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Enzymes, auxiliaries, and cells for the recycling and upcycling of polyethylene terephthalate

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Abstract

Biological recycling and valorization of plastics are promising approaches to solve global plastic waste accumulation. Out of diverse plastic materials, polyethylene terephthalate (PET) is one of the most abundant polymers with rapid development in both biodegradation and product upcycling. In this perspective, we review recent discoveries and engineering of PET-degrading enzymes together with plausible auxiliary pathways, and provide insights on how to construct better parts through systematic bioengineering (metagenome mining, protein design, and directed evolution). Then, we discuss the potential of microbial-based PET degradation and upcycling in either a single host or consortia, as well as bottom-up and top-down methods of microbial consortia engineering using novel synthetic biology tools for enhanced PET circularization.

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Enzymatic degradation, Plastic recycling, Polyethylene terephthalate (PET), Microbial consortia, Protein engineering, Microbiome engineering.

Introduction

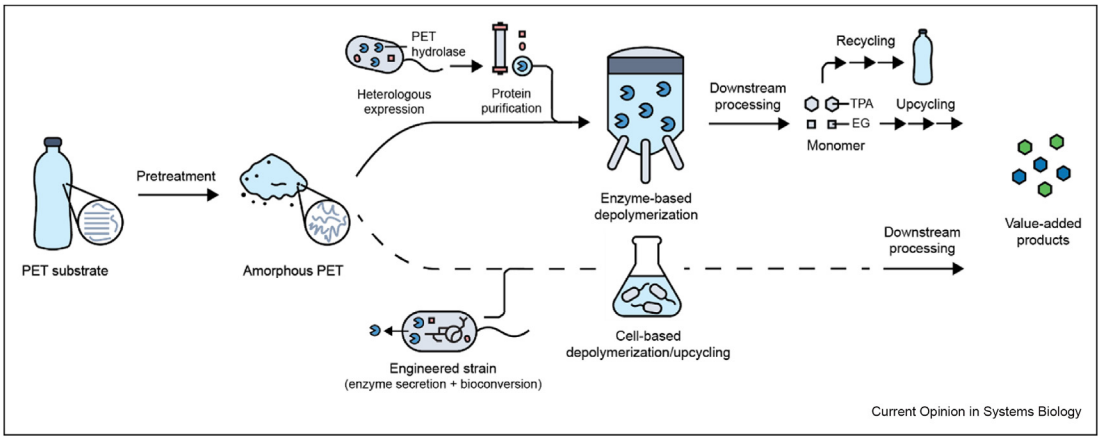
Among various types of plastics, polyethylene terephthalate (PET) stands out as one of the most abundantly used, accounting for 12% of global plastic production in 2021, although only a small fraction is currently recycled [1]. In recent years, enzymatic recycling of PET has witnessed a leap of progress, fueled by its potential in combining distinct advantages of mechanical [2] and chemical recycling [3]. Namely, enzymatic PET hydrolysis exhibits low environmental impact and high retention of product value after recycling. Rapid advancements in the creation of highly active PET-degrading enzymes motivated the development of feedstock pre-treatment, enzymatic PET depolymerization, and product recovery processes to the point where enzymatically recycled products are likely cost-competitive [4] (Figure 1, top). In the first half of the article, we review recent advances in the discovery and engineering of PET hydrolases that lead to such economically viable processes.

Biocatalytic recycling of PET can potentially be accelerated via auxiliary proteins and contained within whole cells (Figure 1, bottom). Cell-based biocatalysis can be cost-effective since enzyme purification steps are bypassed and cofactor regeneration is intrinsic to cells. While cell-based approaches to PET re/upcycling are limited by physiological working temperatures and low tolerance to harsh conditions, they offer opportunities for upcycling of degradation products into new chemicals and materials using endogenous and engineered metabolic pathways. In the second half of the review, we discuss PET hydrolases efficient for PET depolymerization at cell-compatible temperatures, auxiliary systems for PET hydrolysis, and strategies to combine them for cell-based PET circularization and upcycling.

Recent discoveries and engineering of efficient PET hydrolases

Natural bacterial and fungal α/β-hydrolases capable of degrading plant polyesters have been wellsprings of enzymes for PET degradation. Because of similar features of plant polyesters (particularly suberin) with synthetic aromatic polyesters such as PET, these polyester hydrolases can contain intrinsic features useful for PET degradation. These features include 1) wide, lidless substrate binding clefts [5] to accommodate long substrates [6], 2) hydrophobic subsites to facilitate binding to hydrophobic polymers [6,7], 3) flexible catalytic regions adaptable to different substrate structures [8,9], and 4) good stability and activity in extracellular

