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Exploring biotechnology for plastic degradation, recycling, and upcycling for a sustainable future

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ABBREVIATIONS

BC: Bacterial cellulose
CBMs: Carbohydrate-binding modules
CDW: Cell dry weight
ChBD: Chitin-binding domain
EG: Ethylene glycol
HAAs: Hydroxyalkanoic acids
K_D: Dissociation constant
LCC: Leaf compost cutinase
mcl-PHA: Medium-chain-length-PHA
PA: Polyamide
PE: Polyethylene
PET: Polyethylene terephthalate
PhaC: PHA synthase
PhaZ: PHA depolymerase
PHA: Ployhydroxyalkanoate
PHB: Poly(3-hydroxybutyrate)
PHOH: Poly(3-hydroxyoctanoate-*co*-3-hydroxyhexanoate)
PLA: Polylactic acid
PP: Polypropylene
PU: Polyurethane
scl-PHA: Short-chain-length PHA
SMC: Synthetic microbial communities
TA: Terephthalic acid
TPU: Thermoplastic PU

Abstract

The persistent demand for plastic commodities, inadequate recycling infrastructure, and pervasive environmental contamination due to plastic waste present a formidable global challenge. Addressing this "plastic crisis" necessitates an ambitious paradigm shift towards a sustainable plastic value chain based on biodegradable materials. We propose a comprehensive strategy leveraging microbial processes to transform mixed plastics of fossil-derived polymers such as PP, PE, PU, PET, and PS, most notably polyesters, in conjunction with biodegradable alternatives such as PLA and PHA. Sequential enzymatic and microbial degradation of mechanically and chemically pre-treated plastic waste can be orchestrated, followed by microbial conversion into value-added chemicals and polymers through mixed culture systems. Plastics-degrading enzymes can be optimized through protein engineering to enhance their specific binding capacities, stability, and catalytic efficiency across a broad spectrum of polymer substrates under challenging high salinity and temperature conditions. Additionally, novel enzymes capable of degrading recalcitrant polymers can be discovered and characterized. The production and formulation of enzyme mixes can be fine-tuned to suit specific waste compositions, facilitating their effective deployment both *in vitro* and *in vivo* and in combination with chemical technologies. This approach enables the establishment of stable, self-sustaining microbiomes capable of selectively converting liberated plastic monomers into a diverse array of value-added products, including biomass, essential building blocks, and fine chemicals. Any residual material resistant to enzymatic degradation can be reintroduced into the process loop following appropriate physicochemical treatment. The plastic-utilization strategy outlined here, using the complementary strengths of the different fields of catalysis, can contribute to the valorization of mixed plastic waste, as evaluated by a techno-economic analysis.

1. Introduction

<Figure 1>

Global plastic production is projected to double from 400 million tons (Mt) to 800 Mt by 2050, yet only a fraction is currently recycled. Recognizing this urgency, the European Union and China aim to shift towards a circular bioeconomy, outlined in its Plastics Strategy, to manage plastic waste more efficiently. Innovative solutions are crucial for upcycling mixed plastics, including recalcitrant and biodegradable types, into valuable second-generation feedstocks. This endeavor relies on engineered enzyme mixtures for depolymerization and diverse microbial consortia for converting plastic monomers into desired products (**Figure 1**).

Polyethylene terephthalate (PET) and polyurethane (PU) make up over 12.8% of Europe's fossil-based plastic production, serving a multitude of applications (Plastic Europe, 2023). PET's clarity and strength are leveraged in the production of bottles, textiles, and automotive components. Both materials highlight the versatility and importance of polyester plastics in modern industry. Meanwhile, PU is valued for its durability and resistance to various conditions, making it suitable for use in footwear, adhesives, and construction insulation. Their complex properties and prevalent use in multi-layered configurations present significant hurdles for recycling initiatives. At present, PET stands out as a prime example of successful plastic degradation, thanks to the discovery of bacteria capable of breaking down PET and the enzyme PETase (Jia et al., 2024). Recent studies have indeed shed light on the potential for polyurethane (PU) degradation by certain microbes, expanding our understanding of plastic degradation beyond PET. These groundbreaking findings have opened new avenues for the development of more sustainable waste management practices (Utomo et al., 2020). Additionally, novel chemo-catalytic hydrogenolysis methods are envisioned to achieve complete depolymerization of even recalcitrant polyesters.

Efforts are actively underway to develop robust (bio)degradation pathways for non-hydrolysable polyolefin plastics such as polyethylene (PE) and polypropylene (PP), which collectively account for 45% of the plastics waste stream (Plastic Europe, 2023). These materials, prevalent in packaging and agriculture, resist enzymatic breakdown due to their stable C-C bonds lacking any functional groups. Research focuses on utilizing oxidases and enzyme cascades to cleave these resilient bonds (von Haugwitz et al., 2023; Inderthal et al., 2021).

On a more promising note, polylactic acid (PLA) and polyhydroxyalkanoate (PHA) plastics showcase inherent bio-recycling potential without requiring resource-intensive sorting processes (Park et al., 2024). Biobased and biodegradable alternatives like thermoplastic starch (TPS) and PLA represent key innovations in this field. Yet, they currently face challenges as contaminants in existing waste management and recycling systems (Siddiqui et al., 2021). Notably, it was recently reported the embedding of a thermophilic esterase in PLA, thereby enabling novel end-of-life routes (Guicherd et al., 2024).

The European Bioeconomy Alliance (EUBA) aims for 10% of all EU packaging materials to be sourced from biobased origins by 2030. This initiative underscores the goal of integrating fossil and biobased carbon reservoirs in biomaterial synthesis to advance a sustainable and resilient bioeconomy.

2. Hydrolysis of (mixed) plastics

2.1 A short state-of-the-art of plastic-degrading enzymes

The identification and optimization of enzymes with plastic hydrolysis activity offer a promising approach for managing plastic waste (Wei et al., 2020). These enzymes, capable of degrading plastics with heteroatomic bonds, like PET (Wei et al., 2022), polyurethane (polyester-PU) (Liu et al., 2021), and polyamides (PA) (de Witt et al., 2024; Bell et al., 2024) break down polymers into oligomers or monomers. Advancements in protein engineering of these enzymes aim to optimize yield, stability, and activity, and thereby enhancing enzyme efficacy in mixed plastic hydrolysis. By leveraging synergistic enzyme actions, mixed enzymatic hydrolysis provides a cost-effective and eco-friendly method for plastic degradation (Uekert et al., 2023). Tailored enzyme mixes for specific plastic compositions hold the potential for efficient recycling and upcycling of mixed plastic waste, supporting the shift towards a circular economy.

