

Bio-upcycling PET Waste: Advances in Enzymatic Hydrolysis and Biosynthesis of Value-Added Products

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Abstract

With excellent mechanical properties and chemical stability, poly (ethylene terephthalate) (PET), an engineering plastic, is widely applied in textiles and packaging. However, the widespread use and low biodegradability of PET have resulted in significant environmental pollution. Recent advances in PET hydrolase discovery and engineering have driven the rapid advancement of PET bio-recycling, while efficient PET hydrolases can depolymerize PET into monomers under mild conditions, providing a sustainable approach to potentially addressing the plastic pollution issue. However, PET enzymatic hydrolysis still faces some technical challenges, such as poor stability of the hydrolases and low efficiency in degrading high-crystalline PET. Thus, this review summarizes recent advances in strategies to enhance the efficiency of PET enzymatic hydrolysis and explores the interplay of factors affecting PET hydrolysis efficiency. Furthermore, we highlight the progress in metabolic engineering approaches for the biotransformation of PET degradation products into higher value chemicals, providing insights into achieving efficient PET bio-recycling. This review systematically integrates key factors for enhancing the PET enzymatic hydrolysis efficiency and showcases successful examples of PET waste further valorization, providing valuable references and insights for the industrialization of PET bio-upcycling.

Keywords: Poly (ethylene terephthalate); Enzymatic hydrolysis; Poly (ethylene terephthalate) hydrolase; Metabolic engineering; Biotransformation

1. Introduction

The large-scale production and widespread use of plastic products have led to severe global environmental pollution. In 2019, the global annual production of plastic products reached 460 million tons and is estimated to increase to 1.2 billion tons by 2060 (Li, Yue et al., 2023). Plastic products undergo fragmentation or degradation during use or while accumulating in the environment, leading to the formation of microplastics (1 μm –5 mm) or nanoplastics (1 nm–1 μm), which are pervasive in the natural environment and can enter the human body through the food chain or respiratory system (Li, Yue et al., 2023; Winiarska et al., 2024). Notably, studies on human cells have shown that nano- and microplastics can induce harmful biological effects, including cytotoxicity, genotoxicity, inflammation, apoptosis, and oxidative stress (Winiarska et al., 2024). Moreover, improper waste management has led to the presence of approximately 5 trillion plastic fragments, weighing over 260,000 tons, floating on the surface of the oceans (Eriksen et al., 2014), which poses a significant threat to biodiversity.

Among them, poly (ethylene terephthalate) (PET) is a widely used engineering plastic, valued for its strong chemical resistance and mechanical strength. These properties have made it a key material in industries such as packaging and textiles (Han, W. et al., 2024; Mudondo et al., 2023). Specifically, the global production capacity of PET reached 30.5 million tons in 2019, and the PET production volume reached 88.1 million tons in 2022 (Han, W. et al., 2024; Mahler et al., 2025). However, the high demand for PET products and their short lifespan have caused a significant accumulation of PET waste (Zhou et al., 2024b). Meanwhile, the difficulty of PET in natural degradation and improper recycling management has led to significant resource wastage and increased environmental pollution, thus boosting the urgent demand for sustainable PET recycling (Yang et al., 2021).

Currently, the main technologies of PET recovery are: 1) mechanical recycling, which involves the melting and re-spinning of PET, is limited by the purity requirements of PET waste and often results in a quality reduction of the recycled PET products (Barnard et al., 2021; Fei et al., 2020; George and Kurian, 2014; Singh et al., 2021); 2) chemical recycling, encompassing depolymerization methods such as hydrolysis, aminolysis, methanolysis, and glycolysis, which further repurpose valuable monomers for the production of value-added products, thereby facilitating a circular economy (Khan et al., 2022; Laldinpuui et al., 2021). Although successful industrial applications of chemical recycling have been reported (Barnard et al., 2021), chemical method typically requires harsh conditions, including high temperatures and pressures, strong acids, strong bases, or costly industrial catalysts (Zhang, Z. et al., 2022); 3) biological recycling, which involves the enzymatic or microbial cleavage of ester bonds within PET polymer chains, providing notable benefits including mild reaction conditions, decreased energy consumption, and a minimized environmental impact (Soong et al., 2022). Furthermore, enzymatic recycling offers a promising approach for selective depolymerization of complex mixed plastic waste streams, enhancing the efficiency of recycling processes (Bell et al., 2022). However, the enzymatic hydrolysis of PET currently faces several challenges, particularly its limited effectiveness on highly crystalline PET and the insufficient stability of PET hydrolases (Tournier et al., 2020; Zhou et al., 2025). To overcome them, extensive research has focused on improving PET hydrolysis efficiency through substrate pretreatment or enzyme engineering, which significantly boosted the efficiency of clean PET bio-recycling technologies. Carniel et al. comprehensively reviewed the strategies for both chemical and biological depolymerization of PET,

providing a comparative analysis of their respective advantages and limitations (Carniel et al., 2024). Herein, this review specifically focuses on recent progress in enhancing the enzymatic hydrolysis efficiency of PET and emphasizes the latest advances in high-value conversion. By summarizing these critical advancements, this review not only outlines the current state of PET bio-recycling but also highlights the potential for further industrial-scale implementation, offering valuable insights into the development of more sustainable and efficient PET bio-recycling processes.

2. Enzymatic hydrolysis of PET

2.1 PET Hydrolases and Catalytic Mechanism

PET is a semi-crystalline polyester polymer composed of repeating units of ethylene glycol (EG) and terephthalic acid (TPA) (You et al.). Recycling PET into monomeric building blocks via biocatalyst for further high-value product synthesis is widely recognized as the optimal and eco-friendly approach for managing PET waste (Dai et al., 2021a). To date, many PET hydrolases have been identified and engineered to improve the efficiency of PET hydrolysis. These enzymes predominantly belong to the subfamily of carboxylic ester hydrolases (EC 3.1.1.x), including carboxylesterases (EC 3.1.1.1), *IsPETase* (EC 3.1.1.101), lipases (EC 3.1.1.3), cutinases (EC 3.1.1.74), and arylesterases (EC 3.1.1.2) (Han, W. et al., 2024; Sui et al., 2023; Wei and Zimmermann, 2017). Among them, the most renowned is *IsPETase* from *Ideonella sakaiensis* 201-F6, first reported in 2016 for its exceptional ability to depolymerize PET films under ambient conditions (Yoshida et al., 2016). Kim et al. provided a detailed summary of the classification and optimal reaction conditions of PET hydrolases in their review (Kim et al., 2022), and Yao et al. conducted a comparative analysis of their degradation performance (Yao et al., 2024). Therefore, this aspect will not be discussed in further detail here.

PETase, a member of the serine hydrolase family, mediates PET hydrolysis through a well-defined catalytic mechanism that utilizes the conserved serine-histidine-aspartic acid (SHD) catalytic triad to drive the reaction via a two-step process involving acylation and deacylation (Burgin et al., 2024). PET degradation begins with the catalytic serine (Ser) attacking the carbonyl carbon of PET, forming a tetrahedral intermediate (TI, Fig. 1). This intermediate is stabilized by histidine (His), aspartic acid (Asp), and the oxyanion hole, before transitioning to an acyl-enzyme (AE) intermediate. A water molecule then attacks the carbonyl group of the AE, resulting in a second TI, which undergoes deacylation to release carboxylic acid products (Wei et al., 2022). Specifically, among the SHD catalytic triad of *IsPETase*, Ser131 was the covalent nucleophile to attack the carbonyl carbon atom and form a scissile ester bond. The Asp177 residue polarizes the His208 residue to activate the nucleophilic residue Ser131, facilitating its nucleophilic attack on the carbonyl carbon of the substrate (Zhou et al., 2024b). This hydrolysis process yields a mixture of products, including bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), TPA, and EG. Furthermore, MHET can be further converted to TPA by a variety of enzymes, including *IsMHETase* (Yoshida et al., 2016), *Candida antarctica* lipase B (CALB) (Carniel et al., 2017), *Thermus thermophilus* carboxylesterase (TTCE) (Mrigwani et al., 2022), among others, enabling the synthesis of regenerated PET or other high-value products. Since the development of PET circular economy requires complete depolymerization of the polymer into its monomeric constituent TPA, several studies have applied protein engineering strategies to enhance the MHET hydrolysis activity of *IsPETase*, thereby enabling the complete conversion of PET to TPA and EG using a single enzyme (Yin et al.,

121 2022; Yin et al., 2024).

