



# Advances in enzymatic and organismal technologies for the recycling and upcycling of petroleum-derived plastic waste

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Biological catalysts are emerging with the capability to depolymerize a wide variety of plastics. Improving and discovering these catalysts has leveraged a range of tools, including microbial ecology studies, high-throughput selections, and computationally guided mutational studies. In this review, we discuss the prospects for biological solutions to plastic recycling and upcycling with a focus on major advances in polyethylene terephthalate depolymerization, expanding the range of polymers with known biological catalysts, and the utilization of derived products. We highlight several recent improvements in enzymes and reaction properties, the discovery of a wide variety of novel plastic-depolymerizing biocatalysts, and how depolymerization products can be utilized in recycling and upcycling.

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## Introduction

The word 'plastics' is an umbrella term used for hundreds of unique materials. Colloquially, plastics often refer to a smaller subset of high-volume and simple composition materials defined by high-volume production and simple compositions such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyvinylchloride (PVC), polyurethane (PUR), and polystyrene (PS) [1,2]. Despite many existing

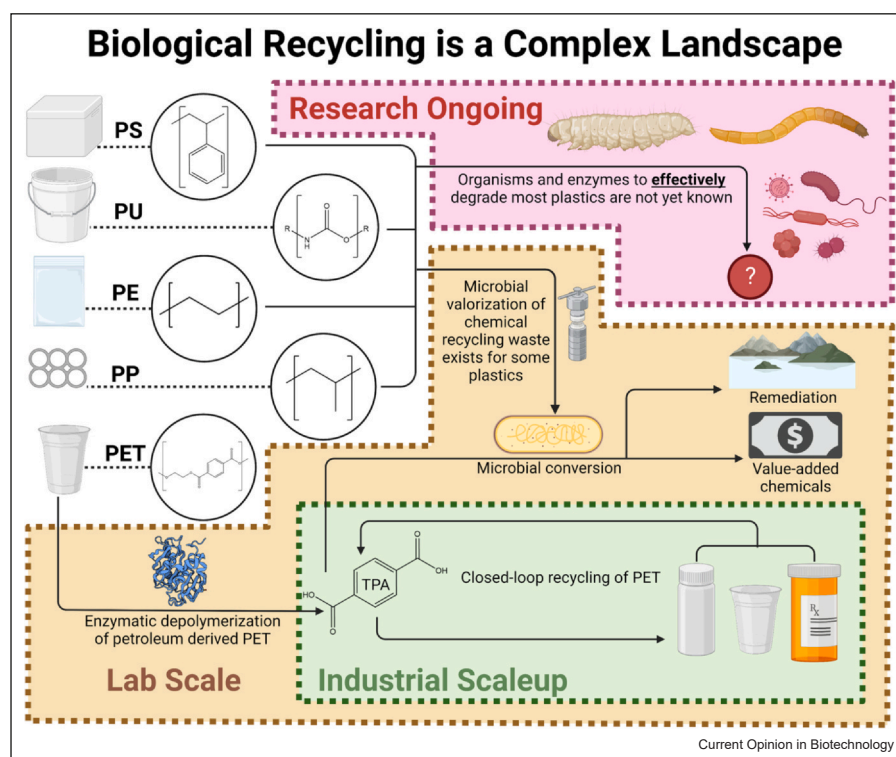
mechanical and chemical plastic recycling technologies available today, the majority of plastic is landfilled or incinerated [3,4]. The sheer growth of this plastic production [5] requires both conventional and novel recycling methods to achieve international emissions and pollution goals [6–9] and a circular economy. In this regard, biological plastic recycling (BPR) is an emerging technology with the potential to address the challenges and limitations of conventional recycling techniques.