Figure 1



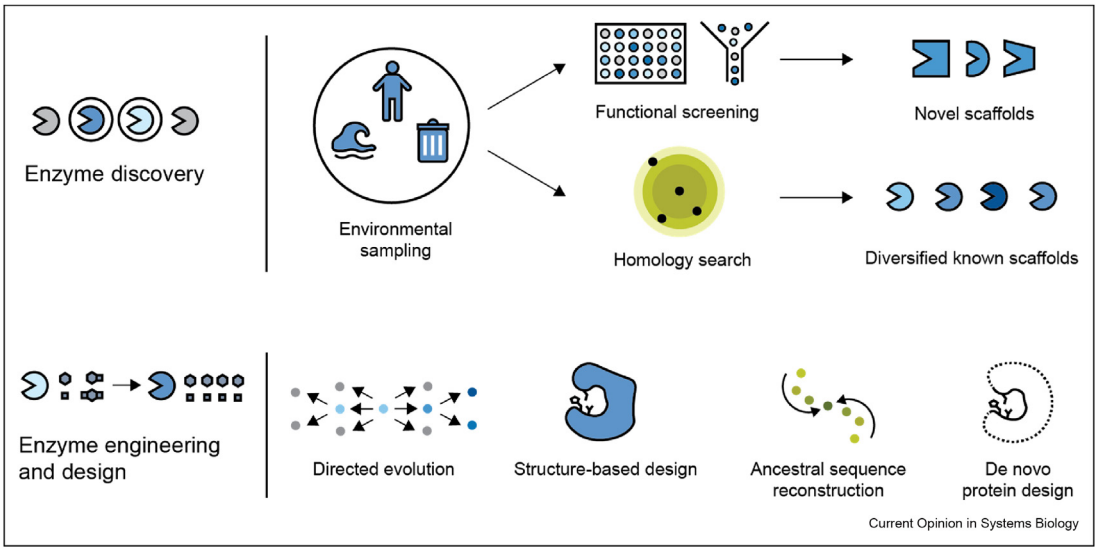
Biocatalytic recycling approaches for polyethylene terephthalate (PET). Post-consumer PET is first mechanically treated to reduce polymer crystallinity for downstream biodegradation. Top, state-of-the-art biocatalytic PET depolymerization relies on purified PET hydrolase enzymes which are functional at high temperatures optimal for degradation. Bottom, an alternative path of PET valorization is a cell-based strategy where engineered recombinant enzymes can be incorporated into engineered microbes. The latter approach can save cost associated with enzyme production/purification and can be linked to upcycling processes, unique to cellular frameworks, to convert PET monomers to value-added products. TPA, terephthalic acid. EG, ethylene glycol.

environments. In the past twenty years, PET hydrolases have been discovered — via environmental sample collection and microbial isolation, as well as metagenomic mining — not only from plant-associated pathogens [10,11] and composts [12,13], but also from PET-containing sediments from a plastic recycling facility [14], marine and terrestrial environments [15,16], and human microbiomes [16]. The widespread distribution of microorganisms containing PET hydrolase genes may point to the ever-growing accumulation of PET in nature, and plastics' role as stable carriers of microorganisms into new environments [17].

Almost all highly active PET hydrolases, including leaf-and-branch compost cutinase (LCC) [12], PHL7 [13], and *Ideonella sakaiensis* PETase (*Is*PETase) [14] were discovered from careful isolation of microbes or metagenomic DNA amplification from environmental samples, coupled with functional screening (Figure 2 and Table 1). These well-characterized scaffolds can serve as queries for metagenome mining of large databases such as MGnify [18] and JGI IMG [19], leading to further discovery of highly active hydrolases such as MG8 from the human saliva metagenome [16]. Initially thought to be present only in a few bacterial phyla [15], the Beckham and McGeehan groups further expanded on the metagenomic investigations of PET hydrolases pioneered by the Streit group. Through this approach, they discovered putative thermostable PET hydrolases with diverse scaffolds relative to conventional PET hydrolases in diverse bacteria [20]. Moving forward, the following multipronged approach was found to be the most general and effective: 1) functional screening of environmental samples, which can lead to discovery of *bona fide* new, active enzyme scaffolds; 2) exploration of large metagenomic databases to expand on the discovered scaffolds; 3) computational tools to enrich for desired properties such as thermostability [20] in selected sequences; and 4) high throughput activity validation and characterization. Beyond efforts in this workflow, ancestral sequence reconstruction was used by the Jackson and Ardevol groups to create putative ancestral enzymes to cutinases/PETase [21]. While the ancestral enzymes are not as active in PET degradation as the state-of-the-art PET hydrolases, the study provided plausible structural explanations of how specific degradation activity against PET, seen in evolutionarily recent PET hydrolases such as *Is*PETase, may arise from promiscuous ancestral cutinases. Notably, the unique traits of ancestral enzymes, such as thermostability, could be informative as plausible engineering trajectories for new enzyme candidates [22] for improved activity at elevated temperatures.

Upon discovery, PET hydrolases were engineered for improved performances through several strategies ranging from mechanistic studies coupled to rational enzyme re-design, high-through screening and directed enzyme evolution approaches, and computation-aided designs (Figure 2). Recent comprehensive reviews have covered engineering efforts of PET hydrolases [23,24]. Efforts have also been made to standardize PET enzymatic degradation protocols to facilitate cross-study comparisons of enzyme performance [25]. Here we highlight approaches used in creating the most efficient PET hydrolases known to date (Table 1).