Enzymatic plastic degradation, especially for PET (**Figure 2**), is a dynamic research area advancing through initiatives like the EU Horizon 2020 project MIX-UP (GA-No. 870294), highlighted in recent expert reviews (Mican et al., 2024; Tournier et al., 2023; Wei et al., 2022). Biological PET recycling was recognized as a top emerging technology in Chemistry by IUPAC in 2023 (Gomollón-Bel, 2023).

<Figure 2>

Although many new enzymes capable of PET hydrolysis (PETase) have been discovered in recent years (Jiménez et al., 2022; Wei et al., 2022), IsPETase from *Ideonella sakaiensis* (Yoshida et al., 2016), leaf-branch compost cutinase (LCC) (Sulaiman et al., 2012; Tournier et al., 2020), and cutinases from *Thermobifida* species (Wei et al., 2014) are still the most commonly used scaffolds for protein engineering (Wei et al., 2022). Recent research has found that the glass transition temperature (T_g) of the PET surface layer, the only polymer layer that can interact with enzymes, is around 40°C in an aqueous environment due to the water plasticization effect (Tarazona et al., 2022). This knowledge helps to determine the conditions under which a mesophilic enzyme can begin to break down PET. Nevertheless, 70°C remains the ideal temperature for enzymatic PET degradation due to its balance of enzyme activity and polymer accessibility (Akram et al., 2024). Higher temperatures can lead to quick polymer recrystallization, hindering depolymerization (Tournier et al., 2020; Wei et al., 2019). As a result, thermostabilizing native PET hydrolases to allow for long-term activity in this temperature range has emerged as a significant trend in customizing industrially applicable biocatalysts. For example, the mesophilic IsPETase with a

melting point (T_m) of 46°C has gradually been thermostabilized (Bell et al., 2022; Brott et al., 2022), and its most stable mutant FastPETase has a Tm of 83°C (Lu et al., 2022). Similar protein engineering strategies were used to improve the stability and activity of LCC (Zeng et al., 2022) and cutinases from *Thermobifida* species (Meng et al., 2023).

Additionally, new thermophilic enzymes like BhrPETase and PES-H1 have been discovered and engineered (Pfaff et al., 2022; Xi et al., 2021). While the former is highly homologous to LCC (94% identity) and has recently been engineered into the so-called TurboPETase, a powerful biocatalyst that allows for rapid and complete PET waste depolymerization at pilot scale (Cui et al., 2024), the latter has a new backbone with a distant phylogenetic relationship to the prominent wild-type enzymes (Pfaff et al., 2022). As a result, it encouraged the MIX-UP partners to solve its crystal structures in complexes with various PET-related ligands, which provided valuable information for its rational design for increased hydrolytic activity (Pfaff et al., 2022). A double mutant of PES-H1 has recently revealed a comparable degradation performance on PET waste to the most prominent LCC-ICCG mutant under industrially relevant conditions, significantly better than other well-known IsPETase derivates (Arnal et al., 2023).

The successful collaboration with structural biologists has also led to the elucidation of complex crystal structures (**Table 1**) of other PET hydrolases and a carboxylesterase with PET oligomer hydrolysis activities (Li et al., 2022; Mican et al., 2024; von Haugwitz et al., 2022). The latter enzyme has been recognized as being extremely useful for accelerating PET waste depolymerization (Belisário-Ferrari et al., 2018), as the accumulation of inhibitory oligo-esters in the late stage of PET hydrolysis has been identified as critical to overall degradation performance and efficacy (Arnal et al., 2023; Wei et al., 2022). Besides, the de/adsorption of the enzymes at the polymer surface have proven to be a determinant for the interfacial biocatalysis process for an efficient degradation of PET (Bååth et al., 2022; Vogel et al., 2021; Xue et al., 2021) and other polyesters such as PLA (Lu et al., 2023a; 2023b).

The efficacy and stability of PETase in large-scale PET biodegradation poses significant challenges. Immobilizing PETase provides a promising solution (**Figure 3**). Compared to their solubilized counterparts, immobilized enzymes offer improved stability, easier separation from reaction mixtures, and hence reusability. Traditional techniques for enzyme immobilization include embedding, formation of insoluble aggregates, and attachment to polymeric surfaces (Hwang and Gu 2013). In contrast to conventional methods, biomimetic mineralization is an innovative approach that involves enzyme immobilization alongside inorganic salt precipitation (Qin et al., 2020). Biomimetic mineralization is valued for its simplicity, high enzyme activity recovery, porosity, large surface area, cost-effectiveness, and enhanced enzyme

stabilization through enzyme-inorganic hybrid nanoflowers (Gawas and Rathod, 2016). The concept builds on the protein-inorganic hybrid system proposed by Gojun et al. (Lei and Zare, 2012), with various hybrid nanoflowers synthesized for efficient immobilization of diverse enzymes (Ke et al., 2016; Cao et al., 2018).

<Figure 3>

In a similar approach, a His-tagged *Is*PETase was used for synthesis of PETase-$Co_3(PO_4)_2$ enzyme-inorganic nanoflowers (Jia et al., 2022) by a MIX-UP partner. Compared to the wild-type enzyme, the immobilized PETase showed 10°C higher optimal catalytic temperature and broader pH tolerance (pH 6.0 to 10.0, instead of pH 6.0 to 8.0). After 12 days, the immobilized enzyme retained 75% of its initial activity, while the free PETase declined to less than 5% activity over the same period. Overall, this nano-immobilization method for PETase demonstrated exceptional enhancements in stability, reusability, streamlining the immobilization process and eliminating costly enzyme purification steps (Jia et al., 2022).

Beyond biological recycling of PET through monomer recovery, enzymatic PET depolymerization can be combined with microbial catabolism of the monomers (Manoli et al., 2024; Bayer et al., 2022; Tiso et al., 2021) as well as chemical transformation (Xue et al., 2024) to produce other value-added products, allowing waste PET to be upcycled.

