic pathway from acetate has been designed computationally [94].

Comparably fewer pathways are available for biobased *de novo* TPA production 15 (Figure 5). For instance, in a metabolically engineered *E. coli* strain, the conversion of *p*-xylene was established by stepwise conversion with *p*-toluate as an intermediate [95]. In a subsequent study in which *P. putida* KT2440 was used as an alternative host, this approach was further improved, yielding a titer of 38.25 g l^{-1} TPA [96]. In addition, Kohlstedt et al. demonstrated the feasibility of linking lignin-related aromatic degradation pathways with PET synthesis [24] (Figure 4b). The genome-reduced *P. putida* strain EM42 was equipped with genetic modifications relevant for the formation of catechol (CAT)-based *cis,cis*-muconate. The

Figure 5


PET as a product
Hemicellulose
Cellulose
Lignin
D-Xylose
D-Galactose
L-Arabinose
D-Mannose
PEN/HEX
Glucose
Catechol
Phenol
Guaiacol
ARO
o-Cresol
D-Arabinose
L-Lyxose
L-Arabinose
Gluconate
2-Ketogluconate
6-P Gluconate
Fructose 6-P
Ribose 5-P
p-Xylene
p-Toluic acid
p-Carboxybenzyl alcohol
p-Carboxybenzaldehyde
XylB
XylA
XylC
Xdh
Gcd
FucI
RhaA
AraA
DTE
D-Xylonic acid
D-Xylulose
D-Ribulose
L-Xylulose
YjhG
YagF
Khk-C
FucK
RhaB
ATP
ADP
H2O
2-Keto-3-deoxy-D-xylonic acid
D-Xylulose-1-P
D-Ribulose-1-P
YjhH
YagE
AldoB
FucA
RhaD
Dihydroxyacetone P
3-P Glycerate
SerC
SerA
2-oxoglutarate
L-glutamate
3-P-Serine
SerB
Phosphate
L-serine
Agt
or
Adh
glyoxylate
glycine
Ammonium
Sdc
Ethanolamine
Hydroxypyruvate
Mao
Kdc
Glycolaldehyde
FucO
YqhD
YjgB
AldA
BetB
3-Step Chemical Conversion
Pdc
Acetaldehyde
Ald
Ethanol
Pyruvate
Acetyl-CoA
Acetate
CitDEF
Malate
AceA
Isocitrate
2-Oxoglutarate
Succinyl-CoA
GhrA
NADPH+H+
NADP+
Glycolate
CatA
CatA2
cis,cis-Muconate
5-Step
Chemical Conversion
Xmo
p-Tolualcohol
p-Tolualdehyde
Bzdh
TsaMB
TsaC
TsaD
EG
PET
Δ, Sb2O3
Chemical
Polycondensation
BHET
H2O
Δ
Chemical
Esterification
TPA
Current Opinion in Biotechnology

PET as a product of coupled bio- and chemo-catalytic processes. Both representative sugars [119] and AROs [16] obtained from lignocellulosic biomass are interesting carbon sources for producing EG or the aromatic PET precursor TPA via metabolically engineered micro-organisms. From all the displayed routes, only EG production via the three-step conversion of ethanol has thus far reached commercial applicability for bio-PET production [84], profiting from recent developments in yeast-based ethanol production [120]. The depicted EG biosynthetic pathways start from D-xylose [25,88–90], D-xylonic acid [91], other hemicellulose-derived pentoses [26], glucose [27,93], or acetate [94]. Additionally, AROs are suitable for direct TPA production from *p*-xylene [95] or for *cis,cis*-muconate production [24]. Toward fully biobased PET, both sugar-based EG and aromatic-derived (dimethyl)terephthalate may be used for BHET production, which subsequently undergoes polycondensation [4]. Enzymes: AceA: Isocitrate lyase; AldA: aldehyde dehydrogenase; Aldo-B: D-xylulose-1-phosphate aldolase; Adh: amino acid dehydrogenase; Agt: aminotransferase; Ald: alcohol dehydrogenase; AraA: L-arabinose isomerase; BetB: aldehyde dehydrogenase; Bzdh: benzaldehyde dehydrogenase; CatA/CatA2: catechol-1,2-dioxygenase; CitDEF: citrate lyase; DTE: D-tagatose epimerase; FucA: L-fuculose phosphate aldolase; FucI: L-fucose isomerase; FucK: L-fuculokinase; FucO: lactaldehyde reductase; GcD: glucose dehydrogenase; GhrA: glyoxylate reductase; Mao: monoamine oxidase; Kdc: α-ketoacid decarboxylase; Khk-C: D-xylulose-1-kinase; Pdc: pyruvate decarboxylase; RhaA: L-rhamnose isomerase; RhaB: L-rhamnulokinase; RhaD: rhamnulose-1-phosphate aldolase; SerA: phosphoglycerate dehydrogenase; SerB: phosphoserine phosphatase; SerC: phosphoserine transaminase; Sdc: serine decarboxylase; TsaC: *p*-carboxybenzyl alcohol dehydrogenase; TsaD: *p*-carboxybenzaldehyde dehydrogenase; TsaMB: toluate methylmonooxygenase; Xmo: xylene monooxygenase; Xdh: D-xylose dehydrogenase; XylA: xylose isomerase; XylB: xylose dehydrogenase; XylC: xylonolaconase; YjgB: aldehyde reductase; YjhG/YagF: D-xylonate dehydratase; YjhH/YagE: 2-keto 3-deoxy D-xylonate aldolase; YqhD: alcohol dehydrogenase.

purified product was subsequently chemically converted into dimethylterephthalate, which, after transesterification to generate BHET, was later used for PET production, paving the groundwork for a novel value chain from lignin to PET [24,40].