Figure 2



Discovery and engineering approaches for PET hydrolases. PET hydrolases can be discovered from diverse environmental samples through functional screening of isolated microbial hosts or microbiomes. Using known hydrolases as guide sequences, further PET hydrolases can be computationally mined from metagenomic databases. Discovered PET hydrolases can be engineered for improved performance. Major approaches in the field are: rational design based on available biochemical information to improve activity and stability; and high-throughput screening and directed evolution of mutagenized enzyme libraries. Both approaches are accelerated through the emergence of *de novo* protein design tools and machine learning algorithms. Ancestral sequence reconstruction can further provide access to PET hydrolases with thermotolerant characteristics.

In the first example, leaf-and-branch compost cutinase (LCC) was engineered via rational design to the point of industrial utility by the André/Duquesne/Marty groups [6]. Through substrate docking and analyses of putative contact surfaces and installation of a stabilizing disulfide bridge, the groups identified quadruple mutants of LCC (particularly LCC^{ICCG}) with increased thermostability (T_m +10 °C compared to wild-type LCC) and improved degradation of pretreated PET waste (82−85% conversion in 20h compared to 53% when wild-type LCC was used). The quadruple mutants of LCC are widely acknowledged as the current best thermostable PET hydrolases.

In the second set of examples, polyester hydrolase PHL7, the recently discovered metagenome-derived PET hydrolase, was engineered via rational design enabled by detailed biochemical characterizations [26,27]. The Zimmermann/Sträter/Sonnendecker groups (who discovered PHL7) obtained a co−crystal structure of PHL7 with the hydrolysis product, terephthalic acid (TPA). Together with molecular simulations and functional measurements, they concluded that subsite I of the hydrolase, where TPA binds, is majorly responsible for the productive binding of the enzyme to PET [26]. Mutagenesis of residues surrounding subsite I led to the identification of $PHL7^{L210\ T/V}$ with better PET hydrolytic activity than the wild-type enzyme. The Liu/Bornscheuer/Wei groups obtained further co−crystal structures of PHL7 with PET substrate analogs and found that small substrates (mono(2-hydroxyethyl)terephthalate or MHET; and bis(2-hydroxyethyl)terephthalate or BHET) can engage in non-productive binding modes within the enzyme active site, consistent with activity data that MHET can inhibit PET hydrolases [28]. Molecular dynamics (MD) simulations coupled with structural data enabled the groups to decipher residues involved in productive binding of oligomeric PET to PHL7, leading to the discovery of a subsite I double mutant, $PHL7^{L92F/Q94Y}$, with excellent PET hydrolytic activity [27]. $PHL7^{L92F/Q94Y}$ is nearly optimally thermostable (current T_m 79°C; ideal T_m 85°C or more [27]) and was shown to be even more active than LCC^{ICCG} in degrading 15% crystalline PET; therefore it is a promising enzyme candidate for industrial-scale PET recycling.

In the last examples, *Is*PETase — the most extensively studied PET hydrolase in recent years — was engineered via machine learning and high-throughput screening to produce FAST-PETase [29] and HotPETase [30], respectively. Built upon ThermoPETase [31] developed by the Lee and Kim groups, FAST-PETase was created by the Alper group via combinatorial evaluations of mutations predicted to confer stability, based on self-supervised machine learning trained on protein structures in the Protein Data Bank. FAST-PETase exhibited one of the highest PET-degradation activities among known PET hydrolases at lower

Table 1

Discovery, enzyme engineering approaches, and key improvements after engineering of selected PET hydrolases.

Wild-type enzyme	Origin	State of the art of engineered variants	Enzyme engineering approaches	Improved properties compared to the wild-type enzyme	References
Thermophilic enzymes					
LCC[a]	Leaf-and-branch compost metagenome	LCC^{ICCG}	Structure-guided engineering	–9.3 °C T_m increase (84.7 to 94 °C) Conversion increase (53% to 82%) for the degradation of post-consumer PET	[6]
PHL7	Compost metagenome	$PHL7^{L210T}$	Structure-guided engineering	2–3°C T_m increase (79.1 to 81–82°C) Activity increase (14% to 39%), achieving better degradation of amorphous PET film (90% to 100%)	[26]
*Ca*PETase	*Cryptosporangium aurantiacum*	*Ca*PETase M9	Structure-guided engineering	–16.7°C T_m increase (66.5 to 83.2°C) 41.7-fold increase of specific activity for the degradation of post-consumer PET powder Versatile for use in both thermophilic and mesophilic conditions	[22]
Mesophilic enzymes					
*Is*PETase	*Ideonella sakaiensis 201-F6*	ThermoPETase	Structure-guided engineering	–8.8°C T_m increase (48.8 to 57.6°C) 14-fold activity enhancement at 40°C over 72 h	[31]
		FAST-PETase	Machine learning	–14.6°C T_m increase (48.7 to 63.3°C) 142-fold more monomers released for the degradation of post-consumer PET at 50°C over 24 h	[29]
		HOT-PETase	Directed evolution	–36.5°C T_m increase (46 to 82.5°C) Four-fold more monomers released for the degradation of post-consumer PET at 40°C over 50 h Versatile for use in both thermophilic and mesophilic conditions	[30]
MG8	Human saliva metagenome	$MG8^{F250A}$	Structure-guided engineering	Similar T_m for wild-type and F250A enzymes (54°C at 0.15M NaCl; 62°C at 4M NaCl) Three-fold increase for BHET hydrolysis; no improvement for crystalline PET degradation	[16]
Mors1	*Moraxella* sp. *TA144*	–	–	–T_m 52°C– 2.5 % weight loss of amorphous PET film in 10 days	[46]