<Table 1>

The scientific community anticipates that developing an industrially viable biocatalytic recycling process for PET will likely be transferable to other commodity plastics with heteroatomic bonds such as PU and PA (Jönsson et al., 2021; Wei et al., 2020). PU can be classified into polyester- or polyether-PU based on the polyols used in their synthesis (Liu et al., 2021). Polyester-PU, with both ester and urethane bonds, is more readily hydrolyzed by ester hydrolases (Liu et al., 2023; Magnin et al., 2019; Schmidt et al., 2017). In contrast, enzymes capable of degrading polyether-PU, which contain only urethane bonds, are less characterized, though recent studies claimed biodegradation using yellow mealworms (*Tenebrio molitor*) (Liu et al., 2022).

Hydrolytic cleavage of urethane bonds is essential to achieve monomeric building blocks from PU that are suitable for closed-loop recycling. Recent advances include the discovery of three novel urethanases (UMG-SP-1, 2, and 3) from a soil metagenome library belonging to the amidase signature protein family (Branson et al., 2023). Crystal structures of UMG-SP-1 and -2 have been resolved with high resolutions, revealing their interactions with carbamate ligands (PDB IDs: 8WDW, 8XTB, 8XTC) (Bayer et

al., 2024). These enzymes expand the arsenal of amidase family members capable of depolymerizing both PU and PA, complementing previous efforts primarily involving less-active enzymes like a polyamidase (NfPolyA) from *Nocardia farcinica* (Gamerith et al., 2016). Furthermore, nylonase C (NylC), a member of another branch of the amidase superfamily, has been identified based on its nylon oligomer hydrolytic activity and has recently been further characterized and engineered (Bell et al., 2024; Negoro et al., 2021, 2023; de Witt et al., 2024). Nonetheless, the most effective NylC variants only caused minor degradation of PA films, emphasizing the need for improved enzymes to allow for industrial applications.

Unlike polymers with hydrolysable backbones, polyolefins, and other non-hydrolysable commodity plastics feature inert C-C bonds resistant to depolymerization by single enzymes (Tai and Harrison 2021; Yeung et al., 2021). Enzymes capable of oxidizing PE with varying polymer characteristics, such as peroxidases, alkane monooxygenases, and laccases, have been studied since the 1990s (Jin et al., 2023). However, their oxidative efficiency and specific biocatalytic mechanisms remain contentious, hindering reproducibility and reliability (Jin et al., 2023; Montazer et al., 2020). For instance, attempts to replicate the degradation of PE using an insect hexamerin from *Galleria mellonella* were unsuccessful (Stepnov et al., 2024).

2.2 Combined enzymatic hydrolysis of mixed plastics (PU, PET, and PVA)

As early as 2012 Ozsagiroglu et al. used a commercial mixture of esterase and protease to measure polyester-PU film degradation, achieving higher degradation compared to esterase alone (Ozsagiroglu et al., 2012). Magnin et al. screened 50 hydrolases and identified an efficient amidase (E4143) and esterase (E3576) capable of hydrolyzing PU bonds, with the dual enzyme system showing improved performance over single enzymes on polycaprolactone polyol-based PU (Magnin et al., 2019). The mixed enzyme approach facilitated ester bond hydrolysis by the esterase, followed by further hydrolysis of oligomers containing urethane bonds by the amidase, indicating the potential for complete PU depolymerization into monomers. More recently, Xin et al. developed a two-enzyme system comprising amidase GatA250 and cutinase LCC for the degradation of polyester-PU. The combined system exhibited superior degradation efficiency for both PU film (42%) and foam (14%) compared to the individual enzymes. Moreover, the LCC-GatA250 system significantly enhanced monomer production, with a 1.80-fold increase in MDA yield (Xin et al., 2024). In a similar fashion, NylC was also shown to be active on poly-ester-amides, showing synergistic activity when combined with the LCC cutinase (de Witt et al., 2024).

Bifunctional biocatalysts, such as PETase-MHETase fusions and *I. sakaiensis* PETase-

Candida antarctica lipase B (CALB) complexes, were reported to significantly enhance PET hydrolysis compared to wild-type enzymes (Knott et al., 2020; Hwang et al., 2022). Computational design led to the development of KL-MHETase and FAST-PETase dual enzyme systems, demonstrating a 2.6-fold faster PET depolymerization rate than FAST-PETase alone (Zhang et al., 2023). These innovations highlight the potential of enzyme fusion and computational approaches in advancing biocatalytic plastic degradation.

Biodegradation of poly(vinyl alcohol) (PVA) typically relies on slow enzymatic processes involving pyrroloquinoline quinone (PQQ)-dependent enzymes, which are costly due to the required cofactor, limiting industrial appeal. Haugwitz et al. addressed this by developing an enzymatic cascade that efficiently degrades modified and unmodified PVAs (von Haugwitz et al., 2023). This cascade (**Figure 4**), utilizing three enzymes with *in situ*-cofactor recycling, sequentially oxidizes the side-chain hydroxyl groups to ketones, performs Baeyer-Villiger oxidation of ketones to esters, and cleaves these bonds by a lipase to depolymerize PVA by forming oligomeric fragments with carboxylate or alcohol end groups. This enzyme cascade, when combined with an extra peroxidase, has recently been demonstrated to depolymerize chemically pre-oxidized short-chain PE wax, releasing medium-sized functionalized molecules such as ω-hydroxy acids and α, ω-carboxylic acids (Bornscheuer et al., 2024; Oïffer et al., 2024). Both proof-of-concept studies highlight the importance and necessity of using multiple enzyme complexes to address non-hydrolysable plastics, indicating that further efforts in enzyme engineering and reaction optimization are required to enable the corresponding industrialized plastic recycling process.

Mixed plastics waste represents a vast and underutilized resource for producing valuable products (Xu et al., 2023). Sullivan et al. introduced a two-stage oxidation and biological funneling strategy capable of breaking down and reforming mixtures of common consumer plastics (Sullivan et al., 2022). Lomthong et al. utilized an enzyme mix from *Laceyella sacchari* LP175 to effectively degrade high-concentration poly(lactide)/thermoplastic starch blends (Lomthong et al., 2022).

Scaling up enzymatic depolymerization of mixed plastics for commercial use requires addressing challenges such as enhancing enzyme stability and activity, reducing production costs, and optimizing reaction conditions (Reifsteck et al., 2023).