Conclusions

The global dependency on the all-around useful product PET faces two major issues arising from conventional fossil fuel-based production [5,6] and the limited possibilities of how to proceed with postconsumer PET in a manner that limits both material loss and environmental concerns [7,8]. Both challenges have been sizable drivers in recent years when establishing alternative routes for both PET synthesis [86] and its valorization [1]. As demonstrated by scientists worldwide, harnessing the outstanding variability and flexibility within natural microbial metabolic pathways is an enormous opportunity toward a sustainable PET industry [15,29,30,66], thereby contributing to the realization of the sustainable development goals of the United Nations [13]. However, biobased routes for PET production and valorization have played an almost nonexistent role in the PET industry thus far. Regarding the current landscape of PET usage (Figure 1), it has become clear that PET production and valorization require innovative approaches and solutions, in which micro-organisms and biotechnology will definitely play important roles.

Data Availability

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

Declaration of Competing Interest

Michael Kohlstedt and Christoph Wittmann have filed patent applications on the use of lignin for bioproduction. Fabia Weiland declares that she has no competing interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zimmermann W: **Biocatalytic recycling of polyethylene terephthalate plastic**. *Philos Trans Ser A Math Phys Eng Sci* 2020, **378**:20190273..
2. Tsironi TN, Chatzidakis SM, Stoforos NG: **The future of polyethylene terephthalate bottles: challenges and sustainability**. *Packag Technol Sci* 2022, **35**:317-325.
3. Dissanayake L, Jayakody LN: **Engineering microbes to bio-upcycle polyethylene terephthalate**. *Front Bioeng Biotechnol* 2021, **9**:656465.
4. Nisticò R: **Polyethylene terephthalate (PET) in the packaging industry**. *Polym Test* 2020, **90**:106707.
5. Lapa HM, Martins LMDRS: **p-xylene oxidation to terephthalic acid: new trends**. *Molecules* 2023, **28**:1922.
6. Chauhan R, Sartape R, Minocha N, Goyal I, Singh MR: **Advancements in environmentally sustainable technologies for ethylene production**. *Energy Fuels* 2023, **37**:12589-12622.
7. Soong YV, Sobkowicz MJ, Xie D: **Recent advances in biological recycling of polyethylene terephthalate (PET) plastic wastes**. *Bioengineering* 2022, **9**:98.
8. Webb HK, Arnott J, Crawford RJ, Ivanova EP: **Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate)**. *Polymers* 2013, **5**:1-18.
9. MacLeod M, Arp HPH, Tekman MB, Jahnke A: **The global threat from plastic pollution**. *Science* 2021, **373**:61-65.
10. Wierckx N, Prieto MA, Pomposiello P, de Lorenzo V, O'Connor K, Blank LM: **Plastic waste as a novel substrate for industrial biotechnology**. *Microb Biotechnol* 2015, **8**:900-903.
11. SYSTEMIQ: Circular PET and Polyester. A Circular Economy Blueprint for Packaging and textiles in Europe, July 2023, 18. https://www.systemiq.earth/wp-content/uploads/2023/07/Circular-PET-and-Polyester-Full-Report-July-2023.pdf .

12. Vollmer I, Jenks MJF, Roelands MCP, White RJ, van Harmelen T, de Wild P, van der Laan GP, Meirer F, Keurentjes JTF, Weckhuysen BM: **Beyond mechanical recycling: giving new life to plastic waste**. *Angew Chem-Int Ed* 2020, **59**:15402-15423.

13. Kumar R, Verma A, Shome A, Sinha R, Sinha S, Jha PK, Kumar R, Kumar P, Shubham, Das S, *et al*.: *Sustainability* 2021, **13**:9963.

14. Nicholson SR, Rorrer JE, Singh A, Konev MO, Rorrer NA, Carpenter AC, Jacobsen AJ, Román-Leshkov Y, Beckham GT: **The critical role of process analysis in chemical recycling and upcycling of waste plastics**. *Annu Rev Chem Biomol Eng* 2022, **13**:301-324.

15. Salvador M, Abdulmutalib U, Gonzalez J, Kim J, Smith AA, Faulon JL, Wei R, Zimmermann W, Jimenez JI: **Microbial genes for a circular and sustainable Bio-PET economy**. *Genes* 2019, **10**:373.