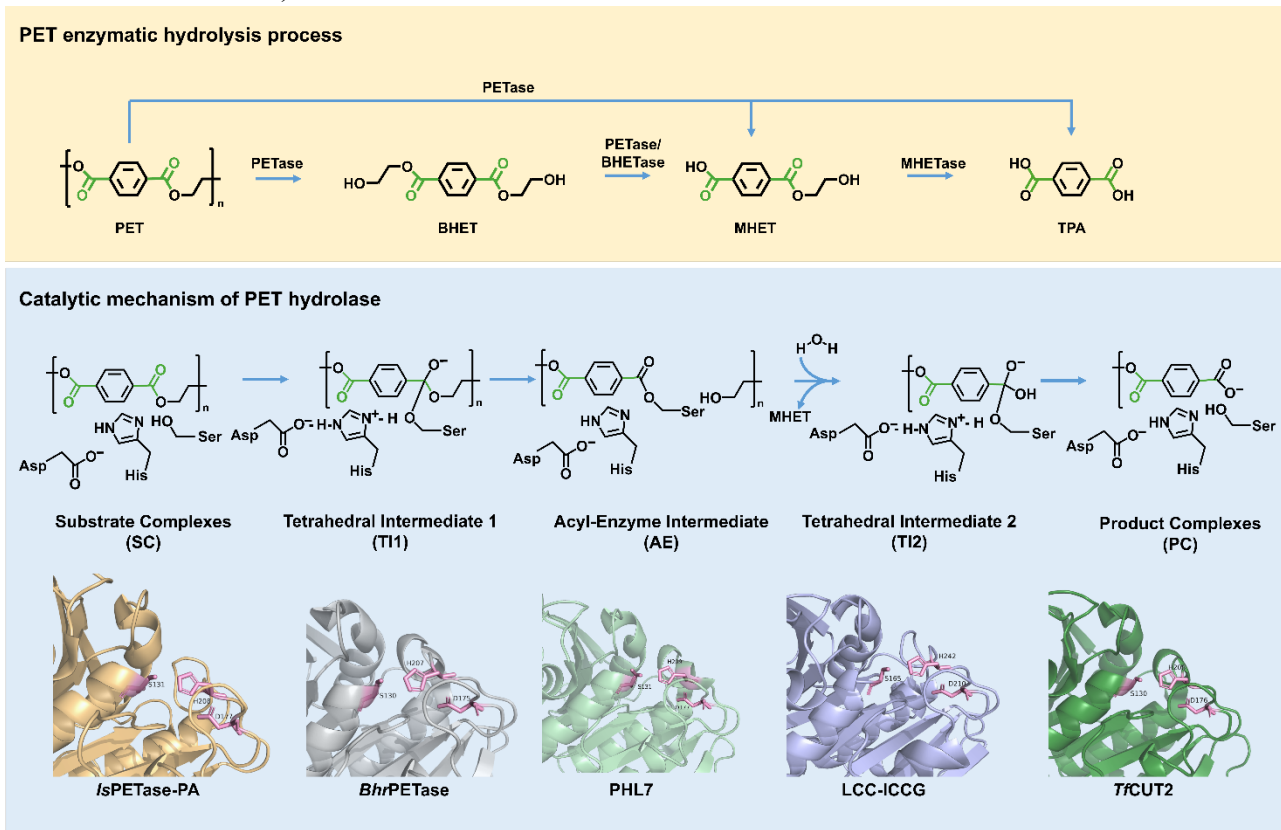


Fig. 1 PET Enzymatic Hydrolysis Process and Catalytic Mechanism.

The structure of the *Is*PETase-PA (Yin et al., 2024) (PDB ID: 8J17), *Bhr*PETase (Cui et al., 2024) (PDB ID: 7EOA), PHL7 (Richter et al., 2023) (PDB ID: 7NEI), LCC-ICCG (Tournier et al., 2020) (PDB ID: 7VVE), and *Thermobifida fusca* cutinase (*Tf*CUT2) (Roth et al., 2014) (PDB ID: 4CG1) are visualized using PyMOL, with the serine-histidine-aspartic acid (SHD) catalytic triad highlighted in pink.

2.2 Strategies on Improving PET Enzymatic Hydrolysis Efficiency

Numerous factors influence the enzymatic degradation of PET, such as crystallinity, chain mobility, molecular size, surface topography, and hydrophobicity. Nikolaivits et al. reviewed mechano-green chemical pretreatment strategies for recalcitrant plastics, including polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP), PET, and polyurethanes (PU), to improve their biodegradation efficiency (Nikolaivits et al., 2021). Herein, we focus specifically on PET and provide a detailed overview of various strategies, including enzyme engineering, substrate pretreatment, and bioprocess optimization, to enhance its enzymatic hydrolysis efficiency.

2.2.1 Enhancement of Enzyme Thermal Stability and Activity

For enzyme engineering, Jayasekara et al. systematically explored strategies for engineering PET hydrolases to enhance their stability and catalytic efficiency (Jayasekara et al., 2023). In a broader context, Huang et al. summarized the techniques and applications of computer-aided enzyme design based on molecular simulation and machine learning approaches, offering valuable insights into the rational design of various enzymes, including PET hydrolases (Zhou and Huang, 2024). Rather, to avoid redundancy, we briefly discuss recent advances specifically aimed at improving the thermostability and catalytic activity of PET hydrolases in this review, in order to provide a broader perspective on how enzyme engineering can enhance the overall efficiency of PET biodegradation.

As a semi-crystalline polyester polymer, PET exhibits distinct thermal properties that significantly influence its enzymatic degradation. Below the glass transition temperature (T_g) at about 65–71 °C, the amorphous chains in PET exhibit rigidity, whereas above T_g the chains transition to a more fluid state, enhancing both their mobility and accessibility to PETases, thereby increasing the overall degradation efficiency (Thomsen et al., 2022). To leverage this temperature-dependent behavior, researchers have focused on enhancing the activities and thermal stability of PET hydrolases through protein engineering strategies, aiming to achieve superior PET degradation efficiency at elevated reaction temperatures (Bell et al., 2022; Cui et al., 2021; Tournier et al., 2020; Yin et al., 2024). Son et al. employed a rational protein engineering approach to design the *IsPETase*^{S121E/D186H/R280A} variant with enhanced thermal stability, which exhibited melting temperature (T_m) from 48.81 °C to 57.62 °C and a 14-fold enhancement in PET degradation activity at 40 °C compared to *IsPETase* (Son et al., 2019). Tournier et al. employed computer-aided enzyme engineering to design LCC-ICCG ($\Delta T_m = 9.3$ °C), which enabled PET depolymerization to reach 90% conversion in less than 10 h, with a mean productivity of 16.7 g_{TPA} L⁻¹ h⁻¹ (Tournier et al., 2020). Bell et al. significantly enhanced the catalytic performance and thermostability of *IsPETase* through the development of an automated directed evolution platform, resulting in HotPETase with a T_m of 82.5 °C, which could operate at the T_g of PET and efficiently depolymerize semicrystalline PET (Bell et al., 2022). Shi et al. developed a fluorescence-based high-throughput screening assay using a newly designed substrate (BHET-OH) to evolve DepoPETase, which exhibited a 23.3 °C higher T_m and a 1407-fold increase in PET degradation activity at 50 °C compared to *IsPETase* (Shi et al., 2023). Xue et al. combined machine learning with evolutionary analysis to rationally redesign LCC-ICCG, obtaining the LCC-ICCG_I6M mutant, which raised the optimal degradation temperature to 75–80 °C and achieved a 3.64-fold increase in depolymerization of 31.3% crystalline PET compared to LCC-ICCG (Ding et al., 2023). However, maintaining the reaction temperature above T_g can induce thermal crystallization of PET, resulting in increased crystallinity and a subsequent reduction in enzymatic degradation efficiency (Tournier et al., 2020; Wei et al., 2022). Therefore, the optimal temperature for enzymatic hydrolysis is slightly below 70 °C, as it balances enhanced chain mobility and enzymatic activity while preventing undesirable crystallization (Lee et al., 2023; Thomsen et al., 2023). In Fig. 2, the T_m and optimal reaction temperatures of different PET hydrolases are summarized, and a clear correlation between the T_m and optimal reaction temperatures can be observed (highlighted in yellow). Conducting the hydrolysis reaction at a temperature 15 °C below the T_m (based on empirical observations) helps to preserve the enzymatic activity of the catalyst (Cui et al., 2024). From this perspective, Cui et al. developed TurboPETase using a computational strategy that combines a protein language model with force-field-based algorithms, achieving balanced thermostability and hydrolytic activity across a broad temperature range (50 °C–65 °C). The TurboPETase enabled near-complete depolymerization of post-consumer PET bottles within 8 h under high industrial substrate loading conditions (200 g kg⁻¹), achieving a maximum PET hydrolysis rate of 61.3 g L⁻¹ h⁻¹ (Cui et al., 2024).

PET surface is significantly limited, posing a major bottleneck in the enzymatic hydrolysis of polyester plastics (Su et al., 2022). To efficiently degrade such water-insoluble polymers, improving enzyme-substrate affinity is essential (Wei et al., 2014). Current approaches to improve enzyme-substrate affinity focus on two primary modification strategies (Table 1): 1) enzyme active-site engineering (e.g., hydrophobization (Silva et al., 2011), charge modulation (Nakamura et al., 2021), substrate-binding pocket enlargement (Bollinger et al., 2020; Chen et al., 2021)), and 2) incorporation of substrate-binding modules (Dai et al., 2021b).

For enzyme active-site modifications, the positive charge introduction has been shown to enhance the enzyme-substrate binding, which contributed to a 6.8 times higher PET degradation efficiency of PET2 7M than that of PET2 WT (Nakamura et al., 2021). Additionally, Chen et al. employed a directional-path modification (DPM) strategy to introduce positively charged amino acids and remodel the binding groove of *Tf*CUT2, achieving nearly a 30-fold enhancement in PET degradation compared to the wild-type enzyme (Chen, X.-Q. et al., 2022).

For the addition of substrate-binding modules, the binding of a cellulose-binding domain (CBM) to *Is*PETase^{EHA} significantly increased its adhesion to the hydrophobic polymer surface, resulting in a 71.5% improvement in catalytic activity compared to the parental enzyme (Dai et al., 2021b). Similarly, Silva et al. engineered cutinase mutations that expanded the substrate-binding site and enhanced its hydrophobicity, achieving a 2-fold increase in TPA released during a 2 h incubation compared to the wild-type enzyme (Silva et al., 2011). Moreover, Chen et al. incorporated a zwitterionic polypeptide composed of alternating glutamic acid (E) and lysine (K) residues at the C-terminus of PETase, which significantly enhanced substrate affinity and resulted in an over 11-fold increase in the release of PET degradation products compared to the wild-type enzyme (Chen et al., 2021).

Additionally, the regulation of enzyme structures through physical means has also been demonstrated to potentially enhance enzyme-substrate affinity. Pellis et al. employed ultrasound to modify the secondary structure of *Thc*_cut1, exposing hydrophobic groups on the enzyme surface. This structural alteration improved enzyme-substrate affinity and accelerated the reaction rate, thereby enhancing PET hydrolysis efficiency (Pellis et al., 2016). However, prolonged exposure may lead to a loss of stability and a decrease in enzyme activity (Brackmann et al., 2023).

Overall, these findings underscore the importance of enhancing enzyme-substrate affinity at the catalytic active site to improve the efficiency of PET enzymatic hydrolysis.