2.3 Increasing selectivity of plastic hydrolysing enzymes using peptide-fusions

Anchor peptides or small material-binding peptides (MBPs) enhance plastic selectivity during degradation by directing plastic-degrading enzymes to target substrates (Mao et al., 2024). Recent studies have focused on identifying anchor peptides with strong

affinity for plastics like PET and PLA (Lu et al., 2023b). Through screening and rational design, peptides with high binding affinity to various plastic surfaces were developed, facilitating enzyme attachment and binding (Mao et al., 2024). Anchor peptide integrations into enzyme formulations enhance degradation rates by improving enzyme retention on plastic surfaces and tailored enzyme mixes for degrading mixed plastics (Lu et al., 2023b). Overall, anchor peptides provide a valuable strategy for achieving plastic selectivity in enzymatic hydrolysis, thereby advancing sustainable plastic waste management.

Blanco et al. engineered small material-binding peptides (MBPs) for surface functionalization of polyesters (Blanco et al., 2023), employing an amphiphilicity-based rational approach. Specifically, two peptides derived from the phasins PhaF and PhaI from *P. putida* KT2440 (designated as MinP and MinI) were evaluated for their binding affinity toward polyhydroxybutyrate (PHB) and poly(hydroxyoctanoate-*co*-hydroxyhexanoate) or (PHOH). *In vivo*, fluorescence studies revealed selective binding toward PHOH. Importantly, MinP and MinI effectively immobilized cargo proteins on the polymer surfaces, demonstrating their utility beyond polyester binding. This study underscores the potential to engineer MBPs with enhanced affinity and broader specificity for diverse plastic substrates facilitating enzymatic degradation (Blanco et al., 2023).

Plastics are often blended with other materials to enhance their properties, creating challenges for efficient recycling. Inspired by natural mechanisms such as cellulose-binding domains, carbohydrate-binding modules (CBMs) were fused with a thermostable variant of leaf compost cutinase (LCC) to improve PET hydrolysis (Graham et al., 2022). Fusion proteins (LCC-YCCG-CBM) outperformed LCC-YCCG alone, increasing monomer release up to fourfold. In a similar study, when the chitin-binding domain (ChBD) from *Chitinolyticbacter meiyuanensis* SYBC-H1 was fused to the C-terminus of LCC-ICCG a 19.6%, improvement in degradation performance was achieved (Xue et al., 2021).

Islam et al. utilized the anchor peptide Tachystatin A2 (TA2) to enhance the degradation of polyester-PU. Fusion with the bacterial cutinase Tcur1278, acting on the ester bonds in this polymer, resulted in a 6.6-fold improvement in nanoparticle degradation compared to Tcur1278 alone, with degradation half-lives reduced from 41.8 h to 6.2 h in diluted suspensions (0.04% w/v) (Islam et al., 2019). Recent protein engineering efforts aimed to enhance the specificity of the material binding peptide Cg-Def for degrading polylactic acid (PLA) in mixed plastic matrices. Initially, a high-throughput screening system (PLABS) validated Cg-Def variants, with the ultimate variant, Cg-Def YH (L9Y/S19H), showing a twofold increase in PLA binding specificity over

polystyrene (PS). Another variant, Cg-Def V2 (S19K/K10L/N13H), demonstrated a 2.3-fold enhancement in PLA binding specificity compared to polypropylene (PP). Combining Cg-Def YH with a PLA-degrading enzyme expedited PLA depolymerization in mixed PLA/PS compared to the enzyme alone (Lu et al., 2023b).

<Table 2>

The precise mechanism of MBP interaction with polymers requires further investigation to enhance targeted polymer binding. While current studies have focused on polymer particle degradation, exploring MBP efficacy across polymers with diverse characteristics, including crystallinity and shape, is crucial. These efforts aim to refine anchor peptides and MBPs for enhanced material-specific binding and polymer-specific degradation, contributing to sustainable plastic recycling.

Hydrophobins, small cysteine-rich proteins from filamentous fungi, are of particular interest for enhancing enzyme performance. They self-assemble into amphiphilic monolayers at interfaces, altering surface wettability (Linder et al., 2005; Ren et al., 2013). Doris et al. (Ribitsch et al., 2013) showed increased hydrolytic activity of keratanase fused with fungal hydrophobic proteins. Similarly, PETase fused with RolA from *Aspergillus oryzae* exhibited a 51% activity boost at 30°C (Tsai, and Lee 2021). HFBII from *Trichoderma reesei* also enhanced polyester hydrolase activity (Kontkanen et al., 2009; Li et al., 2020). These findings suggest HFBII could similarly enhance enzymatic modification of synthetic polymers like PET, promising advancements in enzyme immobilization and industrial applications.

2.4 Chemo-catalytic routes for plastic degradation and recycling

Selective chemical conversion strategies can be used to upcycle polymers into defined products while retaining their inherent value. Established methods like pyrolysis and gasification, while offering high technology readiness levels (TRLs), often result in the loss of valuable chemical functionalities. Transition-metal catalyzed hydrogenolysis stands out as a promising approach for recycling recalcitrant polymers. This process involves breaking polymer bonds with molecular hydrogen, yielding smaller molecules that can integrate into new or existing value chains, promoting circular material economies.

Initial studies by Milstein and Robertson utilized ruthenium-based PNN and PNP complexes to convert polycarbonates (PC), PET, and PLA (Li et al., 2022). Later, they expanded to include tridentate PNO ligands and modified PNN ligands for PET (Fuentes et al., 2015). Early methods required substantial base and high catalyst loadings. In 2018, using molecular ruthenium catalysts, the Klankermayer group

introduced a base-free reductive conversion method for PC, PCL (polycaprolactone), PET, PBT (polybutylene terephthalate), and PLA. This breakthrough achieved complete polymer conversion with minimal catalyst (as low as 0.01 mol%) and demonstrated scalability by treating up to 16 grams of consumer plastic waste (Westhues et al., 2018).

Following initial successes, research has advanced to develop more efficient catalysts (Kindler et al., 2020), expand substrate scope to include PU and PA (Zhou et al., 2021; Zubar et al., 2022), and explore the conversion of composite materials such as fiberglass reinforced epoxy resins (Ahrens et al., 2023). By selectively breaking polymer bonds and integrating hydrogen, this approach transforms waste plastics into valuable building blocks for new materials.

Independent studies done by the groups of Guironnet and Hartwig established multi-step processes for PE conversion, involving initial dehydrogenation followed by isomerization-metathesis to yield propene (Wang et al., 2022; Conk et al., 2022). Klankermayer and Tuba further refined this concept by replacing the energy-intensive dehydrogenation step with milder pyrolysis, producing olefin-rich pyrolysis oils (Farkas et al., 2023). These oils are subsequently converted into valuable products via ruthenium-catalyzed isomerization-metathesis reactions.