[a] Ranked by the melting temperature of the wild-type enzyme.

temperatures (30–40°C), outperforming ThermoPETase by up to 2.5-fold (in terms of monomers released) [29]. HotPETase, also built upon ThermoPETase, was discovered by the Green group through a high-throughput screening workflow using enzymes in cell lysates and activity measurements via ultra-performance liquid chromatography. The workflow enabled the group to screen large enzyme libraries (~2,000 variants in 2 days), after which beneficial mutations can be combined using DNA shuffling. The resulting HotPETase showed excellent PET-degradation activity across different temperature ranges (40–70°C) even outcompeting LCC^{ICCG} at shorter reaction times and high-temperature conditions [30]. Recent advancements in protein folding and design, including deep learning approaches such as ProteinMPNN [32,33], also pose high potential in enzyme engineering especially for thermotolerant characteristics. While these *Is*PETase variants are more prone to thermal inactivation than LCC^{ICCG} due to their lower thermal stability [30], they may serve as good starting points for the development of PET-degradation platforms in whole cells due to their catalytic properties at lower temperatures where the enzyme can be continually regenerated.

Overcoming the physical barrier of degrading semi-crystalline PET

A physical roadblock to biocatalytic recycling of PET is the semi-crystalline nature of consumer-grade PET. In crystalline PET, the polymer chains are packed in tight unit cells (interchain distances of ~5 Å [34]) and assume an extended *trans* conformation, with the OC-CO dihedral angle (Ψ) of the ethylene glycol units of ≈180° [35]. Amorphous PET is less dense [36] and has low degrees of chain orientation, with ~90% of the chains assuming a *gauche* conformation [37] ($\Psi \sim 70°$ [35]). While PET subunit conformations may not affect enzymatic recognition directly [37], the resulting higher polymer density of crystalline PET has proven to be impenetrable by PET hydrolases, necessitating the development of pre-treatment steps to amorphize (semi)crystalline PET.

As the industrially relevant example, the André/Duquesne/Marty groups used extrusion, cryo-grinding, and micronization to convert PET to completely amorphous forms and increase surface areas for efficient enzymatic degradation by LCC variants [6]. Alkaline treatments under ambient temperatures were also shown to reduce PET crystallinity and facilitate enzymatic degradation of PET [38]. Furthermore, the Syrén group reported an intriguing microwave-based pre-treatment method to generate PET oligomers holding the *trans* conformation (typically associated with crystalline PET) [39]. *trans* PET oligomers can be degraded selectively using an engineered $IsPETase^{S238A}$ [39].

State-of-the-art thermostable PET hydrolases such as engineered LCC and PHL7 now have little trouble degrading amorphous PET to completion, but a few considerations remain. While reaction temperatures higher than the glass transition temperature of PET (T_g, ~70°C) can promote mobility of amorphous PET, enzymatic degradation is best performed at temperatures slightly below T_g to avoid extensive thermally induced crystallization of PET at $T > T_g$ [37,40]. It is further known that water can act as a plasticizer and reduce T_g of surface-layer PET in aqueous environments down to mesophilic temperatures (<40°C), suggesting that high mobility at the amorphous surface of PET and enzymatic degradation using mesophilic PET hydrolases are possible. Other thermostable PETases such as TfCut2 were also explored and used as starting point for engineering campaign [41,42]. Recently, the Kim Lab reported a new thermostable PET hydrolase from *Cryptosporangium aurantiacum*, CaPETase, which exhibited near complete decomposition of post-consumer PET at 55°C [22].

Mesophilic and psychrophilic PET hydrolases

Multiple studies have demonstrated the superiority of thermostable PET hydrolases over mesophilic (moderate temperature) and psychrophilic (low temperature) PET hydrolases in degrading PET. It is estimated that the degradation efficiency of wild-type *Is*PETase at 30–40°C is at best 0.2–1.0% of that of thermophilic hydrolases operating at ~70°C [43]. FAST-PETase is at least one order of magnitude more efficient than wild-type *Is*PETase at 30–40°C [29] and can partially overcome the rate deficit compared to thermophilic hydrolases. Nevertheless, improvements to the catalytic efficiency of mesophilic and psychrophilic PET hydrolases are clearly needed for enzymatic PET-degradation at ambient, cell-compatible temperatures to become practically useful.