16. Weiland F, Kohlstedt M, Wittmann C: **Guiding stars to the field of dreams: metabolically engineered pathways and microbial platforms for a sustainable lignin-based industry**. *Metab Eng* 2022, **71**:13-41.

17. Poblete-Castro I, Hoffmann SL, Becker J, Wittmann C: **Cascaded valorization of seaweed using microbial cell factories**. *Curr Opin Biotechnol* 2020, **65**:102-113.

18. Kang MJ, Kim HT, Lee MW, Kim KA, Khang TU, Song HM, Park SJ, Joo JC, Cha HG: **A chemo-microbial hybrid process for the production of 2-pyrone-4,6-dicarboxylic acid as a promising bioplastic monomer from PET waste**. *Green Chem* 2020, **22**:3461-3469.

19. •• Tiso T, Narancic T, Wei R, Pollet E, Beagan N, Schroder K, Honak A, Jiang M, Kenny ST, Wierckx N, *et al*.: **Towards bio-upcycling of polyethylene terephthalate**. *Metab Eng* 2021, **66**:167-178.
Straightforward approach that uses metabolically engineered microbes to upcycle PET monomers into value-added products. The study demonstrates a crucial part of a future bio-based PET industry.

20. •• Werner AZ, Clare R, Mand TD, Pardo I, Ramirez KJ, Haugen SJ, Bratti F, Dexter GN, Elmore JR, Huenemann JD, *et al*.: **Tandem chemical deconstruction and biological upcycling of poly (ethylene terephthalate) to beta-ketoadipic acid by *Pseudomonas putida* KT2440**. *Metab Eng* 2021, **67**:250-261.
Smart value chain to break down PET chemically and feed the obtained monomers to engineered *P. putida* for conversion into a platform chemical at high efficiency, offering promising application options.

21. Kenny ST, Runic JN, Kaminsky W, Woods T, Babu RP, Keely CM, Blau W, O'Connor KE: **Up-cycling of PET (polyethylene terephthalate) to the biodegradable plastic PHA (polyhydroxyalkanoate)**. *Environ Sci Technol* 2008, **42**:7696-7701.

22. Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K: **A bacterium that degrades and assimilates poly(ethylene terephthalate)**. *Science* 2016, **351**:1196-1199.

23. Li WJ, Jayakody LN, Franden MA, Wehrmann M, Daun T, Hauer B, Blank LM, Beckham GT, Klebensberger J, Wierckx N: **Laboratory evolution reveals the metabolic and regulatory basis of ethylene glycol metabolism by *Pseudomonas putida* KT2440**. *Environ Microbiol* 2019, **21**:3669-3682.

24. •• Kohlstedt M, Weimer A, Weiland F, Stolzenberger J, Selzer M, Sanz M, Kramps L, Wittmann C: **Biobased PET from lignin using an engineered *cis*, *cis*-muconate-producing *Pseudomonas putida* strain with superior robustness, energy and redox properties**. *Metab Eng* 2022, **72**:337-352.
First time demonstration of synthetizing bio-based PET from lignin, a globally accumulating waste stream, through a cascaded value chain of chemical and biochemical conversions. The obtained bio-PET exhibited the same material properties as the fossil base market standard.

25. Liu HW, Ramos KRM, Valdehuesa KNG, Nisola GM, Lee WK, Chung WJ: **Biosynthesis of ethylene glycol in *Escherichia coli***. *Appl Microbiol Biotechnol* 2013, **97**:3409-3417.

26. Pereira B, Li ZJ, De Mey M, Lim CG, Zhang HR, Hoeltgen C, Stephanopoulos G: **Efficient utilization of pentoses for bioproduction of the renewable two-carbon compounds ethylene glycol and glycolate**. *Metab Eng* 2016, **34**:80-87.

27. Chen Z, Huang JH, Wu Y, Liu DH: **Metabolic engineering of *Corynebacterium glutamicum* for the *de novo* production of ethylene glycol from glucose**. *Metab Eng* 2016, **33**:12-18.

28. Müller RJ, Schrader H, Profe J, Dresler K, Deckwer WD: **Enzymatic degradation of poly(ethylene terephthalate): rapid hydrolyse using a hydrolase from *T. fusca***. *Macromol Rapid Commun* 2005, **26**:1400-1405.

29. Kawai F, Kawabata T, Oda M: **Current knowledge on enzymatic PET degradation and its possible application to waste stream management and other fields**. *Appl Microbiol Biotechnol* 2019, **103**:4253-4268.

30. Ahmaditabatabaei S, Kyazze G, Iqbal HMN, Keshavarz T: **Fungal enzymes as catalytic tools for polyethylene terephthalate (PET) degradation**. *J Fungi* 2021, **7**:931.

31. Maity W, Maity S, Bera S, Roy A: **Emerging roles of PETase and MHETase in the biodegradation of plastic wastes**. *Appl Biochem Biotechnol* 2021, **193**:2699-2716.

32. Danso D, Chow J, Streit WR: **Plastics: environmental and biotechnological perspectives on microbial degradation**. *Appl Environ Microbiol* 2019, **85**:e01095-19.