Table 1. Summary of Advances in Research on Enhancing Enzyme-Substrate Binding

Category	Method	Mechanism	PET hydrolase	Reaction Condition	Results	Reference
Enzyme active-site hydrophobization	Site-directed mutagenesis	Enhancement of space and hydrophobicity	Tfu_0883	60 °C, 48 h	TPA yield increased 1.5-fold compared to wild-type	(Silva et al., 2011)
	Zwitterionic polypeptide fusion	Enlargement of substrate-binding pocket; Hydrophobic amino acid exposure	IsPETase	40 °C, 96 h	11-fold increase in degradation product release	(Chen et al., 2021)
Enzyme polymer-binding module	Chitin-binding domain from Chitinolyticbacter meiyuanensis SYBC-H1	Enhancement of enzyme adsorption to PET substrates	LCC-ICCG	65 °C, 12 h	improved 19.6% depolymerization degrees	(Xue et al., 2021)
	Cellobiohydrolase I from Hypocrea jecorina (CBM)		Thc_Cut1	50 °C, 72 h	MHET release increased by 5.3-fold	(Ribitsch et al., 2013)
	Polyhydroxyalkanoate depolymerase from Alcaligenes faecalis (PBM)		Thc_Cut1	50 °C, 72 h	TPA release increased by 3.5-fold and MHET release increased by 11.4-fold	(Ribitsch et al., 2013)
	Class II hydrophobins HFB4 and HFB7		Thc_Cut1	50 °C, 24 h	>16-fold increase in PET hydrolysis product release rate	(Ribitsch et al., 2015)
Enzyme pretreatment	Ultrasound	Alteration of enzyme secondary structure; Exposure of hydrophobic groups	Thc_Cut1	60 °C, 6 h	5.2-fold increase of TPA and 6.6-fold increase of MHET released	(Pellis et al., 2016)

2.2.2 Regulation of Substrate Physical Properties

The enzymatic hydrolysis efficiency of PET is significantly influenced by its physical properties, including particle size, surface area, surface hydrophobicity, T_g , crystallinity, and molecular weight. Table 2 provides a comprehensive summary of recent advancements in PET amorphization and the enhancement of enzyme-mediated degradation through physical, chemical, and biological pretreatment strategies.

(1) Crystallinity: The enzymatic hydrolysis of insoluble polyester primarily occurs through surface erosion, meaning the mobility of polyester chains significantly influences the biodegradability of PET by adjusting the affinity and accessibility of ester bonds to enzymes (Wei et al., 2014). PET with low crystallinity or T_g offers more chain flexibility, as the amorphous regions transition from a glassy solid to a viscous liquid (Thomsen et al., 2022). This transformation significantly improves the accessibility of PET chains to enzymes, thereby enhancing PET degradation efficiency. In contrast, high-crystallinity PET forms a tightly packed crystalline structure that restricts polymer chain mobility (Thomsen et al., 2023; Zhou et al., 2025). However, a notable exception was reported by Kawai et al., where wild-type enzymes displayed over 200-fold greater activity toward high-crystallinity PET microfibers (14.1%) compared to low-crystallinity PET films (6.3-8.4%). The difference between the hydrolysis rates of the films and microfiber is attributed to variations in surface dimensions, orientations, and topological features of PET (Kawai et al., 2019), suggesting that a comprehensive consideration of the multifactorial physical properties of PET is necessary to understand their impact on the enzymatic hydrolysis efficiency of PET. Both chemical and physical pretreatments can be used to reduce crystallinity, like alkaline immersion, melt processing, and ball milling (Table 2).

For example, the melt-quenching process converts semicrystalline PET into low-crystalline or amorphous PET by heating the polymer above its T_m . At this temperature, the rigid crystalline regions are disrupted as the polymer transitions into a molten state, leading to the breakdown of the orderly, closely packed chain arrangements. Subsequent rapid cooling of the melt to below the T_g prevents the molecular chains from reorganizing into a regular crystalline structure before solidification, ultimately resulting in the formation of amorphous or low-crystalline PET (Cheng et al., 2024).

For ball milling of PET, some rotational disordering of the polymer chains around their axes leads to an increased spacing between adjacent phenyl rings, while parallel alignment of the molecules is retained. This structural rearrangement induces an "oriented amorphous" state in PET, which enhances the rotational mobility of the polymer segments (Bai et al., 2000). Since the enzymatic hydrolysis of insoluble polyesters primarily proceeds through surface erosion, the mobility of the polyester chains influences the biodegradability of PET, thereby increasing the probability of effective enzyme–substrate interactions (Zhou et al., 2025).

Overall, strategic selection and optimization of these pretreatment methods are crucial for effectively reducing PET crystallinity to maximize enzymatic degradation efficiency.

(2) Particle size: The enzymatic degradation of PET exhibits complex dependencies on material characteristics, with micronization serving as an effective strategy to enhance reaction

kinetics through surface area optimization. Eugenio et al. systematically demonstrated that reducing PET particle size from macroscopic dimensions to micron-scale fragments elevates the initial hydrolysis rate from $0.2 \text{ mmol L}^{-1} \text{ h}^{-1}$ to $1.7 \text{ mmol L}^{-1} \text{ h}^{-1}$, attributed to the increase in accessible ester bond surfaces for PET hydrolase activity (Eugenio et al., 2021). Nonetheless, Brizendine et al. have demonstrated that while reducing the particle size of PET can increase the initial reaction rate, it has a limited impact on the overall conversion rate (Brizendine et al., 2022).

These results highlight that PET enzymatic degradation is governed by synergistic physical properties, whereas pretreatment strategies can alter degradation efficiency through mechanistically diverse pathways. Consequently, comprehensive research is essential to elucidate the relationship between these physical properties and the efficiency of enzymatic degradation. Such research will not only help prevent the overlooking of any critical mechanisms of action but also avoid overemphasizing the impact of any single factor. By delving deeper, we can gain a more precise understanding of how the physical characteristics of PET regulate its biodegradation behavior, thus providing a scientific basis for optimizing the enzymatic degradation process of PET.

(3) Surface Hydrophilicity: In addition to the previously mentioned strategy of enhancing the hydrophobicity of PET hydrolase surfaces, increasing the hydrophilicity of the PET surface also contributes to the enzyme-substrate interaction. Furukawa et al. demonstrated that the addition of an anionic surfactant increased PET hydrolase activity by 120-fold, primarily due to electrostatic attraction between the positively charged enzyme and the negatively charged PET surface formed by the surfactant coating (Furukawa et al., 2018). Similarly, Huang et al. employed a microbe-enzyme synergy strategy to facilitate the colonization of *S. pavanii* JW-G1 on the PET surface, resulting in a significantly rougher and more hydrophilic surface (contact angle reduced from $96.6 \pm 2^\circ$ to $86.3 \pm 2^\circ$) (Huang et al., 2022). Moreover, dimethyl sulfoxide (DMSO) has been introduced to mitigate inhibitory hydrophobic enzyme-enzyme interactions on the plastic surface and to enhance the solubility of produced monomers (Avilan et al., 2023; Erickson et al., 2022a). Notably, Erikson et al. observed that incorporating 10% DMSO into the reaction mixture led to a 3-fold increase in the release of aromatic products during the hydrolysis of amorphous PET film by IsPETase^{DM} (Erickson et al., 2022b).

Notably, several significant breakthroughs for PET biodegradation through innovative enzymatic technologies have markedly accelerated the industrialization of enzymatic PET recycling. Carbios has designed an optimized enzymatic process that achieves 93% depolymerization of post-consumer PET flakes within 24 h at 72 °C, utilizing only 3 g enzyme per kg PET (Tournier et al., 2020). Building on this technological breakthrough, they further constructed their first industrial-scale facility in France, which is expected to become operational in 2025 and will be capable of recycling 50,000 tones PET waste annually (2023). Moreover, Kubu-PM¹² was recently reported to achieve a 90% depolymerization within 8 h under high substrate loading conditions (20% w/w PET), using only 1 g enzyme per kg PET at 70 °C (Seo et al., 2025). Importantly, a critical aspect of these enzymatic processes involves PET amorphization through extrusion and rapid cooling, which significantly reduces crystallinity and thereby enhances enzymatic accessibility.

In summary, crystallinity, surface hydrophilicity and surface area are the most important physical parameters for the PET pretreatment. Reducing crystallinity and increasing hydrophilicity of PET will greatly enhance the enzymatic hydrolysis efficiency. In contrast, the particle size effect on the hydrolysis efficiency is not very straightforward. Meanwhile, the pretreatment process typically affects multiple physical properties of PET simultaneously, making it difficult to evaluate its impact on enzymatic degradation based on a single parameter. Therefore, a comprehensive and systematic analysis is essential to understand the mechanism behind improved PET enzymatic degradation efficiency. Furthermore, there are still lots of unknowns to be addressed like surface chemistry during the enzymatic process of PET, which requires further in-depth investigation in future studies.

331 **Table 2 Summary of Different Pretreatment Methods for Enhancing Enzymatic Hydrolysis Efficiency by Modifying the Physical**
332 **Properties of PET**

Substrate		Pretreatment		Enzymatic hydrolysis		Result		Reference
Pretreatment	Type	Crystallinity	Method	Mechanism	PET hydrolase	Time	TPA yield or concentration	
Physical	PET fiber	36%	Ball milling	Amorphize PET and roughen the surface	LCC-ICCG-YGA	5 days	43.80%	(Zhou et al., 2025)
	PET curtain	30%					60.90%	
	PET flakes	30%	Melt Processing	Reduce crystallinity, molecular weight, and glass transition temperature	LCC-ICCG	3 days	48%–52%	(Patel et al., 2022)
	PET bottle	36%	Moist-solid reaction	Enhanced opportunity of molecular collisions at high-solids reaction conditions	HiC	7 days	20%–25%	(Kaabel et al., 2021)
					HiC	21 days	47%–51%	
	PET textiles	46%	Moist-solid reaction		HiC	6 days	11%–12%	(Kaabel et al., 2023)
Biological	PET bottle	/	Microwave pre-treatment	Increased polymer surface area to improve enzyme accessibility; Change PET conformation; Reduce PET crystallinity	<i>IsPETase</i> ^{S238A}	1 h	the signal intensity of TPA+ MHET increased 1400-fold	(Guo et al., 2023)
	PET film	37%	Microbe-enzyme system	PET surface corrosion	TfC	15 h	50 nM	(Yan, Z.-F. et al., 2021)

Chemical	PET film	7%	Microbe-enzyme system	Enhance PET surface roughness and hydrophilicity	TfC	5 days	2.73-fold increase in degradation rate	(Huang et al., 2022)
	PET fiber	38.8%	Class I hydrophobins HGFI	Transform the hydrophobic surface of PET into hydrophilic	<i>Is</i> PETase	5 days	PET weight loss improved 16.2%	(Puspitasari et al., 2021)
	PET bottle powder	64.8%					PET weight loss improved 11.9%	
	PET flakes	33%	Dissolution/reprecipitation with microwave-assisted	Reduce crystallinity Enhance surface roughness of PET	LCC-ICCG	4 days	Improved PET conversion by 3%	(Attallah et al., 2023)
	PET bottle	27 %	Alkali immersion	Reduce crystallinity	HiC	24 h	0.1% (combined yield of TPA and MHET)	(Giraldo-Narcizo et al., 2023)
	Granular PET	32%	Supercritical CO ₂	Increase internal surface area in PET	HiC	288 h	2-fold increase in TPA yield	(Gurralla et al., 2024)