Innovative polymer design strategies have emerging, creating materials akin to polyolefins but with natural degradation pathways. Mecking et al. pioneered this approach with PE-like materials incorporating keto-functionalities for photolytic or oxidative end-of-life degradation (Baur et al., 2021). They also introduced novel polyesters featuring long-chain ester units for enzymatic hydrolysis biodegradation while mimicking HDPE properties (Eck et al., 2023). Miyake et al. adopted a similar philosophy, designing polyolefin-like multiblock polymers recyclable via ruthenium-catalyzed hydrogenolysis (Zhao et al., 2023).

3. Microbial depolymerization of plastic polymers

Microbial depolymerization of plastic polymers shows promise for sustainable plastic waste degradation through enzymatic activities. However, current research faces significant challenges. Analyzing microbial metabolites and degradation products is difficult, often hindering understanding of degradation mechanisms. Additionally, diverse plastic types, additives, microbial communities, and environmental factors complicate studying microbial depolymerization processes (Montazer et al., 2020).

3.1 Overview of challenges in microbial plastic depolymerization

The biodegradability of synthetic plastics hinges on material properties such as chemical structure, functional groups, molecular weight, crystallinity and hydrophobicity (Thew et al., 2023). For example, the complex spatial structure of plastics impedes microbial access to high molecular weight polymers for metabolic processes. Plastics with minimal branching often exhibit high crystallinity, making them resistant to biodegradation, while amorphous regions being more susceptible to microbial attack (Schubert et al., 2024). The hydrophobic nature of plastics hinders colonization by bacteria or fungi, limiting their use as energy sources (Bertocchini and Arias, 2023).

Research has primarily focused on identifying microbial strains capable of depolymerizing plastics rather than elucidating the specific enzymes involved (Verschoor et al., 2022). Effective polymer-degrading strains and consortia remain limited, primarily due to initial oxidative polymer degradation challenges.

<Figure 4>

Incorporating biodegradable additives such as the plasticizer phthalate, functional additives, oligomers or side chains, can compromise overall biodegradability. Environmental factors such as light, heat, moisture, pH, and microbial activity catalyze structural modifications and the formation of new functional groups, complicating the analysis of degradation intermediates and pathways. Standardized methodologies for assessing and comparing the plastic degradability of microorganisms and enzymes are lacking (Montazer et al., 2020). Moreover, only rarely do studies analyze reveal molecular mechanisms for degradation.

High-throughput screening can identify bacteria with high-degradation rate (Liu et al., 2022) while chemical pretreatment can incorporate oxygen functionalities like hydroxyl, ketone, and epoxide to facilitate microbial attachment and improve degradation (Kong et al., 2022). Future research may focus on converting chemically degraded plastics into high-value chemicals and materials using engineered microbes to maximize resource utilization (Sullivan et al., 2022).

3.2 Chemical pretreatments of plastics improve biodegradation rate and yield

Enhancing PET recycling economics through upcycling involves converting PET monomers into higher-value products. The PET monomers terephthalic acid (TA) and ethylene glycol (EG) were upcycled using various microbes to synthesize valuable compounds such as gallic acid, pyrogallol, muconic acid (Kim et al., 2019), catechol (Kim et al., 2021), vanillin (Sadler and Wallace 2021), β-ketoadipic acid (Werner et al., 2021), and 2-pyrone-4,6-dicarboxylic acid (Kang et al., 2020). Pyrolysis has been

applied successfully for the depolymerization of PET to produce a solid, oil, and gas fraction at 77%, 6%, and 18% by weight, respectively (Kenny et al., 2008). The solids were made up of 51% TA and 20% oligomers, which, when dissolved in sodium hydroxide formed TA (Kenny et al., 2008). The solids fraction dissolved in NaOH to form a sodium terephthalate salt was successfully converted to PHA by *P. umsongensis* GO16 with the polymer making up 27% of cell dry weight of the bacterial cells (Kenny et al., 2008).

High molecular weight polyolefins require pretreatment to biodegrade. Surface modification of PE enhances its interfacial oxidation properties, which is crucial for improving biodegradation. Ultraviolet or microwave irradiation and acid/alkali treatments often suffer from extended treatment durations and suboptimal oxidation efficacy. A more energy-efficient oxidation method was adopted using a Co(acac)2 catalyst, significantly reducing oxidation times to 24 hours (Liu et al., 2022). Unspecific oxygenation of side chains decreased the activation energy required for C-C bond cleavage and depolymerization, thereby accelerating biodegradation. In a subsequent 90-days biodegradation study with *Bacillus velezensis* C5, modified LDPE exhibited a weight loss of up to 24%, illustrating the efficacy of this novel approach (Liu et al., 2022).

The combination of pyrolysis, chemical oxidation and microbial fermentation has been applied to PE (Guzik et al., 2021). Pyrolysis of PE gave rise to a wax, which consists of long chain hydrocarbons (alkanes and alkenes). The wax was subsequently oxidized using a permanganate catalyst at elevated temperatures (100-140℃) to generate mono and di-carboxylic acids. The monocarboxylic acid fraction when used as substrates for *P. putida* KT2440 achieving 83 $g_{biomass}$/l with 65% biomass mcl (medium-chain length)-PHA. The dicarboxylic acid fraction provides building blocks for applications in pharmaceutical industry (Zhao et al., 2022). Guzik et al., first attempted to convert the PE derived wax (C8-C32 hydrocarbons) to PHA without oxidation (Guzik et al., 2014). However, the highest biomass achieved was 0.4 g/l with the cells containing 10% of the cell dry weight as PHA. Instead, oxidation increases substrate conversion to 200-fold improvement in microbial biomass and 6.5-fold improvement in PHA.

3.3 Plastic degradation strategies of bacteria

Plastic degradation relies on multiple microbial traits, including cell surface attachment, enzyme cascade, extracellular enzyme activity for polymer oxidation or hydrolysis, and plastic oligomer uptake and catabolism (von Haugwitz et al., 2023). The isolation and characterization of plastic-degrading bacteria are crucial for understanding

biodegradation mechanisms. Biofilm can facilitate colonization and enhances interactions between bacteria, enzymes, and polymer. For example, *B. velezensis* C5, rapidly biofilmed on untreated PE films, significantly reducing surface hydrophobicity (Liu et al., 2022). *B. velezensis* C5 secretes multiple enzymes to catalyze PE biodegradation, reducing the film's contact angle from 100° to 54° and shedding short-chain alkanes (C24-C29) from the PE polymer skeleton.