BPR encompasses the use of biological catalysts (defined broadly — from proteins to consortia of cells) for the depolymerization of plastics and potential upcycling of generated monomers/oligomers (Figure 1). This broad definition indeed matches the breadth of potential applications. For example, enzymes may serve as great catalysts for industrial depolymerization, whereas organismal catalysts may be better suited for *in situ* bioremediation or for the consolidated upcycling of plastics (Figure 1). In this review, we intend to summarize the current prospects for BPR with a focus on the engineering and discovery of both enzymes and organisms. We will first discuss advances in the biological depolymerization and recycling of PET as it serves as the most mature example in the field and a model for other plastics. After describing the use of biocatalysts to depolymerize the other major commodity plastics, we conclude with enumerating ways in which the depolymerization products of these various plastics can be recycled or upcycled by traditional or biological means. These efforts combine to form a new type of biorefinery around plastics as a circular feedstock (Figure 1).

## Enzymatic depolymerization of polyethylene terephthalate

While BPR of PET is a highly studied approach with industrial demonstrations, it is important to note that this substantial feat is less than 10 years old (Figure 2). A seminal report in 2016 introduced the community to *Ideonella sakaiensis*, a bacterium capable of depolymerizing and assimilating PET via the enzymes PET hydrolase (PETase) (Figure 2a) and monohydroxyethylene-terephthalate (MHET) hydrolase (MHETase) [10,11]. Since this discovery, substantial progress has been made to improve these and other similar biocatalysts as well as optimize the nonbiological parameters for enzymatic PET depolymerization.

Figure 1



*The BPR landscape.* Plastics are a diverse set of polymers, each of which contains different functional groups and properties that influence their recyclability in biological and nonbiological systems. Of the main classes of plastics, PET is the only plastic for which biological recycling has been demonstrated at lab scale and recently industrial pilot scale. The Terephthalic acid (TPA) released by enzymatic PET depolymerization is identical to virgin TPA and can be theoretically recycled repeatedly, this infinite recycling potential is referred to as 'closed-loop recycling'. While not yet being deployed outside of the lab, cell factories, which are able to remediate or upcycle the waste from a variety of chemical recycling processes, have been demonstrated for a variety of polymers, including PE, PP, PS, and PET. Finally, catalysts, which are able to depolymerize PE, PP, PS, and PU, are still under study with many putative enzymes and organisms having been shown to demonstrate limited definitive plastic depolymerization capacity and a larger set of organisms having been shown to have intermediate levels of putative plastic depolymerization capacity.