2.2.3 Alleviation of Product Inhibition

As previously mentioned, the degradation of PET by wild-type PETases generates mixed products, including BHET, MHET, TPA, and EG. Wild-type PET hydrolases typically exhibit limited catalytic efficiency towards MHET (Brizendine et al., 2022; Wei et al., 2022), while *I. sakaiensis* produces a specialized *Is*MHETase to convert MHET into TPA and EG (Wei et al., 2022; Yoshida et al., 2016). Furthermore, the undigested MHET often binds tightly to PET hydrolases in a non-catalytic orientation, resulting in enzyme inhibition and lower catalytic efficiency in MHET hydrolysis (Zhang et al., 2023). In other words, MHET was identified as a competitive inhibitor in PET depolymerization reactions catalyzed by various PET hydrolases, ultimately contributing to the suboptimal efficiency of the overall PET degradation process (Barth et al., 2015a; Barth et al., 2015b; Heinks et al., 2024). However, recent studies further suggest that product inhibition during PET hydrolysis may be enzyme-specific and product-dependent. For example, MHET and EG exhibit inhibitory effects on wild-type *Is*PETase, while TPA may have a slight promoting effect. In contrast, the *Is*PETase^{W159H/S238F} variant shows minimal sensitivity to MHET accumulation, although TPA and EG continue to suppress its catalytic activity (Erickson et al., 2022c). Furthermore, the inhibitory effects of all TPA, MHET and BHET on HiC were assessed at 70 °C, with no evidence of inhibition observed (Eugenio et al., 2022). These findings indicate that product inhibition may involve complex enzyme–product interaction mechanisms beyond classical competitive binding.

Multi-enzyme systems have emerged as a promising strategy for enhancing PET biodegradation by alleviating product inhibition. For example, fused or co-immobilized enzymes offering significant advantages by optimizing substrate channeling and proximity effects between enzymes, which further reduce diffusion limitations between active sites, thereby promoting enzyme synergy and improving overall catalytic efficiency (Chen, K. et al., 2022; Zhang et al., 2023; Zhang and Hess, 2017). A key benefit of multi-enzyme systems lies in their ability to facilitate the synergistic degradation of both PET and its intermediate MHET through the coordinated action of enzymes with complementary substrate specificities (Carniel et al., 2017; Knott et al., 2020; Tarazona et al., 2022). For instance, *Is*MHETase and *Candida antarctica* lipase B (CALB), which catalyze the conversion of MHET to TPA, have been identified as crucial components for achieving complete PET degradation (Hwang et al., 2022; Sagong et al., 2020; Yoshida et al., 2016). Chimeric proteins integrating MHETase and PETase through flexible glycine-serine linkers have demonstrated a 5-fold improvement in PET degradation efficiency and significantly enhanced MHET hydrolysis rates (Knott et al., 2020). Similarly, the synergistic combination of CALB and HiC resulted in a remarkable 7.7-fold increase in TPA yield from PET (Carniel et al., 2017).

To further complement high-efficiency thermophilic PET hydrolases, improving the thermal stability of MHET hydrolases is essential so that both enzymes can work in the same range of temperature to boost the overall PET hydrolysis efficiency. This approach has led to the development of several effective dual-enzyme systems. For instance, the combination of LCC with immobilized *Tf*Cut2 achieved a 2.4-fold increase in PET degradation product yields compared to *Tf*Cut2 alone (Barth et al., 2016). Meanwhile, the system incorporating redesigned KL-MHETase and FAST-PETase demonstrated a 2.6-fold enhancement in PET

depolymerization rate compared to FAST-PETase alone (Zhang et al., 2023). Furthermore, the combination of *Thermus thermophilus* carboxylesterase (TTCE) with LCC resulted in a 30–100% improvement in TPA and EG yields relative to LCC alone (Mrigwani et al., 2022).

In addition to multi-enzyme systems, ultrafiltration has emerged as an effective engineering solution to mitigate product inhibition through the selective removal of soluble metabolites from biocatalytic systems (Andrić et al., 2010). This membrane-based separation enables insoluble PET substrates and enzymes to be retained within the bioreactor compartment, while permeable hydrolysis byproducts are continuously evacuated. Barth et al. demonstrated that the continuous removal of inhibitory hydrolysis products via ultrafiltration membranes enhanced the efficiency of PET biocatalytic hydrolysis by 70% compared to batch hydrolysis (Barth et al., 2015b).

2.2.4 Optimization of Reaction Conditions

(1) Ion Concentration

Protein conformation, solubility, stability, and activity are influenced by a variety of intra- and intermolecular forces, including hydrogen bonding, van der Waals interactions, solvation effects, and polarization. Ions play a crucial role in protein-containing aqueous systems through kosmotropic and chaotropic effects, as well as their interactions with the protein surface and the water molecules within the first hydration shell (Carniel et al., 2021; Zhang and Cremer, 2006). Consequently, the ionic composition and strength of the buffer system significantly impact the enzymatic hydrolysis of PET. For instance, a buffer system for *Tf*Cut2 with high ionic strength (sodium phosphate, 3 M) has been shown to enhance the initial hydrolysis rate by 3.8-fold compared to a low ionic strength buffer (Tris–HCl, 0.07 M) (Barth et al., 2015b). Similarly, a high glycine concentration (150–200 mM) in the buffer can mitigate acidification caused by the accumulation of acidic hydrolysis products, thereby improving the efficiency of PET enzymatic hydrolysis (Yin et al., 2024).

(2) Agitation

As a representative heterogeneous catalytic process, enzymatic PET hydrolysis critically depends on the interfacial interactions between the enzyme and the solid-phase substrate (Nakamura et al., 2021). With the progress of the enzymatic PET hydrolysis, the amount of available substrate decreases, and the overall reaction rate depends on the enzyme penetration and diffusion inside the solid substrate (Eugenio et al., 2022). Thus, agitation of the reaction mixture plays a crucial role in alleviating diffusion limitations and enhancing catalytic efficiency. By reducing the thickness of the diffusional boundary layer at the solid–liquid interface, agitation facilitates mass transfer of both enzymes and hydrolysis products. This not only promotes more effective enzyme–substrate interactions but also prevents the local accumulation of products near the enzyme, thereby accelerating the sequential processes of adsorption, hydrolysis, and desorption at the PET surface (Eugenio et al., 2022; Gan et al., 2002). Barth et al. reported that horizontal shaking or magnetic stirring at 100–150 rpm can enhance the enzymatic hydrolysis rate of *Tf*CUT2 on PET film by approximately 1.8-fold. However, a further increase in stirring speed results in a sharp decline in hydrolysis rate (Barth et al., 2015b). Since the shear deactivation effect can also cause the inactivation and aggregation of enzymes (Colombié et al., 2001), it is necessary to explore an appropriate

stirring speed to balance PET hydrolase stability and kinetics.

(3) pH

pH plays a critical role in modulating the activity and substrate specificity of PET hydrolases. Most PET hydrolases exhibit optimal activity under alkaline conditions. For example, in PETase, the pKa value of His208 in the SHD catalytic triad is 7.21, indicating that under acidic conditions, protonation of His208 impairs the activation of Ser131, thereby reducing the enzymatic activity of the PET hydrolase (Zhou et al., 2024b). Moreover, during the enzymatic hydrolysis of PET, the cleavage of ester bonds produces carboxylic acids, leading to local surface acidification and a significant reduction in pH at the polymer interface. This phenomenon underscores the necessity of maintaining an appropriate reaction pH to ensure optimal enzymatic efficiency and achieve the desired degradation outcomes.

Substrate specificity in PET degradation is significantly influenced by pH, particularly in the hydrolysis of BHET catalyzed by CALB. This pH-dependent behavior enables the selective production of either TPA or MHET (Swiderek et al., 2023). Under acidic conditions, the ionization state of CALB facilitates the formation of a neutral hydrogen bond network, allowing the enzyme to effectively bind both neutral BHET and negatively charged MHET, thereby leading to complete hydrolysis of both ester bonds in BHET. Conversely, alkaline conditions induce conformational changes in CALB, where the MHET anion and ionized residues (e.g., Asp134) destabilize the CALB: MHET complex, thereby preventing further hydrolysis of MHET and resulting in selective cleavage of only one ester bond in BHET (Swiderek et al., 2023). Thus, pH control not only modulates the catalytic efficiency of PET hydrolases but also serves as a critical parameter for regulating substrate specificity, highlighting its central role in optimizing enzymatic degradation processes.

(4) Enzyme concentration

As an interfacial biocatalyst, PET hydrolase exhibits concentration-dependent inhibition, which can be attributed to surface phenomena where excessive enzyme molecules interact with each other after substrate binding, thereby hindering further hydrolysis (Avilan et al., 2023). For example, a comparative study of TPA release rates found that a reaction with 100% enzyme loading produced 5.54 mM TPA, while 50% enzyme loading yielded a similar concentration of 5.24 mM, achieving an 84% reduction in enzyme mass cost without significantly compromising degradation efficiency (Carniel et al., 2021). This inhibition mechanism is further modulated by multiple factors, including incubation time, solution conditions, and available PET surface area (Avilan et al., 2023), highlighting the complex interplay of parameters affecting PET hydrolysis efficiency.

2.3 Economic Feasibility of Enzymatic PET Recycling

As mentioned above, PET bio-depolymerization yields TPA and EG, which serve as essential building blocks for PET repolymerization (Yang et al., 2021). However, techno-economic analysis (TEA) and life cycle assessment (LCA) have shown that even when the feedstock cost is assumed to be US\$0.66 per kg of PET flakes, the minimum selling price (MSP) of purified TPA remains as high as US\$1.93 per kg (Singh et al., 2021). Therefore, despite the environmental advantages of enzymatic PET recycling, achieving cost parity with virgin TPA (US\$0.9-1.5 per kg) remains challenging unless the cost of PET feedstock is further reduced

(Singh et al., 2021).