Three consortia were obtained from petroleum-contaminated soils, utilizing n-hexadecane and LDPE film as substrates. High-throughput sequencing identified dominant species such as *Pseudomonas, Achromobacter, Inquilinus, Brucella,* and *Brevibacillus* accompanying LDPE film degradation (Kumar and Raut, 2015). These species attached to the polymer surface, which was modified by surface-active agents and contribute to PE oxidation using enzymes like AlkB alkane monooxygenase. This oxidation step lowers the energy barrier of PE C-C bonds, facilitating polymer fragmentation. Esterases, lipases, and laccases secreted by *Pseudomonas, Achromobacter,* and *Brevibacillus* cleaved PE into smaller fragments, which are assimilated through hydrocarbon degradation pathways by the microbial community.

3.4 Engineered microbes for enforced plastic degradation

In industry enzyme immobilization is favored over free enzymes due to enhanced stability and reusability (Gennari et al., 2022). Traditional methods like covalent binding and encapsulation risk enzyme denaturation from chemical involvement, leading to reduced activity or loss. Cell surface display systems integrated onto the outer membrane proteins of bacteria provide a whole-cell biocatalysts technology which presents enzymes on microbial cell surfaces (Maghraby et al., 2023). Studies have explored displaying enzymes such as lipase and keratinase (Lee et al., 2005; Lee and Park, 2005; Liu et al., 2010), with PETase successfully in yeast (Chen et al., 2021). Previous work suggests *E. coli* outer membrane fatty acid transporter (FadL), rich in β-structures, is effective for anchoring proteins in enzyme display (Lee et al., 2004).

In a recent study, a novel cell surface display system for a PETase was immobilized on the outer membrane of *E. coli* (Jia et al., 2022) (**Figure 5**). The system introduced hydrophobic proteins that reduced the contact angle with PET surfaces, enhancing PETase affinity for PET. The system retained 73% activity after 7 days at 40°C and maintained 70% activity after 7 catalytic cycles, demonstrating process robustness (Jia et al., 2022).

<Figure 5>

4. Plastic monomer metabolism in microorganisms

Microbial degradation of monomers from chemical, enzymatic, or microbial depolymerization is crucial for bio-recycling plastic waste. Hydrolysates often contain a variety of monomers such as adipic acid, terephthalic acid, and diols like 1,4-butanediol. Various microorganisms, particularly pseudomonads, are leveraged to metabolize these monomers through adaptive laboratory evolution and metabolic engineering.

4.1 Polyethylene terephthalate (PET) monomers

EG, derived from PET, is a prominent example for sustainable plastic recycling, and was argued for as general substrate for biotechnology (Wagner et al., 2023). Pseudomonads like *P. putida* and *P. umsongensis* are promising hosts for plastics upcycling (Tiso et al., 2020; Li et al., 2019; Guzik et al., 2014; Kenny et al., 2008; von Borzyskowski et al., 2023). Although *P. putida* can metabolize EG biologically, it does not use EG as a carbon source for growth (Mückschel et al., 2012). Instead, EG is oxidized to glyoxylate and further to CO_2, enabling electron harvest via redox cofactors like PQQH2, NADH, and cytochrome (Li et al., 2019; Franden et al., 2018; Wehrmann et al., 2017). Mutants of *P. putida* were developed to use EG as their sole carbon source, using the glyoxylate ligase for C-C bond formation (Li et al., 2019).

Bacterial degradation pathways of TA have been elucidated in various organisms (Choi et al., 2005; Narancic et al., 2021; Schläfli et al., 1994; Wang et al., 1995) via dioxygenation to produce protocatechuate (PCA). The most common degradation route is the β-ketoadipate pathway, where PCA is cleaved by PCA-3,4-dioxygenase forming β-carboxy-cis-cis-muconate, which is further degraded to succinate and acetyl-CoA. Other pathways include meta-cleavage by PCA-2,3-dioxygenase and para-cleavage by PCA-4,5-dioxygenase. To use TA as the sole carbon source, *P. putida* requires heterologous expression of the *tph* operon regulated by IclR, e.g., from *P. umsongensis* GO16 (Narancic et al., 2021).

4.2 Polyurethane (PU) and polyamide (PA) monomers

A study by Utomo et al. used a defined microbial mixed culture to bio-convert a mock PU hydrolysate containing adipic acid (AA), 1,4-butanediol (BDO), EG, and 2,4-toluenediamine (TDA) (Utomo et al., 2020). The mixed culture grew well on AA, BDO, and EG but was hindered by TDA (Figure 6). The authors employed specifically engineered microbes capable of utilizing individual PU monomers as the sole carbon and energy source for growth (Ackermann et al., 2021; Li et al., 2020; Li et al., 2019; Espinosa et al., 2020). These strains were shown capable of utilizing the four PU monomers, resulting in a defined mixed culture design. Polyamides (PA) are a class of

synthetic polymers known for their diverse monomer compositions and excellent physical properties, making them widely used in various applications. The monomers of polyamides typically consist of carboxylic acids and amines that polymerize to form amide groups (-CO-NH-) linked into long-chain molecules. For instance, nylon 6,6 is made from adipic acid and hexamethylenediamine through a condensation reaction.

Ackermann et al. highlighted the role of β-oxidation in degrading medium-chain length dicarboxylic acids (mcl-DCAs) like adipic acid present in nylon 6,6 and some PUs (Ackermann et al., 2021). This process required the heterologous expression of the *dcaAKIJP* operon from *Acinetobacter baylyi* and the constitutive expression of β-oxidation genes, enabling *P. putida* to grow on even-chain mcl-DCAs. For uneven-chain-length mcl-DCAs, further expression of *gcdH*, regulated by *gcdR*, was utilized (Ackermann et al., 2024). Li et al. found the PP_2047-51 operon degraded 1,4-butanediol via β-oxidation to acetyl-CoA and glycolyl-CoA or succinate (Li et al., 2020). ALE experiments identified a *secG* mutation that facilitated robust growth on mcl-diols (Ackermann et al., 2024). Meanwhile, *Pseudomonas jessenii* can metabolize 6-amino hexanoic acid or ε-caprolactam (Otzen et al., 2018). Some microorganisms can degrade biogenic amines like putrescine and cadaverine (Kurihara et al., 2005; Luengo and Olivera, 2020), though hexamethylenediamine (HMDA) degradation remains unexplored.