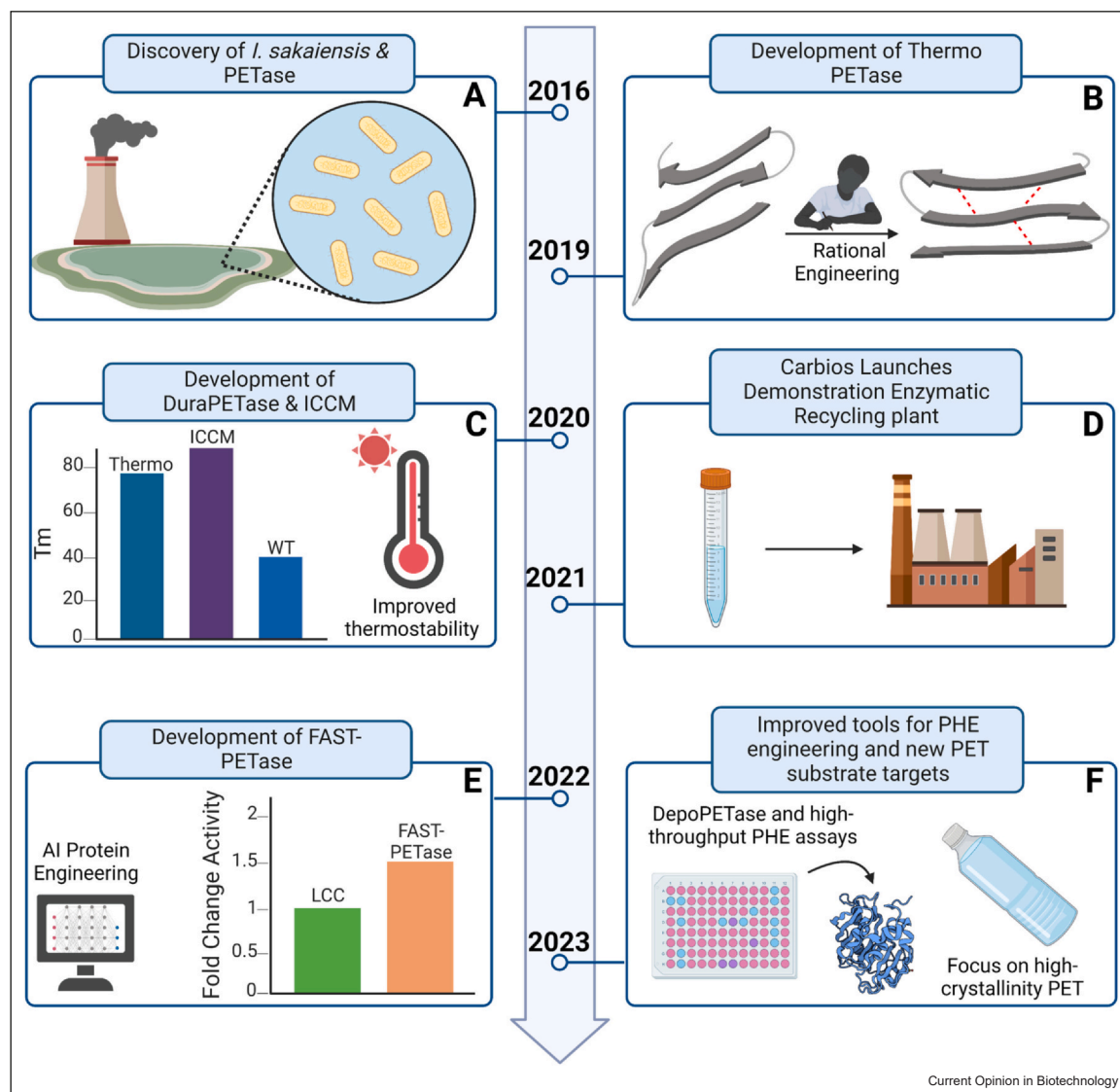
Most of the efforts within the field have focused on identifying and engineering PET-hydrolyzing enzymes (PHEs), the class of enzymes responsible for hydrolyzing the large polymeric chains of PET. The observation that *I. sakaiensis* PETase (isPETase) was very heat-labile led to the first significant engineered PETases, ThermoPETase (Figure 2b) and DuraPETase (Figure 2c). These enzymes exhibited an increased thermostability of 8.81° and 31° increases, respectively, with DuraPETase possessing over 300-fold improvement in activity at mild temperatures [12,13]. Motivated by this potential, three current standout PHEs — ICCM, FAST-PETase, and DepoPETase — have been developed in recent years (Figure 2).

ICCM (Figure 2c) is an engineered variant of the leaf-branch compost cutinase (LCC) developed via the site saturation mutagenesis of active site residues along with other thermostability-enhancing mutations. This

enzyme can hydrolyze 16.7 g of TPA per hour at its ideal reaction temperature of 72°C [14•]. This rate of PET depolymerization was efficient enough to be deployed at industrial pilot scale by the company Carbios (Figure 2d) [15].

Using a convolutional neural net and isPETase as a scaffold, FAST-PETase was developed [16••]. This enzyme incorporated stabilizing mutations from previous engineered PETases in addition to novel AI-predicted beneficial mutations to yield an enzyme that is 1.5-fold more active than ICCM at a lower optimum reaction temperature of 50 °C (Figure 2e). This improvement in enzyme activity was significant considering that ICCM is a highly active, engineered PHE and so relatively small fold changes in activity result in large absolute changes in plastic degradation. Moreover, because the machine learning strategy used in engineering FAST-PETase utilized amino acid microenvironments, the beneficial