Moreover, although extensive efforts have been devoted to enzyme engineering with notable progress (Cui et al., 2024; Seo et al., 2025; Tournier et al., 2020), under current process constraints—such as the need for low-crystallinity substrates, near-neutral pH conditions, and high reaction yields—further enhancement of enzyme performance alone has limited impact on overall process economics or environmental outcomes (Murphy et al., 2025). Instead, more substantial improvements may be achieved through innovations in upstream and downstream operations, including substrate amorphization, acid/base consumption reduction, and more energy-efficient EG recovery strategies (Murphy et al., 2025). In particular, electricity is the dominant energy input in enzymatic PET hydrolysis, with over 90% consumed during the extrusion and cryo-milling of PET flakes into amorphized powders, as well as for steam generation used in EG recovery and sodium hydroxide (NaOH) dosing to maintain pH in the depolymerization reactor. Consequently, replacing fossil-based electricity with renewable energy can reduce greenhouse gas (GHG) emissions by approximately 54%, lowering the overall carbon footprint to levels below those of virgin PET production (Uekert et al., 2023). Meanwhile, in the depolymerization process, the extent of PET breakdown, solids loading, enzyme price, and enzyme loading are all key cost drivers (Singh et al., 2021). Overall, these findings highlight the importance of system-level optimization beyond enzyme engineering to achieve both economic feasibility and environmental sustainability in enzymatic PET recycling.

3. Recent upcycling biotechnologies and PET valorization

To further enhance economic feasibility, it is of significant research interest to integrate process optimizations—aimed at improving enzymatic hydrolysis efficiency and reducing operational costs—with downstream conversion of PET degradation products (TPA and EG) into higher-value compounds.

3.1 Upcycling of Terephthalic Acid

PET hydrolases typically exhibit maximum degradation efficiency under slightly alkaline conditions (Murphy et al., 2025; Yoshida et al., 2016), resulting in the formation of soluble terephthalic acid salts (e.g., Na₂TPA). This process necessitates subsequent acidification to precipitate TPA, generating substantial volumes of saline wastewater as a byproduct (Li, A. et al., 2023; Zhou et al., 2024a). Thus, the direct utilization of plastic hydrolysates as carbon and energy sources for microbial conversion into value-added molecules and materials has emerged as a promising alternative, offering both economic and environmental advantages (Tiso et al., 2021).

3.1.1 Metabolic pathways:

Multiple bacterial species, including *Comamonas* sp. E6 (Sasoh et al., 2006), *Rhodococcus jostii* RHA1 (Hara et al., 2007), *Pseudomonas umsongensis* sp. GO16 (Narancic et al., 2021), *Pseudomonas stutzeri* (Liu et al., 2021), and *Ideonella sakaiensis* (Yoshida et al., 2016), exhibit catabolic pathways for TPA, which facilitate the successful biotransformation of TPA into a variety of high-value products, such as polyhydroxybutyrate

(PHB), polyhydroxyalkanoate (PHA), vanillin, β -Keto adipic acid (β -KA), protocatechuate (PCA), gallic acid (GA), pyrogallol (PG), muconic acid (MA), vanillic acid (VA), glycolic acid (GLA), 2-Pyrone-4,6-dicarboxylic acid (PDC), adipic acid (AA), catechol and lycopene.

Typically, TPA is initially converted into 3, 4-dihydroxy-1, 5-cyclohexadiene-1, 4-dicarboxylic acid (DCD) through the catalyzed by terephthalate 1,2-dioxygenase (TphAabc). Subsequently, DCD is oxidized by 4-hydroxythreonine-4-phosphate dehydrogenase (TphB) to produce PCA. PCA serves as a key intermediate in the upgrading of TPA to various high-value products, which can be converted into acetaldehyde and further transformed to acetyl-CoA via acetaldehyde dehydrogenase, entering the central carbon metabolism (Fig. 3):

(1) PCA can be converted into VA by catechol O-methyltransferase, which then be reduced into vanillin by carboxylic acid reductase;

(2) PCA can also be converted into GA by p-hydroxybenzoate hydroxylase and further transformed into pyrogallol by gallate decarboxylase;

(3) PCA can be directly converted into catechol by protocatechuate decarboxylase, which then can be converted into MA by catechol 1,2-dioxygenase or be converted into PG by phenol hydroxylase. Further, MA can be reduced into AA by enoate reductase;

(4) PCA can be converted into pyruvate, which is subsequently transformed into acetyl-CoA via the pyruvate dehydrogenase E1 component.

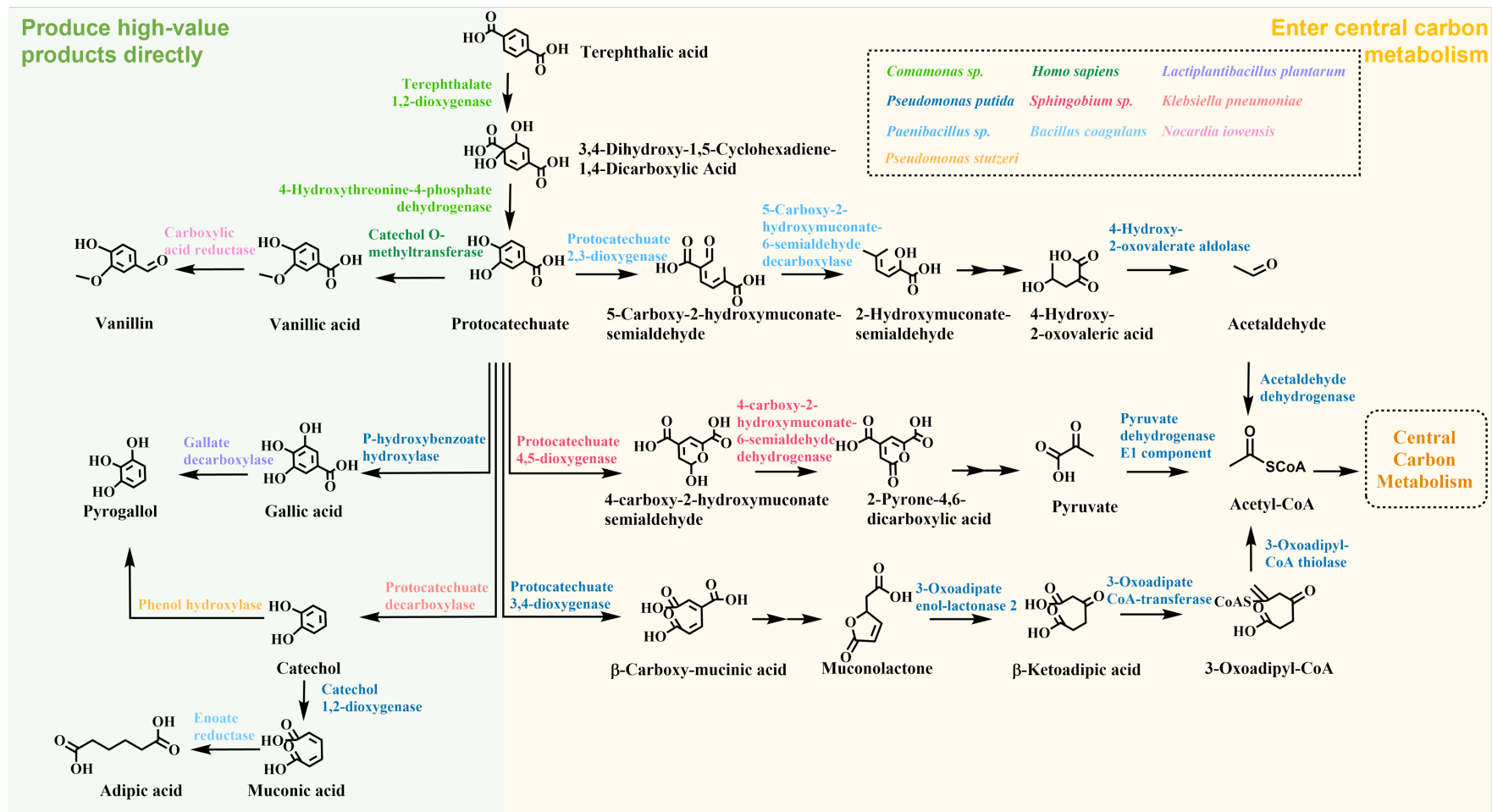


Fig. 3 Biotransformation Pathways of TPA

3.1.2 Metabolic products

(1) Flavor compounds:

Vanillin, the key flavor component of vanilla bean extract widely used in food and cosmetics, reached global demand of 37,000 tons in 2018 (Sadler and Wallace, 2021) and is projected to drive a \$724.5 million market by 2025 (Martău et al., 2021). VA, serves as a direct precursor for vanillin, which can be synthesized from PCA via O-methyltransferase (OMT) with subsequent conversion to vanillin catalyzed by carboxylic acid reductase (CAR) (Kim et al., 2019). Previous research has employed *E. coli* MG1655 equipped with a designed enzymatic pathway as the host, which achieves maximum vanillin titers of $744 \mu\text{M} \pm 100 \mu\text{M}$ from 1 mM TPA. However, biotransformation with lower concentrations of TPA (300–400 μM) derived from post-consumer plastic bottles results in the production of only 68 μM vanillin (Sadler and Wallace, 2021), highlighting the need for more efficient PET enzymatic depolymerization and sufficient TPA availability for effective bioconversion.

(2) Anti-oxidative compounds:

PCA, a multifunctional phenolic acid, demonstrates pharmaceutical efficacy against various diseases (Lubbers and Vries, 2021) and industrial utility in food/polymer sectors (Antony and Wasewar, 2020). Thus, the global PCA market was valued at 13 million in 2020 and is projected to reach US\$ 15 million by 2027 (Research, 2021).

GA, is widely used as an antioxidant food additive in oils, dairy, bakery products and various other food products (Liu, L. et al., 2022). With a broad range of industrial applications, the market size of GA is forecast to reach \$85.15 Billion by 2030 (IndustryARC).

PG, a versatile antioxidant, is widely used across food, cosmetics, and pharmaceutical industries (Wang et al., 2018), with its global market size was valued at approximately \$105 million and is expected to reach around \$160 million by 2032 (Sharma, 2023).