33. Fernández CDB, Castillo MPG, Pérez SAQ, Rodríguez LVC: *Microbial degradation of polyethylene terephthalate: a systematic review*, *Sn Appl Sci* 2022, **4**:263.

34. Gao RR, Sun CM: **A marine bacterial community capable of degrading poly(ethylene terephthalate) and polyethylene**. *J Hazard Mater* 2021, **416**:125928.

35. Hachisuka SI, Nishii T, Yoshida S: **Development of a targeted gene disruption system in the poly(ethylene terephthalate)-degrading bacterium *Ideonella sakaiensis* and its applications to PETase and MHETase genes**. *Appl Environ Microbiol* 2021, **87**:e0002021.

36. Gercke D, Furtmann C, Tozakidis IEP, Jose J: **Highly crystalline post-consumer PET waste hydrolysis by surface displayed PETase using a bacterial whole-cell biocatalyst**. *Chemcatchem* 2021, **13**:3479-3489.

37. Kosiorowska KE, Moreno AD, Iglesias R, Leluk K, Mironczuk AM: **Production of PETase by engineered *Yarrowia lipolytica* for efficient poly(ethylene terephthalate) biodegradation**. *Sci Total Environ* 2022, **846**:157358.

38. Moog D, Schmitt J, Senger J, Zarzycki J, Rexer KH, Linne U, Erb T, Maier UG: **Using a marine microalga as a chassis for polyethylene terephthalate (PET) degradation**. *Microb Cell Factor* 2019, **18**:1-15.

39. Weimer A, Kohlstedt M, Volke DC, Nikel PI, Wittmann C: **Industrial biotechnology of *Pseudomonas putida*: advances and prospects**. *Appl Microbiol Biotechnol* 2020, **104**:7745-7766.

40. Kohlstedt M, Starck S, Barton N, Stolzenberger J, Selzer M, Mehlmann K, Schneider R, Pleissner D, Rinkel J, Dickschat JS, *et al*.: **From lignin to nylon: cascaded chemical and biochemical conversion using metabolically engineered *Pseudomonas putida***. *Metab Eng* 2018, **47**:279-293.

41. Brandenberg OF, Schubert OT, Kruglyak L: **Towards synthetic PETtrophy: engineering *Pseudomonas putida* for concurrent polyethylene terephthalate (PET) monomer metabolism and PET hydrolase expression**. *Microb Cell Factor* 2022, **21**:119.

42. •• Sullivan KP, Werner AZ, Ramirez KJ, Ellis LD, Bussard JR, Black BA, Brandner DG, Bratti F, Buss BL, Dong X, *et al*.: **Mixed plastics waste valorization through tandem chemical oxidation and biological funneling**. *Science* 2022, **378**:207(-+).
Inspiring demonstration of a multistep valorization of mixed plastics waste using engineered microbes. Delivers a blueprint for future plastics valorization.

43. Liu P, Zheng Y, Yuan YB, Zhang T, Li QB, Liang QF, Su TY, Qi QS: **Valorization of polyethylene terephthalate to muconic acid by engineering *Pseudomonas putida***. *Int J Mol Sci* 2022, **23**:10997.

44. Wilkes RA, Waldbauer J, Caroll A, Nieto-Domínguez M, Parker DJ, Zhang LC, Guss AM, Aristilde L: **Complex regulation in a**

Comamonas **platform for diverse aromatic carbon metabolism**. *Nat Chem Biol* 2023, **19**:651(-+).

45. Roberts C, Edwards S, Vague M, León-Zayas R, Scheffer H, Chan G, Swartz NA, Mellies JL: **Environmental consortium containing Pseudomonas and Bacillus species synergistically degrades polyethylene terephthalate plastic**. *Msphere* 2020, **5**:1110-1128.

46. Qi XH, Ma Y, Chang HC, Li BZ, Ding MZ, Yuan YJ: **Evaluation of PET degradation using artificial microbial consortia**. *Front Microbiol* 2021, **12**:778828.

47. Bao T, Qian Y, Xin Y, Collins JJ, Lu T: **Engineering microbial division of labor for plastic upcycling**. *Nat Commun* 2023, **14**:5712.

48. Rillig MC, Kim SW, Zhu YG: **The soil plastisphere**. *Nat Rev Microbiol* 2023,1-11.

49. • Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, Acosta DJ, Alexander BR, Cole HO, Zhang Y, *et al*.: **Machine learning-aided engineering of hydrolases for PET depolymerization**. *Nature* 2022, **604**:662-667.
Successful implementation of artificial intelligence to derive superior enzymes for PET breakdown.

50. •• Tournier V, Topham CM, Gilles A, David B, Folgoas C, Moya-Leclair E, Kamionka E, Desrousseaux ML, Texier H, Gavalda S, *et al*.: **An engineered PET depolymerase to break down and recycle plastic bottles**. *Nature* 2020, **580**:216(-+).
Enabling study on engineering PET depolymerases for efficient breakdown of plastic bottles.