Figure 2



*The timeline of enzymatic PET depolymerization.* Enzymatic PET depolymerization has advanced rapidly in the past 7 years: **(a)** the discovery of *Ideonella sakaiensis* and associated PETase and MHETase enzymes kicked off the current wave of research on the enzymatic depolymerization of PET. **(b)** ThermoPETase was the first significantly engineered PETase variant and was developed based on rational engineering that focused on improving the stability of the main alpha-beta-hydrolase beta-sheet based on other more stable hydrolases in the alpha-beta-hydrolase family. **(c)** The development of DuraPETase and ICCM (LCC variant F243I/D238C/S283C/N246M) were the first highly thermostable PHEs, both of which were developed with a combination of computational and rational design strategies. **(d)** ICCM was a sufficiently active PHE that the company Carbios launched the first industrial-scale demonstration plant for enzymatic recycling using that enzyme. **(e)** FAST-PETase was developed using machine learning and was able to make significant improvements in activity compared with other engineered PHEs being 1.6-fold more active than ICCM and led to the discovery of a suite of beneficial mutations that are portable to other PHEs such as ICCM. **(f)** Research continues to improve enzymatic recycling of PET with high-throughput assays for PET depolymerization being used to develop DepoPETase and new technologies such as moist solid reaction, accessory enzymes, surfactants, and hydrophobins being used to tackle more recalcitrant substrates such as high-crystallinity PET.

mutations identified in this study are demonstrated to be portable to other PHE scaffolds such as LCC and ICCM and can further improve their functions [16••].

Most recently, DepoPETase (Figure 2f) has been developed using a novel directed evolution approach [17].

Like FAST-PETase, DepoPETase is most active at 50° and the enzyme performs similarly to FAST-PETase. The novelty of this enzyme stems from the directed evolution technique used during its development. Specifically, the researchers used TPA-OH as a high-throughput fluorescent marker that can be produced

Table 1

**Organisms for the depolymerization of non-PET plastics.** List of organisms recently shown to depolymerize PE, PP, PS, PU, or some combination thereof, including any enzymes heterologously shown to be active on the reported substrate. Organisms with matching values in the consortia column were either discovered or utilized together for plastic depolymerization.

Organism	Polymers	Enzymes (if known)	Part of consortia	Citation (s)
<i>Rhodococcus ruber</i>	PE	-	-	[27]
<i>Acinetobacter guillouiae</i>	PE	-	-	[28]
<i>Klebsiella pneumoniae</i> MK-1	PE	Multicopper oxidase	-	[29]
<i>Sterigmatomyces halophilus</i> SSA1575	PE	-	C1	[30]
<i>Meyerozyma guilliermondii</i> SSA1547	PE	-	C1	[30]
<i>Meyerozyma caribbica</i> SSA1654	PE	-	C2	[30]
<i>Galleria mellonella</i>	PE, PS	Hexamerin and prophenoloxidas	-	[31,35,36]
<i>Zophobas atratus</i>	PE, PP, and PS	-	-	[32,37]
<i>Tenebrio molitor</i>	PE, PP, and PS	-	-	[32,37]
<i>Pseudomonas aeruginosa</i> nov. bt DSCE-CD03	PE, PP	-	C2	[33]
<i>Enterobacter cloacae</i> nov. bt DSCE01	PE, PP	-	C2	[33]
<i>Enterobacter cloacae</i> nov. bt DSCE02	PE, PP	-	C2	[33]
<i>Bacillus thuringiensis</i>	PS	-	C3	[38]
<i>Klebsiella aerogenes</i>	PS	-	C3	[38]
<i>Citrobacter freundii</i>	PS	-	C3	[38]
<i>Serratia marcescens</i>	PS	-	C3	[38]
<i>Stenotrophomonas maltophilia</i>	PS	-	C3	[38]
<i>Pseudomonas aeruginosa</i> spp.	PS	-	-	[38]
<i>Enterococcus faecalis</i>	PS	-	-	[38]
<i>Enterobacter asburiae</i>	PS	-	-	[38]
<i>Acinetobacter</i> sp. AnTc-1	PS	-	-	[39]
<i>Pseudomonas lini</i> JNU01	PS	-	-	[40]
<i>Acinetobacter johnsonii</i> JNU01	PS	Alkane-1-monooxygenase	-	[40]
<i>Cladosporium</i> sp. P7	PU	-	-	[44]
<i>Fusarium</i> sp. IA2	PU	-	-	[45]
<i>Aspergillus</i> sp. MM36	PU	-	-	[45]
<i>F. oxysporum</i> BPOP18	PU	-	-	[45]
<i>Bacillus velezensis</i> GUIA	PU	FAD-binding oxidoreductase	-	[47]