Catechol, an aromatic organic compound with potent antioxidant effects, serves as a critical intermediate in chemical manufacturing, including plastics, pharmaceuticals, and agrochemicals (Balderas-Hernández et al., 2014; Belmekki et al., 2024). The global catechol market size was valued at \$148 million in 2023 and is forecast to a readjusted size of \$219 million by 2030 (Research, G.I., 2024).

Lycopene, an acyclic open-chain unsaturated carotenoid renowned for its exceptional antioxidant properties, has found widespread applications across nutraceuticals, pharmaceuticals, cosmetics, agriculture, and numerous other industries (Li et al., 2020; Li, Yanxin et al., 2023). Due to its well-documented health benefits and wide range of applications, the global lycopene market reached a value of \$161.0 million in 2023 (RESEARCH, G.V., 2024).

Given the growing market demand and functional value of these multifunctional antioxidants, employing sustainable biotechnological methods to convert environmental plastic waste into high-value compounds not only offers a promising solution to environmental pollution but also enables value-added recycling, aligning with principles of circular economy and sustainable development. Kim et al. employed engineered *E. coli* strain PCA-1 with TphAabc and TphB expression and successfully produced 2.8 mM PCA from TPA in PET hydrolysate. Additionally, metabolic enzymes were introduced to synthesize GA (2.7 mM), PG

(1.1 mM), catechol (2.7 mM), MA (2.7 mM), and VA (1.4 mM) from TPA—via PCA as the key intermediate, achieving relatively high molar conversion yields ranging from 32.7% to 92.5% (Kim et al., 2019). Moreover, Kim et al. demonstrated chemo-enzymatic PET depolymerization and TPA-to-catechol biotransformation, achieving 99.5% conversion (5.97 mM catechol from 6 mM TPA in 12 h) using whole-cell *E. coli* (Kim, H.T. et al., 2021). For lycopene biotransformation, *Rhodococcus jostii* RPET S13 co-produced 3.2 mg L⁻¹ lycopene and 1.5 g L⁻¹ lipid from post-consumer PET, reaching a 20% carbon conversion rate. Fed-batch fermentation further increased yields to 22.6 mg L⁻¹ lycopene and 2.08 g L⁻¹ total lipids (Diao et al., 2024).

(3) Chemical product monomers:

MA (2,4-hexadiendioic acid) is a six-carbon dicarboxylic acid featuring conjugated double bonds, widely used in food additives, pharmaceuticals, and chemical production, particularly in AA synthesis and as a monomer for resins and bio-plastics (Almqvist et al., 2021; Blaga et al., 2024). Specifically, MA can be chemically converted through single-step hydrogenation to **AA**, one of the monomers in the common polymer nylon-6,6, which represents a multi-billion-dollar global market (Sobottka et al., 2024). Upon addition of TPA (166 mg L⁻¹) to alginate hydrogels supporting *E. coli*_pPCA1_pAA4 cells, AA conversion reached 79%, corresponding to a production of 115 mg L⁻¹. Then the TPA derived from PET bottle alkaline hydrolysis resulted in 65 mg L⁻¹ AA (Valenzuela-Ortega et al., 2023), demonstrating the feasibility of producing high-value AA from waste plastic via biotechnological approaches.

Furthermore, TPA can be bioconverted into other high-value polymer precursors, such as **β-KA** for the synthesis of nylon-6,6 analogs, **PDC** for the production of high-performance plastics, and **PHB**, a biodegradable and biocompatible polymer widely used in packaging, medical devices, and agricultural applications. Table 3 summarizes the strains and yields of these value-added products.

Table 3 The high-value utilization of monomers in PET degradation

Feedstock	Concentration	Strain	Product	Production
EG+TPA	40 mM TPA and 40 mM EG	<i>P. umsongensis</i> GO16	Polyhydroxyalkanoate (PHA) (Tiso et al., 2021)	0.15 g L ⁻¹
	50 mM TPA and 50 mM EG	<i>Rhodococcus jostii</i>	Lycopene (Diao et al., 2024)	3.2 mg L ⁻¹
TPA	3.10 (±0.01) mM	<i>Escherichia coli</i>	2-pyrone-4,6-dicarboxylic acid (PDC) (Kang et al., 2020)	2.30 ± 0.04 mM
	3.4 mM	<i>Escherichia coli</i>	PCA (Kim et al., 2019)	2.8 mM
	3.4 mM	<i>Escherichia coli</i>	Pyrogallol (PG) (Kim et al., 2019)	1.1 mM
	2.9 mM	<i>Escherichia coli</i>	Gallic acid (GA) (Kim et al., 2019)	2.7 mM
	3.0 mM	<i>Escherichia coli</i>	Catechol (Kim et al., 2019)	2.7 mM
	3.2 mM	<i>Escherichia coli</i>	Muconic acid (MA) (Kim et al., 2019)	2.7 mM
	3.4 mM	<i>Escherichia coli</i>	Vanillic acid (VA) (Kim et al., 2019)	1.4 mM
	1 mM	<i>Escherichia coli</i>	Vanillin (Sadler and Wallace, 2021)	744 µM±100 µM
	20.6 g L ⁻¹	<i>P. putida</i> KT2440	β-ketoadipic acid (βKA) (Werner et al., 2021b)	15.1 g L ⁻¹
	166 mg L ⁻¹	<i>Escherichia coli</i>	Adipic acid (AA) (Valenzuela-Ortega et al., 2023)	115 mg L ⁻¹
	10 g L ⁻¹	<i>Yarrowia lipolytica</i>	Polyhydroxybutyrate (PHB) (Liu et al., 2021)	11.56 wt% of cell dry weight PHB
	45 mM	<i>Escherichia coli</i>	2,4-dihydroxybutyric acid (DHB) (Frazão et al., 2023)	6.75 mM
EG	10 g L ⁻¹	<i>Escherichia coli</i>	Tyrosine (Panda et al., 2023)	2 g L ⁻¹
	28.6 mM	<i>G. oxydans</i> KCCM 40109	Glycolic acid (GLA) (Kim et al., 2019)	28.5 mM

3.2 Upcycling of Ethylene Glycol

The separation of EG from PET hydrolysate is hindered by its high viscosity, high water solubility, and high boiling point of 197.6 °C, which results in elevated energy consumption during the separation process and consequently high cost of directly recycling EG as a feedstock for regenerated PET (Zhang et al., 2024). Alternatively, EG can be assimilated into the central metabolic pathways (e.g., glycolysis, the tricarboxylic acid cycle) by microbes. Furthermore, the central metabolites such as acetyl-CoA or pyruvate can be readily redirected to synthesize value-added products (Zhang, C. et al., 2022), such as amino acids, GLA, PHA, and other high-value products. If the high conversion yield is achieved, such an upcycling route of EG may lead to higher economic benefit than direct recycling for PET production.

3.2.1 Metabolic pathways:

EG is typically assimilated through the intermediate glycolaldehyde by lactaldehyde reductase or alcohol dehydrogenase (Ren et al., 2025; Zhang et al., 2015), which subsequently can be metabolized to acetyl phosphate (AcP), GLA, and D-arabinose 5-phosphate to produce acetyl-CoA, then enter the central metabolic network for bulk chemicals biosynthesis, or be transformed into D-threose to produce L-2,4-hydroxybutyrate (Fig. 4):

EG assimilation pathway *via* glycolaldehyde:

(1) Glycolaldehyde can be catalyzed into AcP via the action of acetyl phosphate synthase, utilizing inorganic phosphate. The resulting AcP serves as a precursor in the formation of acetyl-CoA through phosphate acetyltransferase (PTA), which constitutes the most direct pathway to acetyl-CoA while maintaining carbon conservation and exhibiting ATP independence (Lu et al., 2019);

(2) Glycolaldehyde can be converted to GLA, which can subsequently be metabolized to the key intermediate product 2-phosphoglycerate and ultimately transformed into product L-tyrosine (4-hydroxyphenylpyruvate) (Panda et al., 2023). Meanwhile, 2-phosphoglycerate can also be metabolized to pyruvate-by-pyruvate kinase, which can subsequently be converted to acetyl-CoA;

(3) Glycolaldehyde can be converted into D-arabinose 5-phosphate by arabinose 5-phosphate aldolase. This intermediate is further transformed into AcP, then into acetyl-CoA, which enters the central carbon metabolism (Liu et al., 2024; Wagner et al., 2023);

(4) In addition to entering central carbon metabolism, glycolaldehyde also can synthesize the versatile platform molecule DHB through 5 sequential reaction steps. Succinctly, two molecules of glycolaldehyde are fused via the catalytic action of a D-threose aldolase to form D-threose molecule, then be oxidized by a D-threose dehydrogenase to produce D-threono-1,4-lactone, which can be converted to its corresponding sugar acid either spontaneously or through a reaction catalyzed by a D-threono-1,4-lactonase. Next, D-threonate undergoes dehydration by a D-threonate dehydratase to produce 2-oxo-4-hydroxybutyrate (OHB), which is reduced to yield DHB in a reaction catalyzed by an OHB reductase (Frazão et al., 2023).

EG assimilation pathway *via* acetaldehyde:

EG can also be converted to acetaldehyde via propanediol dehydratase, followed by its transformation into acetyl-CoA. Nonetheless, the cytotoxicity of acetaldehyde necessitates the

630 implementation of metabolic engineering interventions to enhance the efficiency of microbial
631 biotransformation ([Trifunović et al., 2016](#)).