51. • Sonnendecker C, Oeser J, Richter PK, Hille P, Zhao Z, Fischer C, Lippold H, Blázquez-Sánchez P, Engelberger F, Ramírez-Sarmiento CA, *et al*.: **Low carbon footprint recycling of post-consumer PET plastic with a metagenomic polyester hydrolase**. *ChemSusChem* 2022, **15**:e202101062.
Interesting work on obtaining novel PET hydrolases from metagenomes. Opens up a rich natural source to obtain novel enzymes from various habitats.

52. Fujiwara R, Sanuki R, Ajiro H, Fukui T, Yoshida S: **Direct fermentative conversion of poly(ethylene terephthalate) into poly(hydroxyalkanoate) by**. *Sci Rep* 2021, **11**:19991.

53. Li A, Sheng Y, Cui H, Wang M, Wu L, Song Y, Yang R, Li X, Huang H: **Discovery and mechanism-guided engineering of BHET hydrolases for improved PET recycling and upcycling**. *Nat Commun* 2023, **14**:4169.

54. Son HF, Cho IJ, Joo S, Seo H, Sagong HY, Choi SY, Lee SY, Kim KJ: **Rational protein engineering of thermo-stable PETase from *Ideonella sakaiensis* for highly efficient PET degradation**. *ACS Catal* 2019, **9**:3519-3526.

55. Bell EL, Smithson R, Kilbride S, Foster J, Hardy FJ, Ramachandran S, Tedstone AA, Haigh SJ, Garforth AA, Day PJR, *et al*.: **Directed evolution of an efficient and thermostable PET depolymerase**. *Nat Catal* 2022, **5**:673(-+).

56. Son HF, Joo S, Seo H, Sagong HY, Lee SH, Hong H, Kim KJ: **Structural bioinformatics-based protein engineering of thermo-stable PETase from *Ideonella sakaiensis***. *Enzym Microb Technol* 2020, **141**:109656.

57. Cui YL, Chen YC, Liu XY, Dong SJ, Tian YE, Qiao YX, Mitra R, Han J, Li CL, Han X, *et al*.: **Computational redesign of a PETase for plastic biodegradation under ambient condition by the GRAPE strategy**. *ACS Catal* 2021, **11**:1340-1350.

58. Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, El Omari K, *et al*.: **Characterization and engineering of a plastic-degrading aromatic polyesterase**. *Proc Natl Acad Sci USA* 2018, **115**:E4350-E4357.

59. Arnal G, Anglade J, Gavalda S, Tournier V, Chabot N, Bornscheuer UT, Weber G, Marty A: **Assessment of four engineered PET degrading enzymes considering large-scale industrial applications**. *ACS Catal* 2023,13156-13166.

60. Pose-Rodriguez J, Ménière Y, Rudyk I, Dossin M, Grilli M, Marsitzky D, Meiser W, Philpott J, Rossatto C, Tassinari T, *et al*.: **Patents for Tomorrow's Plastics Global Innovation Trends in Recycling, Circular Design and Alternative Sources**. European Patent Office; 2021.

61. Knott BC, Erickson E, Allen MD, Gado JE, Graham R, Kearns FL, Pardo I, Topuzlu E, Anderson JJ, Austin HP, *et al*.: **Characterization and engineering of a two-enzyme system for plastics depolymerization**. *Proc Natl Acad Sci USA* 2020, **117**:25476-25485.

62. von Haugwitz G, Han X, Pfaff L, Li Q, Wei HL, Gao J, Methling K, Ao YF, Brack Y, Mican J, *et al*.: **Structural insights into (tere) phthalate-ester hydrolysis by a carboxylesterase and its role in promoting PET depolymerization**. *ACS Catal* 2022, **12**:15259-15270.

63. Chen ZZ, Duan RD, Xiao YJ, Wei Y, Zhang HX, Sun XZ, Wang S, Cheng YY, Wang X, Tong SW, *et al*.: **Biodegradation of highly crystallized poly(ethylene terephthalate) through cell surface codisplay of bacterial PETase and hydrophobin**. *Nat Commun* 2022, **13**:7138.

64. Erickson E, Gado JE, Avilán L, Bratti F, Brizendine RK, Cox PA, Gill R, Graham R, Kim DJ, König G, *et al*.: **Sourcing thermotolerant poly(ethylene terephthalate) hydrolase scaffolds from natural diversity**. *Nat Commun* 2022, **13**:7850.

65. Dierkes RF, Wypych A, Pérez-García P, Danso D, Chow J, Streit WR: **An ultra-sensitive *Comamonas thiooxidans* biosensor for the rapid detection of enzymatic polyethylene terephthalate (PET) degradation**. *Appl Environ Microbiol* 2023, **89**:e01603-22.

66. Gao R, Pan HJ, Kai L, Han K, Lian JZ: **Microbial degradation and valorization of poly(ethylene terephthalate) (PET) monomers**. *World J Microbiol Biotechnol* 2022, **38**:89.

67. Wang YZ, Zhou YM, Zystra GJ: **Molecular analysis of isophthalate and terephthalate degradation by *Comamonas testosteroni* YZW-D**. *Environ Health Perspect* 1995, **103**:9-12.