Aromatic diamines, known for their ecological toxicity, present challenges due to their complex and unmapped microbial metabolic pathways. Key diamine monomers in PU include 4,4'-methylenedianiline (MDA) and 2,4'-toluene diamine (TDA). Espinosa et al. advanced the understanding of TDA degradation in *Pseudomonas* sp. TDA1, demonstrating its ability to use TDA as a sole carbon/nitrogen source, and proposed its degradation pathway through genomic and transcriptomic analyses (Espinosa et al., 2020; Puiggené et al., 2022).

The degradation of 2,4-TDA from PU involves oxidation, decarboxylation, and deamination of the methyl group, leading to the formation of 4-aminocatechol. This compound is subsequently transformed via the catechol metabolic pathway into 4-amino-2-hydroxyadipic acid, ultimately undergoing complete mineralization. Interestingly, this microbial process shares similarities with toluene metabolism, suggesting common mechanisms for breaking down aromatic compounds in microbial environments. In contrast, the microbial degradation pathway of MDA is less explored. Liu et al. isolated *Cladosporium* sp. P7, which utilizes MDA as its sole carbon source. They proposed a metabolic pathway for MDA degradation resembling the biphenyl pathway, involving initial oxidation by dioxygenase and dehydrogenase enzymes. This leads to the formation of AABD (3-amino-6-(4-aminobenzyl) benzene-1,2-diol), which

undergoes further enzymatic transformations culminating in complete mineralization, supporting microbial growth (Liu et al., 2023). Understanding this pathway can offer insights into potential bioremediation strategies for MDA-contaminated environments.

<Figure 6>

5. Genetic technologies for improving microbes

5.1 Advancements in genetic engineering tools in non-model organisms

Advances in genetic engineering tools for microorganisms are pivotal for programming new traits in targeted species and strains, enhancing both existing platforms and creating new ones. Key advancements include the development of the Standard European Vector Architecture (SEVA) 4.0 vector platform, extending capabilities beyond *E. coli* to non-model Gram-negative bacteria like *Pseudomonas* spp. and *Halomonas* spp. (Li et al., 2019; Tan et al., 2011) (**Figure 7a**). This updated platform integrates enhanced features for genome editing, gene expression, chromosomal integration, and transposon mutagenesis, simplifying the design of strains with novel traits (Martínez-García et al., 2023).

The Golden Standard assembly method, part of MIX-UP, enables modular cloning across a broad spectrum of bacteria. These innovations streamline the DNA assembly process (Damalas et al., 2020; Lammens et al., 2022). Golden Standard facilitates the assembly of complex genetic circuits with up to twenty transcriptional units, validated for DNA assembly, portability, and phenotype engineering in α-, β-, γ-, and δ-Proteobacteria (Blázquez et al., 2023). A dedicated web portal supports community-driven development by providing resources for designing constructs and sharing parts and vectors.

5.2 Optimization of *Pseudomonas putida* as adhesin display platform and DNA diversification strategy

Innovations in leveraging the naturally-occurring properties of *P. putida* include enhancing its suitability as a chassis for displaying synthetic adhesins (Martínez-García et al., 2020). This approach offers the possibility of directing cell binding to solid surfaces presenting specific molecular motifs recognized by adhesin, thereby altering the tropism of the cell catalyst towards target materials (Fraile et al., 2021). Genetic modification of *P. putida*'s genome to remove flagella and fimbriae that hinder adhesin interaction allows display adhesins on their outer membranes (**Figure 7b**), facilitating precise cell binding to designated surfaces and supporting rapid nanobody generation,

crucial for enhancing cell-to-cell and cell-to-surface interactions (Al-Ramahi et al., 2021).

Diversifying regulatory sequences like promoters and intergenic regions into *P. putida* can optimize gene activity. Such an approach was developed using cytosine deaminases (CdA) fused to bacteriophage T7 RNA polymerase (RNAPT7), leveraging high mutagenic rates to induce DNA segment-specific variability (Velázquez et al., 2022) (**Figure 7c**). This genetic platform evolved defined DNA portions in vivo without altering the rest of the genome. Integrating RNAPT7-CdA fusions with transposons housing complex genetic devices, were used to explore diverse chromosomal locations and construct *P. putida* strains responsive to green light for biofilm attachment on plastic surfaces (Hueso-Gil et al., 2023) (**Figure 7d**).

The advancements in genetic technologies achieved through MIX-UP highlight the value of collaborative efforts in developing versatile tools that find applications across diverse fields of biotechnological research.

<Figure 7>

6. Mixed plastic monomers to value-added products

With advances in depolymerizing plastics, especially complex waste streams, microbes can be useful in the bioconversion of mixed plastic hydrolysates into value-added products (Tiso et al., 2022; Yan et al., 2022) such as surfactants hydroxyalkanoyloxy-alkanoic acids and rhamnolipids, or biopolymers like cyanophycin and functionalized PHAs. Downstream efforts also focus on novel applications across packaging, textiles, automotive, and biomedical sectors. Diversifying products and refining separation processes aim to maximize biopolymer production value, fostering sustainable alternatives to traditional plastics and advancing circular economy initiatives.

6.1 Challenges and new concepts of mixed plastic monomers upcycling to biopolymers

Prior work in upcycling mixed plastics focused on developing bacteria capable of processing multiple plastic-derived monomers simultaneously. However, increasing the number of heterologous genes raises metabolic burden, reducing bacterial fitness. An alternative is using Synthetic Microbial Communities (SMCs) division of labor (Bao et al., 2023). Recent advances in model-assisted metabolic engineering have provided an overview of metabolic modeling approaches for SMC characterization (Gudmundsson and Nogales 2021, García-Jiménez et al., 2021). Three primary approaches for engineering SMCs include: i) bottom-up: assembling individual systems to create more

complex consortia, requiring a deep understanding of individual components; ii) top-down: simplifying complexity using evolutionary engineering, not requiring prior knowledge of community functionality; iii) middle-out: functional enrichment by transferring components from SMCs constructed through either bottom-up or top-down methods (León and Nogales, 2022).