Beyond *IsPETase*, several mesophilic and psychrophilic PET hydrolases have recently been characterized [44,45]. Notable examples — as they were demonstrated to be more active than *Is*PETase at low/warm temperatures — are the aforementioned CaPETase, Mors1, and MG8. Mors1, a hydrolase from the Antarctic bacteria *Moraxella* sp. Strain TA144, contains an additional disulfide bond not present in other PET hydrolases, which may allow the enzyme to retain good activity at lower temperatures. At 25°C, Mors1 caused ~1.3-fold more PET weight loss than wild-type *Is*PETase [46]. Our group discovered MG8, originated from the human saliva metagenome, through mining of human metagenomes. MG8 is drastically more active than wild-type *Is*PETase (46-fold more product released, measured from combined MHET + TPA) and an engineered PETase variant DuraPETase [47] (1.6-

fold more product released) at 37°C [16]. Both Mors1 and MG8 likely possess higher active site flexibility than thermophilic hydrolases, which is a hallmark for enzyme adaptation for function at low temperatures [8]. MD simulations revealed that Mors1 have high flexibility at loop regions surrounding the active site [46]. MG8 contains an elongated (three additional residues) catalytic histidine loop not found in other known PET hydrolases, and the longer catalytic loop was shown to be critical to the enzyme's efficient function [16]. These PETase discovery and engineering efforts underscore an evolving platform of biochemical PET depolymerization (Figure 2).

Biological auxiliary parts to promote PET degradation

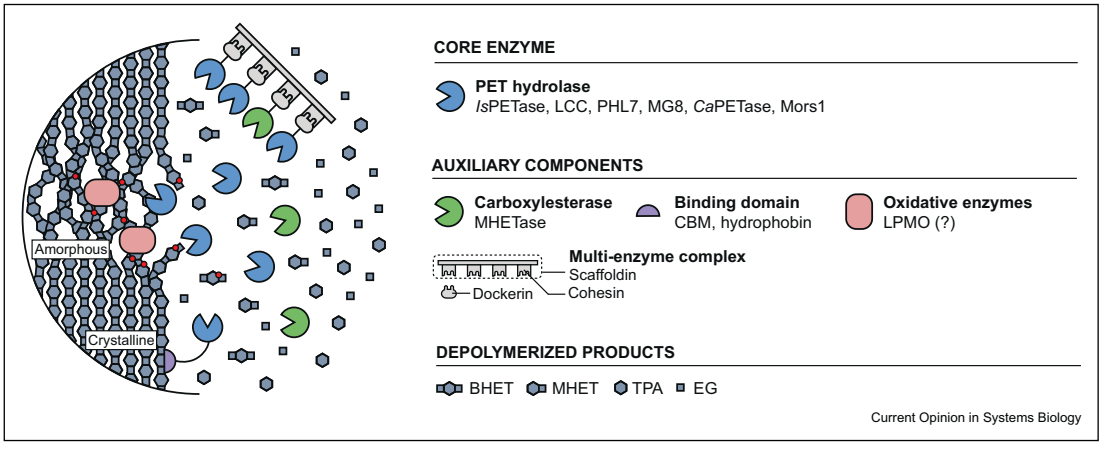
In nature, multi-enzyme assemblies are used to degrade recalcitrant polymers, such as lignocellulose and chitin, and might be repurposable for synthetic polymer degradation. Cellulosomes, for instance, contain a scaffoldin subunit which recruits multiple cellulolytic enzymes via dockerin-cohesin modules [48]. Scaffoldin further contains a carbohydrate-binding module (CBM), enabling targeting of the cellulosome to substrates. The synergistic efforts of different domains and components allow the breaking down of crystalline and hydrophobic cellulose, motivating researchers to apply a similar concept to PET degradation. Here we review limited successes in the applications of auxiliary parts to PET hydrolases for accelerating PET degradation (Figure 3).

The most well-established helpers to PET hydrolases are lidded carboxylesterases, which drive PET degradation through the removal of MHET, a PET-degradation intermediate and an inhibitor of PET hydrolases. Carboxylesterases from *Thermobifida fusca KW3* and *I. sakaiensis*, known as TfCa and MHETase, respectively, were shown to promote PET degradation by *T. fusca* cutinase and *Is*PETase [14,28]. MHET-mediated inhibition is relieved when degradation is performed at higher temperatures. Therefore, a carboxylesterase is not needed for thermostable PET hydrolases and thermophilic PET degradation, but is a useful partner for PET degradation at low/warm, cell-compatible temperatures.