68. Sasoh M, Masai E, Ishibashi S, Hara H, Kamimura N, Miyauchi K, Fukuda M: **Characterization of the terephthalate degradation genes of *Comamonas* sp strain E6**. *Appl Environ Microbiol* 2006, **72**:1825-1832.

69. Narancic T, Salvador M, Hughes GM, Beagan N, Abdulmutalib U, Kenny ST, Wu HH, Saccomanno M, Um J, O'Connor KE, *et al*.: **Genome analysis of the metabolically versatile *Pseudomonas putida* GO16: the genetic basis for PET monomer upcycling into polyhydroxyalkanoates**. *Microb Biotechnol* 2021, **14**:2463-2480.

70. Choi KY, Kim D, Sul WJ, Chae JC, Zylstra GJ, Kim YM, Kim E: **Molecular and biochemical analysis of phthalate and terephthalate degradation by *Rhodococcus* sp strain DK17**. *FEMS Microbiol Lett* 2005, **252**:207-213.

71. Hara H, Eltis LD, Davies JE, Mohn WW: **Transcriptomic analysis reveals a bifurcated terephthalate degradation pathway in *Rhodococcus* sp strain RHA1**. *J Bacteriol* 2007, **189**:1641-1647.

72. Kim HT, Kim JK, Cha HG, Kang MJ, Lee HS, Khang TU, Yun EJ, Lee DH, Song BK, Park SJ, *et al*.: **Biological valorization of poly (ethylene terephthalate) monomers for upcycling waste PET**. *ACS Sustain Chem Eng* 2019, **7**:19396-19406.

73. Borrero-de Acuna JM, Gutierrez-Urrutia I, Hidalgo-Dumont C, Aravena-Carrasco C, Orellana-Saez M, Palominos-Gonzalez N, van Duuren JBJH, Wagner V, Glaser L, Becker J, *et al*.: **Channelling carbon flux through the *meta*-cleavage route for improved poly(3-hydroxyalkanoate) production from benzoate and lignin-based aromatics in *Pseudomonas putida* H**. *Microb Biotechnol* 2020,2385-2402.

74. Mückschel B, Simon O, Klebensberger J, Graf N, Rosche B, Altenbuchner J, Pfannstiel J, Huber A, Hauer B: **Ethylene glycol metabolism by *Pseudomonas putida***. *Appl Environ Microbiol* 2012, **78**:8531-8539.

75. Franden MA, Jayakody LN, Li WJ, Wagner NJ, Cleveland NS, Michener WE, Hauer B, Blank LM, Wierckx N, Klebensberger J, *et al*.: **Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization**. *Metab Eng* 2018, **48**:197-207.

76. Poulsen JS, Nielsen JL: **Proteomic characterisation of polyethylene terephthalate and monomer degradation by *Ideonella sakaiensis***. *J Proteom* 2023, **279**:104888.

77. Schada von Borzyskowski L, Schulz-Mirbach H, Troncoso Castellanos M, Severi F, Gomez-Coronado PA, Paczia N, Glatter T, Bar-Even A, Lindner SN, Erb TJ: **Implementation of the beta-hydroxyaspartate cycle increases growth performance of *Pseudomonas putida* on the PET monomer ethylene glycol**. *Metab Eng* 2023, **76**:97-109.

78. Kalathil S, Miller M, Reisner E: **Microbial fermentation of polyethylene terephthalate (PET) plastic waste for the production of chemicals or electricity**. *Angew Chem-Int Ed* 2022, **61**:e202211057.

79. Welsing G, Wolter B, Hintzen HMT, Tiso T, Blank LM: **Upcycling of hydrolyzed PET by microbial conversion to a fatty acid derivative**. *Methods Enzymol* 2021, **648**:391-421.

80. Diao J, Hu Y, Tian Y, Carr R, Moon TS: **Upcycling of poly (ethylene terephthalate) to produce high-value bio-products**. *Cell Rep* 2023, **42**:111908.

81. Sadler JC, Wallace S: **Microbial synthesis of vanillin from waste poly(ethylene terephthalate)**. *Green Chem: Int J Green Chem Resour: GC* 2021, **23**:4665-4672.

82. Schaerer LG, Wu RC, Putman LI, Pearce JM, Lu T, Shonnard DR, Ong RG, Techtmann SM: **Killing two birds with one stone: chemical and biological upcycling of polyethylene terephthalate plastics into food**. *Trends Biotechnol* 2023, **41**:184-196.

83. Carniel A, Santos AG, Chinelatto Júnior LS, Castro AM, Coelho MAZ: **Biotransformation of ethylene glycol to glycolic acid by *Yarrowia lipolytica*: a route for poly(ethylene terephthalate) (PET) upcycling**. *Biotechnol J* 2023, **18**:2200521.

84. Pang JF, Zheng MY, Sun RY, Wang AQ, Wang XD, Zhang T: **Synthesis of ethylene glycol and terephthalic acid from biomass for producing PET**. *Green Chem* 2016, **18**:342-359.