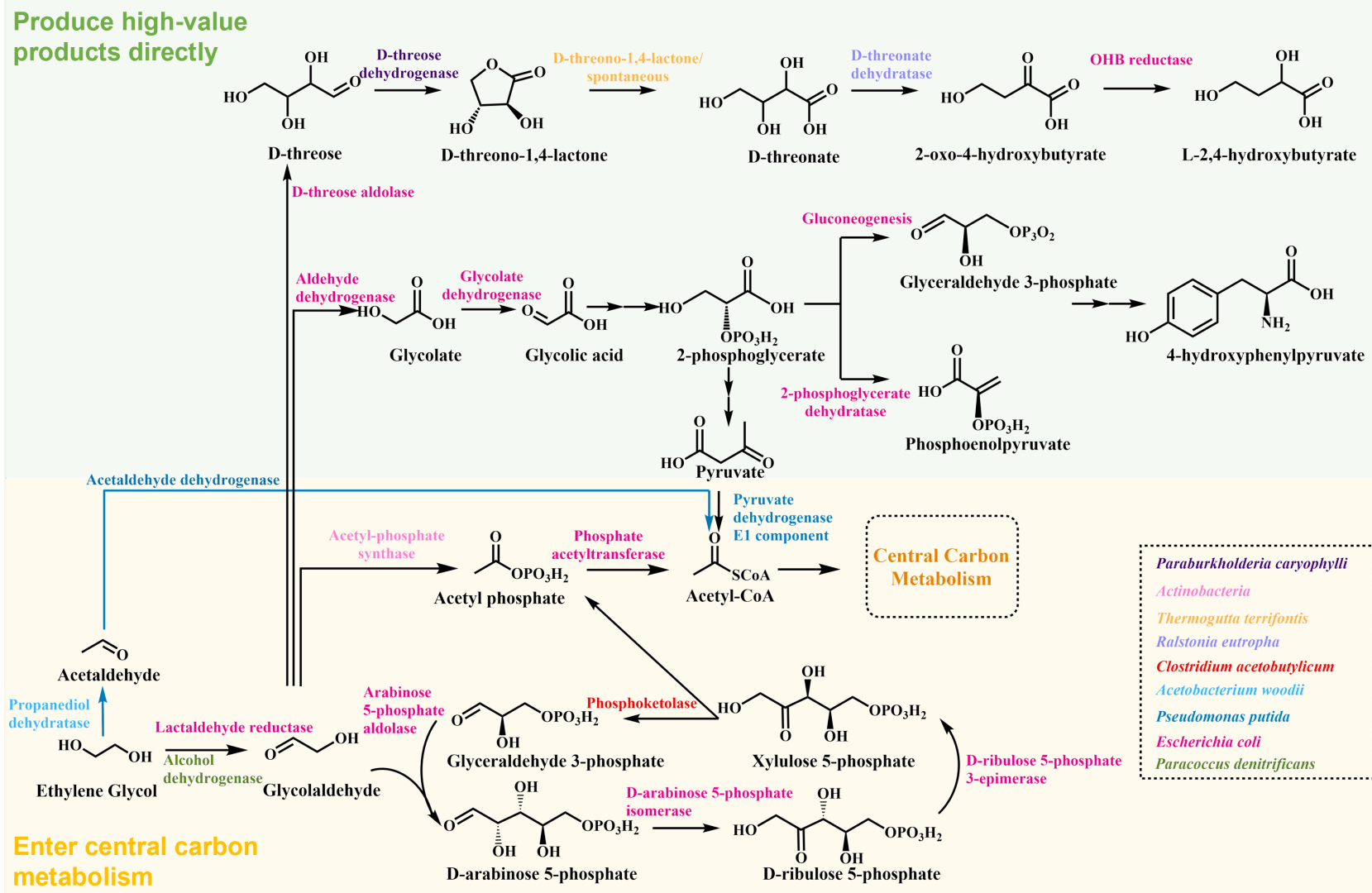


Fig. 4 Biotransformation Pathways of EG

3.2.2 Metabolic products

(1) Amino acid:

L-tyrosine (Tyr) is an essential aromatic amino acid required for protein biosynthesis in all organisms, which is widely applied in dietary supplements, pharmaceuticals, chemical and cosmetics industries (Jo et al., 2019; Moon et al., 2018). The global tyrosine market size was valued at around \$1.5 billion in 2023 and is projected to reach approximately \$2.7 billion by 2032 (Dataintelo, 2024). Panda et al. utilized engineered *E. coli* to transform EG into *L*-tyrosine, achieving a yield of 2 g L⁻¹ of *L*-tyrosine from 10 g L⁻¹ of EG under optimal fermentation conditions, even surpassing the performance observed with glucose under identical experimental conditions (Panda et al., 2023).

DHB, an α -hydroxy acid, serves as a precursor for the methionine analogue 2-hydroxy-4-(methylthio)butyrate, which is regarded as an animal nutrition supplement to improve the nutritional value of animal feed (Dong et al., 2023). Frazão et al. reported a five-step synthetic metabolic pathway that enables the carbon-conserving biosynthesis of the versatile platform molecule DHB starting from EG as previously mentioned. Engineered *E. coli* could produce 6.75 mM (or 0.8 g L⁻¹) of DHB from 320 mM EG at a yield of 0.15 mol mol⁻¹ (Frazão et al., 2023), offering a promising approach to convert sustainable carbon sources like EG into a valuable industrial compound.

(2) Chemical product monomer:

GLA, the simplest α -hydroxy acid, is widely used in the production of various chemicals for adhesives, metal cleaning, and dye additives (Hua et al., 2018; Lachaux et al., 2019). Due to the growing demand for GLA, the global market demand for GLA was \$93.3 million (Hua et al., 2018), while biotechnological production of this simple organic acid from renewable resources has received substantial interest. Kim et al. utilized *Gluconobacter oxydans* KCCM 40109 as a whole-cell catalyst to convert 30.6 g L⁻¹ of EG in PET hydrolysate to 31.4 g L⁻¹ of GLA with a molar yield of 91.6%, comparable to the 90.1% yield obtained using reagent-grade EG (Kim, D.H. et al., 2021), highlighting the potential of engineered microbial systems for the efficient conversion of EG into various high-value products.

Polyhydroxyalkanoates (PHAs), with their advantages of biodegradability and biocompatibility, are suitable for applications in agriculture, marine environments, and medicine (Harada et al., 2021). The global PHA market size was \$30.6 million in 2023 and is projected to grow from \$38.8 million in 2024 to \$301.7 million in 2032 (Insights, 2024), but the high cost currently limits its large-scale industrial application (Bhola et al., 2021). Franden et al. engineered *Pseudomonas putida* KT2440 to produce 32.19 $\pm$ 2.2% of its dry cell weight as medium-chain-length polyhydroxyalkanoate (mcl-PHA) at a yield of 0.06 $\pm$ 0.00 g of mcl-PHA per g of EG consumed (Franden et al., 2018), providing a promising approach for the production of biodegradability PHA from waste streams.

3.3 Economic Feasibility in PET high-valued Bioconversion Applications

The versatility of TPA/EG in bioconversion highlights their potential not only as a feedstock for recycled PET but also as a precursor for the synthesis of various high-performance polymers. Assessing the economic feasibility of their utilization requires careful consideration of market dynamics and production costs. Notably, the fundamental

physicochemical differences between TPA and EG inherently determine their most suitable downstream utilization strategies and economic value.

TPA, characterized by its aromatic rigidity and low solubility in acidic conditions, can be efficiently recovered from PET depolymerization solutions via acid-induced precipitation. This behavior stems from its weakly acidic carboxylic groups ($\text{pK}_{\text{a}1} \approx 3.51$, $\text{pK}_{\text{a}2} \approx 4.82$), which promote the transition from a highly soluble dianionic form (TPA^{2-}) to an insoluble neutral molecule under $\text{pH} < 3$, promoting rapid crystallization. This low-cost and straightforward recovery process enables TPA to be effectively reused as a monomer for PET repolymerization, making it an economically advantageous route. From a metabolic engineering perspective, considering that some assimilation pathways suffer from carbon loss, cofactor imbalances, and limited conversion rates—resulting in low yields and high operational costs—the bioconversion of TPA into high-value chemicals requires the selection of target products with significantly higher market value than TPA to ensure economic viability. Table 4 provides a comparative summary of market values and metabolic efficiencies for representative products derived from TPA/EG, helping to assess the feasibility of their bioconversion routes. As illustrated in Table 4, although some bioproducts like catechol exhibit a higher market value (\$266/kg) than TPA (\$194/kg), the high cost and inefficiency of microbial conversion make such routes economically impractical. Moreover, in cases where the target product is less valuable than TPA, such as AA, the economic rationale for bioconversion becomes even less viable.

In contrast to TPA, EG is highly soluble and more challenging to recover through conventional separation methods, typically requiring energy-intensive distillation. However, its simple two-carbon structure and compatibility with various microbial metabolic pathways make EG an ideal candidate for direct bioconversion. By circumventing the need for purification, EG-containing depolymerization solutions can be directly utilized as fermentation substrates, streamlining the process and reducing operational costs. Furthermore, EG's metabolic flexibility enables the production of a range of higher value-added bioproducts while enabling the synthesis of value-added bioproducts.

In summary, maximizing the economic and environmental benefits of PET depolymerization requires a product-specific strategy. TPA is most efficiently recycled via direct recovery and reuse, while EG offers greater potential for integrated biotransformation. Notably, bioconversion should be selectively applied only when it adds clear economic value, ensuring optimal resource utilization in PET upcycling processes.

Metabolite	TPA/EG Consumption (mol)	ATP Consumption (mol)	NAD(P)H Consumption (mol)	CO₂ Release (mol)	Molar conversion Efficiency (%)	Price* (\$/kg)
TPA						194
EG						167
VA(Kim et al., 2019)	1 TPA	0	0	1	87.5	742
PCA (Kim et al., 2019)	1 TPA	0	0	1	87.5	815
GA (Kim et al., 2019)	1 TPA	0	−1	1	87.5	995
PG (Kim et al., 2019)	1 TPA	0	−1	2	75	813
Catechol (Kim et al., 2019)	1 TPA	0	0	2	75	266
AA (Valenzuela-Ortega et al., 2023)	1 TPA	0	−1	2	75	60.4
Vanillin (Sadler and Wallace, 2021)	1 TPA	0	−1	1	87.5	406
GLA (Kim et al., 2019)	1 EG	0	2	0	100	436
L-Tyrosine (Panda et al., 2023)	6.6 EG	5.6	9.9 (anaerobic) / 7.7 (aerobic)	4.3	68.18	919

*The product prices were obtained from the Sigma-Aldrich website and are for reference only, as chemical prices may vary depending on the source, application, and specification. Further market investigation is required for accurate cost estimation.

3.4 Biotransformation Methods of PET Enzymatic Hydrolysis Products

Current research employed three approaches for the high-value transformation of PET degradation products (Fig. 5): (1) bio-transformation following the separation or purification of PET hydrolysates (Werner et al., 2021a), (2) simultaneous PET degradation and bio-transformation (Liu et al., 2021), and (3) direct bio-transformation using the reaction solution after PET degradation (Sadler and Wallace, 2021).