from PHE-derived TPA via Fenton chemistry [17]. This methodology utilizes a fluorescent signal adaptable to many high-throughput studies of PET depolymerization instead of the chromatography-based methods used to develop other evolved PETases such as HotPETase [18]. In the end, ICCM, FAST-PETase, and DepoPETase are all extremely effective at depolymerizing PET under their optimal conditions and can depolymerize many forms of PET packaging in under 24 hours [14•,16••,17]. Despite these advantages, these enzymes are all still limited in their ability to directly utilize high-crystalline PET.

While PHEs are the main enzymes responsible for the depolymerization of PET, their activity can be augmented both in natural and synthetic systems with other proteins. MHETase is an enzyme from *I. sakaiensis* that depolymerizes MHET and aids in more rapid, complete depolymerization of PET to TPA by cleaving MHET and BHET during enzymatic reactions. MHETase also exhibits exo-PETase functionality on PET pentamers with rates up to 0.5  $\mu\text{m}$  per hour [19]. This functionality is especially important in biological conditions wherein a MHETase gene knockout in *I. sakaiensis* reduced the amount of PET depolymerization from 6.9 mg to 1.4 mg in the study under equivalent conditions [20]. In

addition to MHETase, researchers have recently discovered BHETases that likewise assist in the depolymerization of PET. Two engineered variants of the BHETases, ChryBEHTase and BsEst, were able to improve the TPA yield of DepoPETase and FAST-PETase between 1.6- and twofold [21]. Prior efforts have also demonstrated an improvement enabled by physically linking PETase and MHETase [11].

Beyond enzyme improvements, efforts have been made to improve the overall process and complete depolymerization of PET. As noted above, PHEs typically struggle with raw, highly crystalline PET (Figure 2f) [22] and a variety of approaches have been considered to improve this rate. First, cosurface display of PETase and the hydrophobin HFBI was able to increase the depolymerization of highly crystalline PET (crystallinity > 45%) by 328.8-fold at 30°. A similar approach marked with aqueous inclusion of the hydrophobin RolA along with PETase enabled 26% weight loss of PET bottles, a substrate with known difficulty and high crystallinity [23,24]. Second, the use of moist solid reactions in which plastic and enzyme are milled together in a moist environment enabling a much higher surface contact has shown progress [25•]. As an example, this process equalized the ability of *Humicola insolens* cutinase to



depolymerize both amorphous and crystalline PET while also improving enzyme efficiency by 15-fold. Third, surfactants have been used to improve the performance of PHEs via the Sabatier principle with the inclusion of cetyltrimethylammonium bromide improving the turnover rate of LCC by fivefold [26]. These efforts all demonstrate how enzyme discovery, subsequent engineering, and accessory enzymes/process conditions can be combined to allow for depolymerization of PET.

### Expanding biological plastic depolymerization to new polymers

In contrast to PET, there is significantly less known about the presence (or capability) of biological catalysts to target other major plastics (Table 1). Additionally, these polymers are all more difficult targets for BPR due to either a lack of functional groups or stronger polymer backbones. PE and PP are the most common aliphatic plastics with PE being the largest-volume plastic produced globally [2]. The bacterium *Rhodococcus ruber* is considered to be a model organism in the study of PE catabolism with depolymerization occurring at a rate of 1.2% polymer mass per year and validated using  $^{13}\text{C}$ -labeled PE-based assimilation [27•]. Beyond *R. ruber*, several other organisms can colonize on or putatively depolymerize PE (Table 1), including *Acinetobacter guilouiae*, *Klebsiella pneumoniae* MK-1, and a variety of termite-derived yeast strains [28–30]. Despite this documented activity, the enzymes responsible for both PE depolymerization and catabolism are largely unknown. The current proposed mechanism involves alkane hydroxylation and alcohol degradation as key steps preceding hydrolysis. This hypothesis is further supported by an increase in abundance of the enzyme classes responsible for these chemistries across organisms with known and putative PE depolymerization capability.