85. **European Bioplastics, BIOPLASTICS — Facts and Figures**. *Eur Bioplastics* 2023,, https://docs.european-bioplastics.org/publications/EUBP_Facts_and_figures.pdf.

86. Cui YA, Deng C, Fan LQ, Qiu YJ, Zhao LM: **Progress in the biosynthesis of bio-based PET and PEF polyester monomers**. *Green Chem* 2023, **25**:5836-5857.

87. Wang YH, Xian M, Feng XJ, Liu M, Zhao G: **Biosynthesis of ethylene glycol from D-xylose in recombinant *Escherichia coli***. *Bioengineered* 2018, **9**:233-241.

88. Cabulong RB, Valdehuesa KNG, Ramos KRM, Nisola GM, Lee WK, Lee CR, Chung WJ: **Enhanced yield of ethylene glycol production from D-xylose by pathway optimization in *Escherichia coli***. *Enzym Microb Technol* 2017, **97**:11-20.

89. Alkim C, Cam Y, Trichez D, Auriol C, Spina L, Vax A, Bartolo F, Besse P, François JM, Walther T: **Optimization of ethylene glycol production from D-xylose via a synthetic pathway implemented in *Escherichia coli***. *Microb Cell Factor* 2015, **14**:1-12.

90. Chae TU, Choi SY, Ryu JY, Lee SY: **Production of ethylene glycol from xylose by metabolically engineered *Escherichia coli***. *AIChE J* 2018, **64**:4193-4200.

91. Zhang ZX, Yang Y, Wang YK, Gu JJ, Lu XY, Liao XY, Shi JP, Kim CH, Lye G, Baganz F, *et al.*: **Ethylene glycol and glycolic acid production from xylonic acid by *Enterobacter cloacae***. *Microb Cell Factor* 2020, **19**:1-16.

92. Lu X, Yao Y, Yang Y, Zhang Z, Gu J, Mojovic L, Knezevic-Jugovic Z, Baganz F, Lye G, Shi J, *et al.*: **Ethylene glycol and glycolic acid production by wild-type *Escherichia coli***. *Biotechnol Appl Biochem* 2021, **68**:744-755.

93. Pereira B, Zhang H, De Mey M, Lim CG, Li ZJ, Stephanopoulos G: **Engineering a novel biosynthetic pathway in *Escherichia coli* for production of renewable ethylene glycol**. *Biotechnol Bioeng* 2016, **113**:376-383.

94. Bourgade B, Humphreys CM, Millard J, Minton NP, Islam MA: **Design, analysis, and implementation of a novel biochemical pathway for ethylene glycol production in *Clostridium autoethanogenum***. *ACS Synth Biol* 2022, **11**:1790-1800.

95. •• Luo ZW, Lee SY: **Biotransformation of *p*-xylene into terephthalic acid by engineered *Escherichia coli***. *Nat Commun* 2017, **8**:15689.
Demonstration of a novel pathway to produce the industrial PET monomer terephthalic acid through fermentation.

96. Luo ZW, Choi KR, Lee SY: **Improved terephthalic acid production from *p*-xylene using metabolically engineered *Pseudomonas putida***. *Metab Eng* 2023, **76**:75-86.

97. Kenny ST, Runic JN, Kaminsky W, Woods T, Babu RP, O'Connor KE: **Development of a bioprocess to convert PET derived terephthalic acid and biodiesel derived glycerol to medium chain length polyhydroxyalkanoate**. *Appl Microbiol Biotechnol* 2012, **95**:623-633.

98. Liu P, Zhang T, Zheng Y, Li Q, Su T, Qi Q: **Potential one-step strategy for PET degradation and PHB biosynthesis through co-cultivation of two engineered microorganisms**. *Eng Microbiol* 2021, **1**:100003.

99. Kim HT, Ryu MH, Jung YJ, Lim S, Song HM, Park J, Hwang SY, Lee HS, Yeon YJ, Sung BH, *et al.*: **Chemo-biological upcycling of poly(ethylene terephthalate) to multifunctional coating materials**. *ChemSusChem* 2021, **14**:4251-4259.

100. Cao ZJ, Fu XZ, Li H, Pandit S, Noa FMA, Öhrström L, Zelezniak A, Mijakovic I: **Synthesis of metal-organic frameworks through enzymatically recycled polyethylene terephthalate**. *ACS Sustain Chem Eng* 2023, **11**:15506-15512.

101. Zhang YB, Chen YH, Cao G, Ma XY, Zhou JG, Xu WL: **Bacterial cellulose production from terylene ammonia hydrolysate by *Taonella mepensis* WT-6**. *Int J Biol Macromol* 2021, **166**:251-258.

102. Yoshida S, Hiraga K, Taniguchi I, Oda K: ***Ideonella sakaiensis*, PETase, and MHETase: from identification of microbial PET degradation to enzyme characterization**. *Enzym Plast Degrad* 2021, **648**:187-205.

103. Zhong-Johnson EZL, Voigt CA, Sinskey AJ: **An absorbance method for analysis of enzymatic degradation kinetics of poly (ethylene terephthalate) films**. *Sci Rep* 2021, **11**:928.