The separation of PET degradation products for subsequent bio-transformation allows both processes to operate under their respective optimal conditions, thereby enhancing the overall efficiency of high-value PET biotransformation. However, although TPA generated from PET degradation can be easily separated *via* acidification, the two-stage reaction process incurs additional buffer consumption, resulting in resource wastage and secondary environmental pollution (Kim et al., 2019; Zhou et al., 2024a). Meanwhile, as mentioned before, the recovery of EG through distillation is highly energy-intensive (Yan, L. et al., 2021). Therefore, EG is more suitable for direct bioconversion into high-value products within the reaction solution.

Utilizing the PET depolymerization solution directly for subsequent biotransformation offers notable advantages, including reduced downstream purification costs and minimized solvent usage. However, a major challenge lies in the incompatibility of optimal conditions for PET depolymerization and microbial bioconversion. For instance, PET hydrolysis often requires alkaline conditions, whereas most microbial hosts for biotransformation prefer neutral to slightly acidic environments. Further pH adjustment of the depolymerization solution can result in the formation of high-salinity byproducts, which may severely inhibit microbial growth and compromise the overall efficiency of the bioprocess (Panda et al., 2023). Moreover, high-concentration PET hydrolysate also has high osmolarity. For example, the biomass of *Rhodococcus jostii* strain PET was reduced by approximately half in the presence of 250 mM simulated PET hydrolysate (TPA + EG), compared to 100 mM (Diao et al., 2023). And the presence of potentially toxic additives in post-consumer PET, such as co-monomers, dyes, and plasticizers, may also exert substantial inhibitory effects on microbial growth (Diao et al., 2024). This highlights the need to select chassis strains with adequate tolerance or to improve strain robustness via adaptive laboratory evolution (ALE), thereby ensuring the feasibility of high-value PET bioconversion. Meanwhile, precise control of TPA and EG concentrations in the reaction buffer is also essential. Elevated EG levels can exert inhibitory effects on the growth of engineered strains (Chi et al., 2024), while insufficient concentrations may not support adequate cellular metabolism (Liu et al., 2021), both of which can lead to decreased yields of the desired product. Therefore, ensuring efficient PET degradation to obtain sufficient products for biotransformation, while adapting the biotransformation host to the PET degradation solution environment, would contribute to the clean and efficient high-value biotransformation of PET.

Simultaneous PET degradation and bio-transformation require efficient hydrolysis of PET by PET hydrolases under conditions compatible with bio-transformation (Liu et al., 2021). However, PET hydrolases exhibit higher degradation efficiency near the T_g of PET (65–70 °C), and the relatively low product concentrations generated by PET hydrolysis at room temperature

limit the efficiency of bio-transformation (Liu et al., 2021). Therefore, there is a need to develop thermophilic strains as hosts and advance corresponding genetic manipulation techniques for bio-transformation, or to enhance the hydrolysis efficiency of PET hydrolases under mild conditions through enzyme engineering to further improve the efficiency of high-value conversion of PET waste. However, due to the high T_g of PET, achieving efficient degradation at ambient temperature remains technically challenging at present.

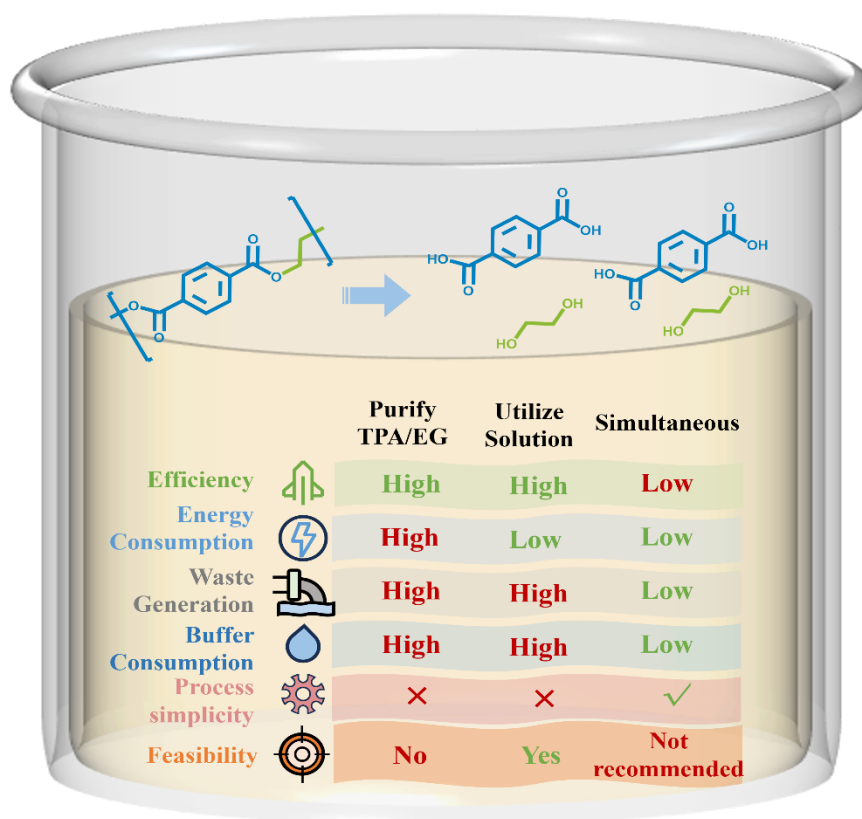


Fig. 5 Biotransformation Methods of PET Enzymatic Hydrolysis Products

4. Concluding remarks

Currently, PET biodegradation has garnered significant attention due to its environmentally sustainable advantages, with rapid progress being driven by the discovery and engineering of highly efficient PET hydrolases. The further biotransformation of PET hydrolysate into high-valued products for applications in food, pharmaceuticals, and chemicals holds significant environmental and economic benefits. Meanwhile, it is essential to thoroughly evaluate the technical feasibility and economic value of these products based on their metabolic pathways, market demand and intended applications.

Enhancing the PET enzymatic hydrolysis efficiency can be approached from two complementary perspectives: optimization of enzyme performance and improvement of substrate physicochemical properties. In recent years, enzyme engineering has been extensively applied to enhance the catalytic activity and thermostability of PET hydrolases, resulting in substantial advancements in PET hydrolysis performance. Given that the T_g of PET ranges from 65 °C to 71 °C, improving the efficiency of PET enzymatic hydrolysis requires careful consideration of the reaction temperature during enzyme design. Specifically, it is necessary to balance the fluidity of PET chains at high reaction temperatures to enhance degradation efficiency while avoiding increased crystallinity caused by PET aging. Therefore, thermophilic PET hydrolases offer inherent advantages due to their naturally high thermostability, which enables them to efficiently and stably degrade PET at temperatures near its T_g without the need for extensive thermal stabilization engineering, making thermophilic enzymes ideal candidates for efficient PET hydrolysis.

On the other hand, the physical characteristics of the substrate also play a crucial role in determining enzymatic degradation efficiency. For example, crystallinity, particle size, surface hydrophobicity, and surface roughness of PET collectively influence enzyme-substrate interactions. Thus, the development of efficient and stable PET hydrolases, when combined with optimized PET pretreatment strategies, can significantly enhance overall degradation outcomes. Recent studies have successfully demonstrated that the synergistic integration of enzyme engineering and substrate pretreatment offers significant potential for the large-scale implementation of clean and sustainable PET bio-recycling technologies, providing a promising strategy to mitigate plastic pollution.

Furthermore, considerable efforts have been directed toward utilizing PET hydrolysates for the synthesis of high-value compounds through cell factories and metabolic engineering strategies, thereby contributing to the valorization of plastic waste within a circular bioeconomy framework. Notably, developing strategies to degrade PET into high-purity monomers is a fundamental prerequisite for the biological valorization of PET degradation products. Due to the substrate specificity of PET hydrolases, their degradation products typically consist of a mixture of BHET, MHET, and TPA. Recently, strategies such as the engineering of PET hydrolases and the application of dual-enzyme systems have been employed to achieve complete degradation of PET into TPA, demonstrating promising results at the laboratory scale. However, the effective integration of these strategies under industrial conditions remains challenging due to factors such as the complexity of real PET waste streams,

potential enzyme inhibition by degradation intermediates, and the high costs associated with enzyme production and recycling. Moreover, biotransformation processes often involve the consumption of chemical inputs, ATP, and carbon flux, which can introduce additional energetic and material costs. Therefore, before designing bio-upcycling pathways for PET-derived substrates, it is critical to conduct a preliminary evaluation of both the conversion efficiency and the economic viability of the target products to avoid investing resources into the production of low-value compounds, which would ultimately undermine the feasibility of the overall process.

Additionally, regarding the utilization strategies of PET hydrolysates, the direct utilization of PET degradation solutions for biotransformation offers significant advantages in terms of environmental sustainability and energy efficiency. In particular, the separation of EG from the PET degradation solution requires energy-intensive distillation, thus directly utilizing EG in the degradation solution for bioconversion can effectively reduce energy consumption and minimize the need for additional buffer solutions. However, while this approach is economically and environmentally attractive, it requires optimization of the reaction condition compatibility between the two stages and critically depends on achieving efficient enzymatic hydrolysis of PET to ensure sufficient concentrations of degradation products for downstream conversion. Moreover, current studies that employ PET-derived EG directly as a substrate for engineered strain cultivation often report relatively low product yields, possibly due to inhibitory effects associated with the high osmotic pressure from high salt concentrations or high TPA/EG concentration present in the degradation solutions. Future studies should focus on optimizing engineered strains through metabolic engineering strategies such as adaptive laboratory evolution, in order to improve product yields when utilizing PET-derived EG as a substrate.

In conclusion, compared to chemical and physical methods, PET bio-upcycling – characterized by mild reaction conditions with non-toxic catalysts – represents a cleaner, more efficient, and environmentally friendly strategy for addressing plastic pollution. This approach not only mitigates the environmental impact of plastic waste, but also offers novel opportunities for its resourceful utilization, ultimately achieving the goal of transforming waste into valuable resources.

CRediT authorship contribution statement

Yu Zhou: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization; Jiaying Zhang: Conceptualization, Writing – review & editing, Supervision; Shengping You: Writing – review & editing, Supervision; Congqiang Zhang: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition; Wei Qi: Supervision.

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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Data availability

Data will be made available on request.

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