There are multiple potential routes for polyolefin depolymerization. In contrast to the hydroxylation/hydrolysis process described above, the hexamerin and prophenoxidase salivary enzymes from *Galleria mellonella* can likewise depolymerize PE *in vitro* [31•]. Other insects such as *Zophobas atratus* and *Tenebrio molitor* larvae have been shown to depolymerize PP consuming 0.961 and 2.11 mg of PP per larvae, respectively, over the course of 35 days and with reductions in polymer molecular weight of 9% and 20%, respectively, after passing through the larvae using enzymatic activity determined to be microbiome-dependent [32]. The depolymerization capacity of gut-associated microbiomes has also been seen with microbes isolated from cow dung in which a consortia of *Pseudomonas aeruginosa* and *Enterobacter cloacae* species could depolymerize PE and PP by 64.25% and 63.00% over the course of 160 days [33].

PS is another carbon–carbon backbone plastic, but it differs from PE and PP with the addition of benzyl groups as sidechains. The styrene monomer itself is well-degraded by a variety of bacteria owing to well-characterized metabolic pathways such as the styABCD operon [34]. In recent years, the biodepolymerization of PS has sustained research interest by studying the insects and associated microbes that serve to depolymerize PS plastic in its fully polymerized form. For example, the larvae of *G. mellonella*, *T. molitor*, and *Z. atratus* have all been shown to depolymerize PS with *G. mellonella* showing the most impressive activity demonstrating a 56.12% mass loss after 7.25 days [35–37]. Studies in *T. molitor* and *Z. atratus* likewise showed reduced PS molecular weight and that these host organisms underwent significant microbiome remodeling, thus implicating these microbes as the causative agents of depolymerization. In addition to whole-insect systems, microbes from insects are able to depolymerize PS, including a consortia isolated from *T. molitor* and even individual organisms such as *Acinetobacter* sp. ANtC-1 [38,39]. Environmentally isolated microbes such as *Pseudomonas lini* JNU01 and *Acinetobacter johnsonii* JNU01 also demonstrate PS depolymerization with the alkane-1-mono-oxygenase as a key enzyme seen to function also when expressed heterologously [40].

PU is the second most-produced plastic with a non-carbon-to-carbon backbone after PET. Urethane backbone bonds are compositionally similar to amide and ester bonds and, as such, can be cleaved by a variety of known protein classes such as proteases, lipases, amidase, and esterase enzymes. Major advances in PU biodepolymerization have been made via the development of several model substrates. As an example, carbamates derived from TDI-based PUR have been used to identify two novel PU-hydrolyzing enzymes from PU-contaminated soil [41]. Likewise, a set of commercial hydrolases was screened using model low-molecular-weight urethanes and polyester–PUR dispersions to identify an efficient amidase and esterase pair suitable for weight loss of up to 33% after 51 days [42].

Beyond mining for PU-hydrolyzing enzymes, traditional enrichment culturing and cell adaptation can identify PU-depolymerizing consortia with enhanced production of esterase and amidase enzymes [43–46]. As with some of the other plastics described above, other unique mechanisms for the biodepolymerization exist, including from the deep-sea bacterium *Bacillus velezensis* GUIA that has been shown to degrade PU and the biodegradable plastic polybutylene adipate terephthalate using the oxidoreductase Oxr-1 [47•]. While the BPR of plastics beyond PET is not as well-characterized, these overall examples serve to demonstrate useful starting points for further engineering that will hopefully expand to PE, PP, PS, and PU.