CBMs, particularly type A, can bind to hydrophobic, flat polymer surfaces [49], and were used as fused domains to PET hydrolases to promote enzyme adsorption to PET surface. In addition to CBMs, hydrophobins [50] and polyhydroxyalkanoate-binding modules [51] have been explored as PET-binding domains. While some successes were demonstrated (summarized in Ref. [23]), a recent study suggested that CBM-PET hydrolase fusions may not be an effective strategy in high-solid loading conditions (>10 wt%), which are relevant to industrial applications [52]. Moreover, too tight binding of PET hydrolases to PET is known to obstruct the enzyme turnover and reduce their efficiency. To this end, a study conducted by the Yanyan Wang and Zefang Wang research groups demonstrated a yeast co-display strategy to tether *Is*PETase and *Trichoderma reesei* hydrophobin at the cell surface [53]. Displayed hydrophobins at the cell surface enabled proximal placement of PET hydrolases near PET, but the enzyme movement and catalysis were not as restricted as when they were fused directly to hydrophobins. The whole-cell biocatalyst showed >300-fold improvements in turnover rates compared to when purified *Is*PETase was used, highlighting how PET-binding proteins can be used in conjunction with cells to degrade PET.

Other viable strategies inspired by natural multi-enzyme complexes are the use of scaffolds and

Figure 3



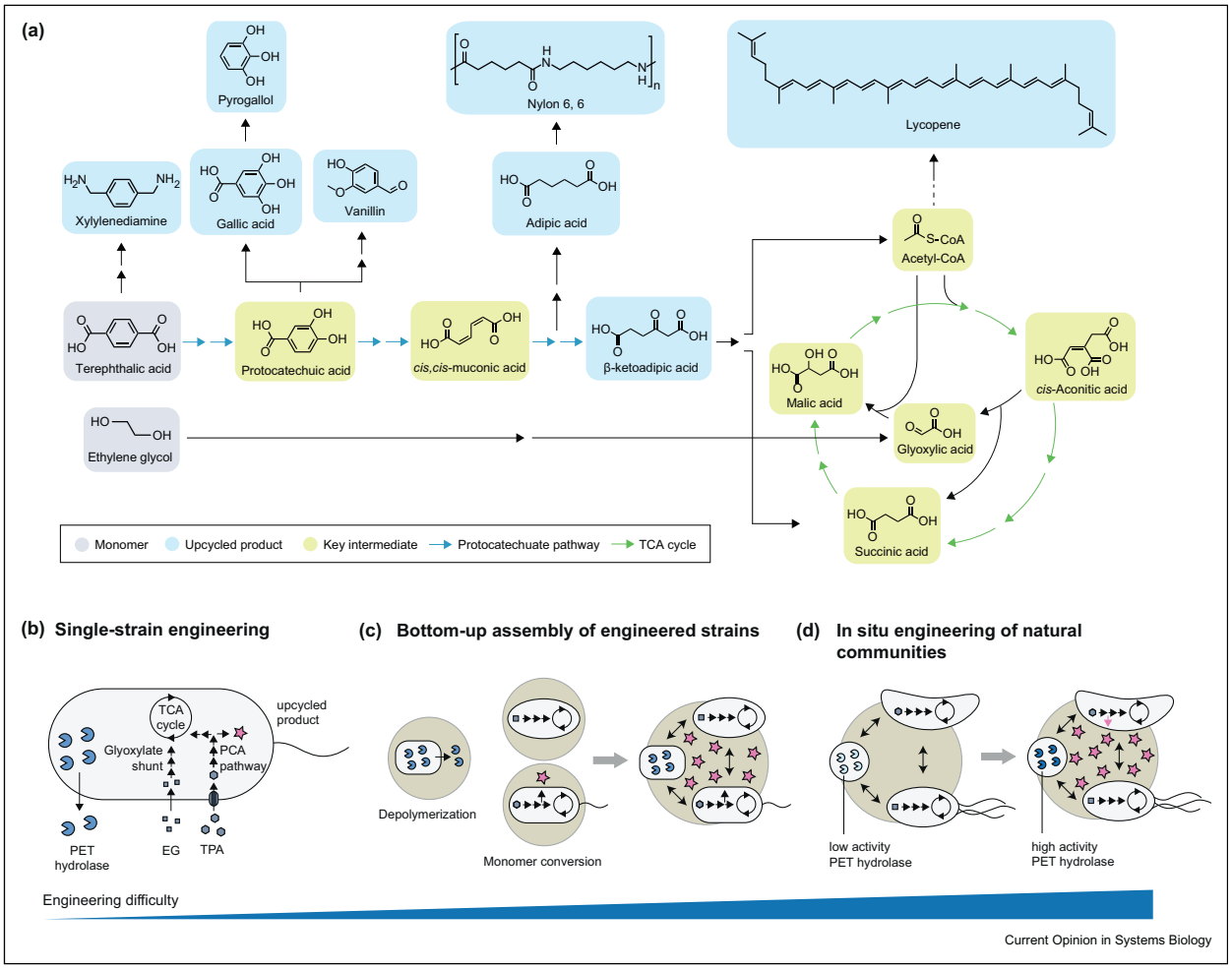
Biological PET degradation can be enhanced by auxiliary modules. Local concentrations of core PET-degrading enzymes on PET surface can be increased via modular binding partners or scaffold proteins, and via incorporation of hydrophobic-moiety binding motifs such as carbohydrate-binding modules or hydrophobins to core enzymes. Oxidative enzymes may be used in conjunction with PET hydrolases to cause further breakage to crystalline PET. Carboxylesterases can relieve inhibition of PET hydrolases by degradation intermediates and promote further PET degradation.