104. Lee SH, Seo H, Hong H, Park J, Ki D, Kim M, Kim HJ, Kim KJ: **Three-directional engineering of *Is*PETase with enhanced protein yield, activity, and durability**. *J Hazard Mater* 2023, **459**:132297.

105. Müller RJ: **Biological degradation of synthetic polyesters - enzymes as potential catalysts for polyester recycling**. *Process Biochem* 2006, **41**:2124-2128.

106. Herrero Acero E, Ribitsch D, Steinkellner G, Gruber K, Greimel K, Eiteljoerg I, Trotscha E, Wei R, Zimmermann W, Zinn M, *et al.*: **Enzymatic surface hydrolysis of PET: effect of structural diversity on kinetic properties of cutinases from *Thermobifida***. *Macromolecules* 2011, **44**:4632-4640.

107. Wei R, Breite D, Song C, Gräsing D, Ploss T, Hille P, Schwerdtfeger R, Matysik J, Schulze A, Zimmermann W: **Biocatalytic degradation efficiency of postconsumer polyethylene terephthalate packaging determined by their polymer microstructures**. *Adv Sci* 2019, **6**:1900491.

108. Sagong HY, Son HF, Seo H, Hong H, Lee D, Kim KJ: **Implications for the PET decomposition mechanism through similarity and dissimilarity between PETases from *Rhizobacter gummiphilus* and *Ideonella sakaiensis***. *J Hazard Mater* 2021, **416**:126075.

109. Carr CM, de Oliveira BFR, Jackson SA, Laport MS, Clarke DJ, Dobson ADW: **Identification of BgP, a cutinase-like polyesterase from a deep-sea sponge-derived actinobacterium**. *Front Microbiol* 2022, **13**:1194.

110. Carr CM, Keller MB, Paul B, Schubert SW, Clausen KSR, Jensen K, Clarke DJ, Westh P, Dobson ADW: **Purification and biochemical characterization of SM14est, a PET-hydrolyzing enzyme from the marine sponge-derived *Streptomyces* sp. SM14**. *Front Microbiol* 2023, **14**:1170880.

111. Bollinger A, Thies S, Knieps-Grünhagen E, Gertzen C, Kobus S, Höppner A, Ferrer M, Gohlke H, Smits SHJ, Jaeger KE, Novel A: **Polyester hydrolase from the marine bacterium *Pseudomonas***

***aestusnigri* - structural and functional insights**. *Front Microbiol* 2020, **11**:114.

112. Sagong HY, Kim S, Lee D, Hong H, Lee SH, Seo H, Kim KJ: **Structural and functional characterization of an auxiliary domain-containing PET hydrolase from *Burkholderiales* bacterium**. *J Hazard Mater* 2022, **429**:128267.

113. Gamerith C, Vastano M, Ghorbanpour SM, Zitzenbacher S, Ribitsch D, Zumstein MT, Sander M, Acero EH, Pellis A, Guebitz GM: **Enzymatic degradation of aromatic and aliphatic polyesters by P. pastoris expressed cutinase 1 from Thermobifida cellulosilytica**. *Front Microbiol* 2017, **8**:938.

114. Ronkvist ÅM, Xie WC, Lu WH, Gross RA: **Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate)**. *Macromolecules* 2009, **42**:5128-5138.

115. Xi XX, Ni KF, Hao HL, Shang YP, Zhao B, Qian Z: **Secretory expression in *Bacillus subtilis* and biochemical characterization of a highly thermostable polyethylene terephthalate hydrolase from bacterium HR29**. *Enzym Microb Technol* 2021, **143**:109715.

116. Sulaiman S, Yamato S, Kanaya E, Kim JJ, Koga Y, Takano K, Kanaya S: **Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using a metagenomic approach**. *Appl Environ Microbiol* 2012, **78**:1556-1562.

117. Richter PK, Blázquez-Sánchez P, Zhao ZY, Engelberger F, Wiebeler C, Künze G, Frank R, Krinke D, Frezzotti E, Lihanova Y, *et al.*: **Structure and function of the metagenomic plastic-degrading polyester hydrolase PHL7 bound to its product**. *Nat Commun* 2023, **14**:1905.

118. Pfaff L, Gao J, Li ZS, Jäckering A, Weber G, Mican J, Chen YP, Dong WL, Han X, Feiler CG, *et al.*: **Multiple substrate binding mode-guided engineering of a thermophilic PET hydrolase**. *ACS Catal* 2022,9790-9800.

119. Periyasamy S, Karthik V, Kumar PS, Isabel JB, Temesgen T, Hunegnaw BM, Melese BB, Mohamed BA, Vo DVN: **Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review**. *Environ Chem Lett* 2022, **20**:1129-1152.

120. van Aalst ACA, de Valk SC, van Gulik WM, Jansen MLA, Pronk JT, Mans R: **Pathway engineering strategies for improved product yield in yeast-based industrial ethanol production**. *Synth Syst Biotechnol* 2022, **7**:554-566.