### Upcycling and recycling of degraded plastic

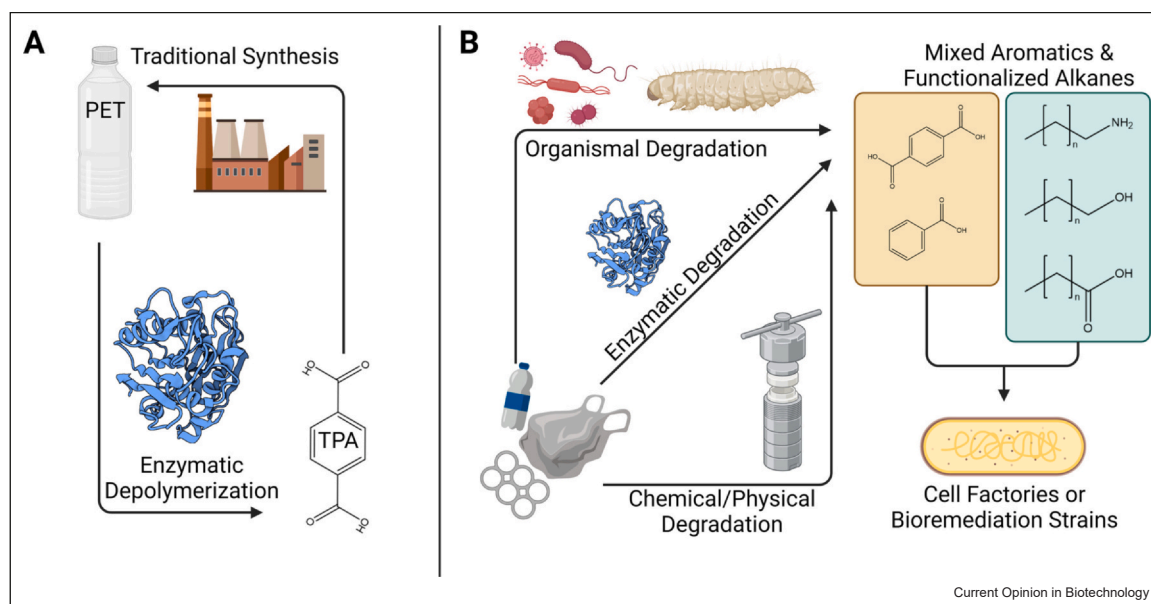
The initial discovery and engineering of biological catalysts for the depolymerization of plastics is certainly raising the prospects for BPR. However, there are factors to consider on the downstream part of this process as it relates to upcycling and recycling. These secondary considerations include many questions such as: (1) what are the downstream monomers liberated from biological depolymerization of plastics? (2) What products provide the lowest energy input and highest yields from these monomers? (3) Are the liberated small molecules homogeneous or heterogeneous? (4) How well will these monomers separate away from the depolymerization reactions? (5) Are there contaminating compounds or heteroatoms that prevent use of these monomers for repolymerization applications? (6) What is the impact of mixed plastic and/or multilayer components in the process? (7) Does upcycling or recycling make more economic sense?

In some cases, especially when the liberated monomer is a drop-in replacement for the original petroleum-derived monomer, an obvious choice is to recycle into new plastics (Figure 3a). This approach seems most feasible for PET depolymerization as the products TPA and EG are chemically identical to their petroleum-derived counterparts. The biological depolymerization–chemical repolymerization cycle for PET has been demonstrated

multiple times at lab scale, including examples where TPA derived from ICCM reactions was used to remake PET [14•] and again when dyed PET packaging was degraded using FAST-PETase to repolymerize into clear, virgin-quality PET with a yield and purity of 94.9% and 97%, respectively [16••]. Outside of the laboratory, companies such as Carbios are already working to implement these processes at industrial scale using LCC PHEs [15]. The main barrier to further deployment of industrial-scale enzymatic PET recycling is cost. Techno-economic analysis for enzymatically recycled PET has estimated the potential for this recycling process to be competitive at \$1.93/kg [48]. This same analysis suggests that while enzymatic recycling benefits from lower energy requirements than virgin PET synthesis, price reductions in enzyme production, improvements in solid loading/pretreatment, and the cheaper separation of depolymerization products are areas that need improvement before enzymatic recycling of PET can become truly cost-competitive.