oxidative enzymes to assist with PET degradation. The Han group used scaffoldin protein to assemble CBM3 with *Is*PETase and *Candida antartica* lipase B and achieved a 6.5-fold increase in weight loss on crystalline PET [54]. The Guo group showed that lytic polysaccharide monooxygenase (LPMO) from white-rot fungus *Pycnoporus coccineus* (*Pc*AA14A) can improve the efficiency of PET degradation by *Is*PETase, though the degradation promotion stemmed from PET-binding properties of LPMO (which promoted accumulation of PETase on PET) instead of its oxidative activity [55]. All in all, the use of oxidative enzymes to degrade recalcitrant polymers including PET is conceptually promising, but plagued by the lack of definitive characterizations of enzymes and reactions involved.

Assembly of PET bioconversion parts into the microbial host

One challenge in the PET recycling system is the valorization of degraded products into fine chemicals or functional materials. To date, only a few examples have been developed via *in vitro* biocatalytic upcycling of PET beyond TPA and ethylene glycol (EG) hydrolyzate. Jiang, Wei, and Dong groups demonstrated a reconstitution of PET hydrolyzate into calcium terephthalate [56], a raw material for battery electrodes. Using TPA and MHET as starting materials, the Kanjapur group combined carboxylic acid reductase (CAR) and ω-transaminase (TA) reaction cascades to enzymatically produce p-(aminomethyl)-benzoic acid and p-xylylenediamine [57]. Alternatively, cell-based strategies (Figure 4 and Table 2)

Figure 4



Cell-based assembly for PET degradation and upcycling. Incorporation of PET-degradation modules provided a simple path for conversion of PET into valuable products. **(a)** Metabolism of terephthalic acid (TPA) and ethylene glycol (EG) into industrially relevant products (blue boxes) or central carbon metabolism. TPA usually gets catabolized into protocatechuic acid (PCA) which can be branched into the desired products (e.g. vanillin, gallic acid, and adipic acid) or further converted into *cis*,*cis*-muconate, and β-ketoadipate, then routed into the Embden-Meyerhof-Parnas (EMP) pathway. EG, on the other hand, can be incorporated into glyoxylate shunt of the EMP pathway. See Table 2 for detailed information. **(b–d)** These reaction cascades could be assembled into microbial cell factories using multiple strategies: **(b)** assimilation of all pathways into a single organism; **(c)** division of metabolic labor into multiple hosts, then combining into a synthetic consortium; or **(d)** incorporation of PET-degrading/utilizing pathways into natural microbial consortia via synthetic biology tools.

Table 2

Selected upcycling strategies of PET and degraded monomers.

Substrate	Product	Biochemical systems	Enzymes/pathways	References
PET hydrolyzate[a]	Vanilin (10.3 mg/L)	*E. coli*	tphAabc, dcddh, comt, sfp	[63]
PET hydrolyzate[b]	Lycopene (1.3 mg/L)	*Rhodococcus jostii* strain PET (RPET)	A carotenoid biosynthetic pathway (crtEBI, Δcrtl-b)	[60]
PET hydrolyzate[b]	Polyhydroxyalkanoate (PHA) (637.30 mg/L)	Co-culture of *Pseudomonas putida*	A TPA cluster; A PHA biosynthetic pathway (phaG, alkk, phaC1, phaC2, ΔphaZ, ΔfadBA, ΔfadBAxE)	[68]
PET hydrolyzate[b]	Muconic acid (4.73 g/L)	Co-culture of *Pseudomonas putida*	Cell for TPA-to-catechol conversion: tpa, aroY, ecdB, ΔcatA, ΔcatRBC. Cell for catechol to muconic acid conversion: catA, ΔcatRBC	[68]
TPA	Hydroxyalkanoyloxy-alkanoates (35 mg/L)	*Pseudomonas umsongensis* GO16	rhlA	[78]
TPA	*p*-xylylenediamine (69 % yield)	*In vitro*	CAR, TA, PPK12, GDH, PPase	[57]
TPA	Adipic acid (115 mg/L)	*E. coli*	tphA1, tphA2, tphB2, dcddh, aroY, kpdB, catA, and bcER	[64]
TPA	Protocatechuic acid (431.5 mg/L)	*E. coli*	tphAabc, tphB	[65]
TPA	Gallic acid (459.3 mg/L)	*E. coli*	tphAabc, tphB, pobA	[65]
TPA	Pyrogallol (138.7 mg/L)	*E. coli*	tphAabc, tphB, pobA, lpdC	[65]
TPA	Muconic acid (383.7 mg/L)	*E. coli*	tphAabc, tphB, aroY, catA	[65]
TPA	Vanillic acid (235.4 mg/L)	*E. coli*	tphAabc, tphB, hsOMT	[65]
MHET	*p*-(aminomethyl)benzoic acid (70 % yield)	*In vitro* enzyme cascade	CAR, TA	[57]
BHET	β-ketoadipic acid (15.1 g/L)	*Pseudomonas putida* KT2440	ΔhsdMR, tphA1, tphA2, tphA3, tphB, tphE6, fpvA, tpaKRHA1, glcDEFG, pp_3749, ΔgclR, PETase, MHETase	[62]
EG	Glycolic acid (98.6 % molar yield)	*Gluconobacter oxydans* KCCM 40109	–	[65]

[a] PET was depolymerized with enzymatic process using engineered LCC enzymes.

[b] PET was depolymerized with alkaline lysis.

provide a versatile route for PET upcycling in several aspects, including the ability to regenerate energy and cofactor and bypassing enzyme purification step into *in vivo* recombinant protein expression in a one-pot manner. With TPA and EG as starting carbon sources for cellular utilization, these monomers could be catabolized via the protocatechuic acid (PCA) pathway or glyoxylate shunt to be converted into other desired compounds through central carbon metabolism [58,59]. These technologies could be developed by mining the microbes with functional enzymes or pathways for TPA/EG degradation [60] or by engineering known pathways into domesticated organisms [61]. Recently, upcycling through engineered microbes provided sustainable production of diverse chemicals such as β-ketoadipate, adipic acid, vanillin, pyrogallol, and lycopene [60,62–65].

In the bottom-up approach of synthetic biology, construction of an engineered microbial strain that is capable of every task is arduous and could be highly challenging. Introducing novel functions to a microbe, in the form of recombinant enzymes, may lead to disruption of existing functions necessary for PET degradation. Therefore, different engineered strains could be constructed in parallel and assembled into a synthetic microbial consortium with segregated modules embedded in different organisms. The PET-degrading enzymes were previously shown to be secreted into the extracellular matrices [66,67] which provide additional modes of engineering. A recent work from Lu and Collins groups showed that TPA and EG catabolic activities could be divided into two *Pseudomonas putida* co-culture and yielded faster assimilation of PET hydrolyzates compared to that of monoculture [68]. However, maintaining synthetic microbial consortia remains challenging as they usually lead to ecological failure due to natural competition across species [69]. Therefore, novel synthetic biology tools to stabilize the synthetic consortia could be implemented to sustain the microbial co-cultures. Recent work from Alper and Nelson groups demonstrated that tripartite microbial consortia could be stably maintained using hydrogel-immobilized parts, also termed Engineered Living Materials (ELMs) [70]. Additionally, mining for stable consortia with partial properties (e.g. substrate utilization) could be a shortcut where microbiome engineering tools could be applied to encompass new functions [71,72].

Finally, novel tools in synthetic biology have accelerated the engineering potential of domesticated organisms. Novel platforms such as Adaptive Laboratory Evolution (ALE) could be implemented to improve performance in certain reaction environments [73,74]. With diverse tools to engineer and regulate organisms (including non-model) such as thermophilic *Clostridium thermocellum* [75] and *Geobacillus* species [76], one can express and maintain cell-based systems at elevated temperatures to improve the biochemical reactions that occur at the initial PET-degradation step. To minimize carbon yield losses, novel carbon-conserving pathways could also be implemented for improved conversion efficiency [77]. Altogether, cell-based systems may provide an economical route for PET cycling platforms — both degradation and upcycling — empowered by protein discovery and engineering, processing engineering, and synthetic biology tools.

Outlook

Multiple PET plastic recycling approaches are currently employed at an industrial scale, with biocatalytic recycling poised to be a key future player. Approaches that use diverse waste streams and tolerate impure feeds will allow more PET forms to be recycled. To this end, the selectivity of PET hydrolase enzymes toward PET among mixed materials provides a clear advantage over other recycling tools. While efficient PET hydrolases now exist for industrial and commercial uses [6,25], future work can improve the catalytic efficiency and speed of the enzymes (which will reduce process costs); ensure enzymes can withstand contaminants from diverse feedstocks; and crack the problem of degrading crystalline PET, which will reduce cost and energy use associated with pretreatment steps. Auxiliary parts associated with PET hydrolases are currently underdeveloped and can be further explored to assist with depolymerization.

PET hydrolases can be used in living cells equipped with degradation auxiliaries and enzymatic cascades to convert degradation products into a variety of chemicals and materials. Further discovery and engineering of mesophilic and psychrophilic PET hydrolases will pave way for such an endeavor. As cells have limited tolerance toward chemical changes in their growth conditions, building smart genetic circuits to not only promote PET depolymerization, but also detoxifying degradation (by)products and reducing the metabolic burden will be crucial. The use of microbial consortia with division of labor and specialization is a promising approach to create sustainable PET-degrading cells. Opportunities are abound for new genetic and genome engineering tools which enable maintenance of stable natural and synthetic co-cultures, and the incorporation of new functionalities into natural consortia and non-model microbes.

Declaration of competing interest

T.W. and C.U. have filed a patent covering the use of MG8 and its variants for PET degradation. All other authors declare no competing interests.

Data availability

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

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