More complex biological recycling technologies have also been demonstrated for PET. Reductive enzyme cascades have been shown to convert TPA into para-xylylenediamine at a yield of 69%, which can subsequently be used to make new polymers such as polyamides, polyimides, and PURs, thus upcycling/ converting TPA into a more high-value product and

Figure 3



*Downstream applications of biological plastic depolymerization reactions.* Potential downstream applications of BPR are dependent on their chemical outputs. (a) Enzymatic depolymerization of plastics such as PET is able to be directly recycled into new PET as the TPA released during enzymatic depolymerization is identical to its petroleum-derived counterpart. (b) The biological degradation of polymers such as PE, PP, and PS will likely produce a mixture of aromatic and alkane products, regardless of the utilization of biological or nonbiological recycling method. These outputs are not able to be easily regenerated into their parent plastics, but they can be utilized by engineered microbes to convert the plastic derivatives into new commodity chemicals or remediate the waste by converting it into biomass.

diversifying the economic prospects for this biological process [49•]. The use of biological systems for the direct valorization of PET has likewise been explored. A great example of this process is the direct conversion of PET to the biodegradable bioplastic polyhydroxyalkanoate (PHA) by *I. sakaiensis* with a 15% conversion yield [50]. Organisms such as *Acinetobacter baylyi* and *Comamonas testosteroni* are being investigated or engineered for their ability to degrade TPA and other cyclic aromatic compounds to produce commodity chemicals [51,52].

In contrast, the carbon backbone plastics (especially PE and PP) are likely not amenable for direct recycling back into original polymers. The cleavage of the backbone of PE, PP, and PS currently requires the introduction of novel functional groups and heteroatoms (including oxygen). Likewise, the downstream products tend to be a heterogeneous mixture, leading to cellular valorization as a more viable route (Figure 3b). The most impressive example of these microbial cell factories are microbes that have been engineered to valorize the by-products of chemical recycling techniques such as pyrolysis and thermal liquefaction. The yeast *Yarrowia lipolytica* was recently used to convert thermally depolymerized PP into long-chain fatty acids with a conversion rate over 80% [53]. In a similar fashion, *Pseudomonas putida* was first engineered to convert BHET, a model short-chain substrate of PET depolymerization, into beta-ketoadipate at titers of 15.1 g L<sup>-1</sup> with 76% of the mass coming from BHET [54]. A further engineered strain of *P. putida* can assimilate mixed plastic waste from chemical oxidation of PET, PE, and PS to beta-ketoadipate or PHA with over 70% of the mass coming from the plastic-derived feedstocks [55••]. Advances in the conversion of mixed plastic waste will be paramount to the future success of BPR as the majority of consumer products and plastics today are composed of mixed materials and/or are poorly sorted.

## Conclusion and future perspectives

Advances in the biological depolymerization of plastics have greatly expanded the potential for using and up-cycling plastic as part of a plastics biorefinery. The rapid and continuing advances in PET depolymerization, including recent scale-up attempts, are bolstering the ability to discover, engineer, and improve these systems. However, these recent advances should not overshadow the challenges. Many of the identified catalysts are mostly more obscure cellular systems that lack industrial applications and synthetic tools. Moreover, the catalytic enzymes have yet to be fully elucidated. However, there is promise as recently discovered enzymes such as Oxr-1 for the degradation of PU, AlkB for the degradation of PS, and hexamerin/prophenoloxidases for the depolymerization of PE represent great launchpads for enzyme

engineering. Certainly, high-throughput assays such as those used to engineer DepoPETase will speed the engineering and development process.

While biological depolymerization of PET has proven quite successful, efforts to create a biological catalyst for polymers such as PE, PP, PS, and PU lag far behind. The depolymerization of these plastics will likely require complex reaction cascades composed of multiple enzymes and organisms and will require catalysts that are amenable to the heterogeneous depolymerization products. Moreover, these complexities are compounded when considering that many postconsumer packaging and other plastic products are derived from multiple polymer types along with nonplastic additives such as dyes, plasticizers, and even forever chemicals such as per- and polyfluoroalkyl substances. On the flipside, BPR schemes may actually be able to ameliorate these challenges and depolymerize these normally contaminating molecules that intercede in traditional chemical and mechanical recycling.

## Data Availability

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

## 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.

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