The MIX-UP project primarily used the bottom-up approach FLYCOP (FLexible sYnthetic Consortium OPtimization). FLYCOP optimizes microbial consortia configurations for specific objectives. An updated version of FLYCOP is being developed and used for upcycling PET into mcl-PHA. FLYCOP suggests that optimal mcl-PHA production relies on microbial relationships rather than monomer metabolism by individual cells (García and Nogales, 2018)

For plastic pyrolysis oil waste (PPOW), a SMC was used in a two-phase system to remove high concentrations of biocides. This removal process was monitored online without invasive sampling, demonstrating excellent PPOW degradation ability. Additionally, a back-propagation neural network model was applied to predict O_2 depletion and to optimize experimental conditions. This approach addresses plastic resource utilization and contributes to advancing a bio-circular economy for enzymatically unassailable plastic waste (Jia et al., 2024).

6.2 PET monomers to PHA

The regulation of carbon metabolism and oxidative stress tolerance was explored using engineered *P. putida* KT2440 derivatives with varying PHA depolymerase (PhaZ) expression levels. Notably, PhaZ expression showed significant effects on cell size, PHA accumulation, extracellular 3-hydroxy alkanoic acids production. Enhanced resistance to oxidative stress correlated with increased PHA hydrolysis (Nogales and Prieto 2022; Escapa et al., 2012). A model-driven strategy using the genome-scale metabolic model iJN1411was used to improve the utilization of lignin and plastic monomers by *P. putida* KT2440 (Nogales et al., 2020). The cell *pha* gene cluster was deleted and a new minimum set of genes required for *pha* production was overexpressed, including *phaC1* (poly(3-hydroxyalkanoate) polymerase), *phaG* (a 3-hydroxyacyl-ACP thioesterase), and *alkK* (a medium chain-fatty acid CoA ligase). The engineered strain accumulated up to 46% mcl-PHA per biomass in a balanced carbon/nitrogen medium using enzymatically hydrolyzed PET as a feedstock (Manoli et al., 2024).

Wild type *P. umsongensis* GO16 has a native ability to metabolize both PET monomers TA and EG (Kenny et al., 2008). This strain and was engineered to demonstrate PET

upcycling to PHA conversion of TA and EG to multiple biobased polyesters (Tiso et al, 2021). Strain GO16 can also accumulate both scl- and mcl-PHA (Narancic et al, 2021). It was demonstrated that scl- and mcl-PHAs are simultaneously accumulated in the strain and appear to be blended in the PHA granules (Cerrone et al, 2023). The resulting blend has improved viscoelasticity, as mcl-PHA act as a plasticizer for scl-PHA.

6.3 Product diversification of PHA into versatile biopolymers

Tailoring the polymer composition of *P. putida* strains using synthetic biology techniques using genes from scl-PHA producers *Cupriavidus necator*, *Rhodospirillum rubrum*, and *P. pseudoalcaligenes* via Golden Gate/MoClo technology was studied. This approach circumvented endogenous PHA synthesis regulation and revealed an inverse relationship between PhaC synthase and granule size distribution in the host. The inclusion body protein IbpA was also identified to be positively correlated with PhaC levels and to be crucial for PHA accumulation (Manoli et al., 2023).

To enhance the functionality of natural PHA for targeted molecule delivery, Blanco et al. utilized phasins' polymer-binding and surfactant properties. A MinP tag affixed enzybiotics, such as the antipneumococcal lysin Cpl-711, onto PHA-based materials, preserving their enzyme-based antimicrobial activity. This fusion protein (M711) was then immobilized onto PHA nanoparticles (Blanco et al., 2023). Meanwhile, research by Campano et al. demonstrated the incorporation of PHA particles into bacterial cellulose, controlled via precise colonization processes (Campano et al., 2022). This self-propelled assembly results in biodegradable films with exceptional properties. These new materials demonstrate 4.25 times higher Young’s modulus and exhibit oxygen permeability three times lower than PET films even at eight times lower film thickness. (Campano et al., 2022). Additionally, Rivero-Buceta et al. illustrated the application of similar strategies when they combined antimicrobial-functionalized PHA with bacterial cellulose to create tailored hydrogels for wound healing applications (Rivero-Buceta et al., 2020).

6.4 Plastic monomers for alternative biopolymer and surfactant production

Cyanophycin (poly-L-arginine-poly-L-aspartate) is a valuable product with applications in pharmaceuticals, bioplastics, and agriculture. Cyanophycin synthetase genes introduced into *P. putida* polymerize L-arginine and L-aspartate into cyanophycin (Wiefel et al., 2011). Recently, a codon-optimized cyanophycin synthetase gene from *Anabaena sp.* PCC 7120 was expressed in the *P. putida* KT2440 PET2bio strain, designed to consume EG and TA simultaneously (Shingwekar et al., 2023). In shake flask cultivations a cyanophycin titer of 14 mg/L was achieved (Figure 6b). A carbon-

limited fed-batch process elevated the cyanophycin titer to 1.4 g/L.

Hydroxyalkanoic acids (HAAs) are amphiphilic dimers of hydroxy fatty acids with surfactant properties (Tiso et al., 2017). They demonstrate remarkable versatility as platform chemicals, from diesel-like fuels (Meyers et al., 2019), biohybrid fuels (Hellmuth et al., 2023), and fine chemicals like 1,3-diols (Beydoun and Klankermayer 2019). HAAs are also explored as building blocks for novel polyurethanes and as internal plasticizers in materials engineering (Tiso et al., 2021). Olefins have been synthesized from HAA congeners using modified Grubbs-Hoveyda catalysts (Tiso et al., 2020). Tiso and colleagues initially produced HAAs in *P. umsongensis* GO16 pSB01, using TA and EG from PET enzymatic hydrolysis, achieving 35 mg/L with a production rate of 5 mg/L/h (Tiso et al., 2021) (Figure 6c).

Rhamnolipids are microbial-derived biosurfactants, particularly from *Pseudomonas* species, prized for their versatile applications across industries. Their biodegradability, low toxicity, and versatility position rhamnolipids as sustainable alternatives to synthetic surfactants. Recent efforts have focused on engineering recombinant microorganisms to produce rhamnolipids from renewable carbon sources. Studies by (Utomo et al., 2020) genetically modified *Pseudomonas* strains with plasmid pPS05 containing *rhlA* and *rhlB* genes (Tiso et al., 2016), to explore rhamnolipids production using different plastic monomers. In the polyurethane (PU) approach utilizing AA, BDO, EG, and TDA, a mixed culture achieved complete substrate utilization, yielding 70 mg/L of rhamnolipids. Conversely, employing a single strain in the PET-based approach with 30 mM of EG and TA resulted in 385 mg/L of rhamnolipid synthesis (Figure 6d).

6.5 Synthesis of thermoplastic PUs (TPUs) from plastic waste monomers

Recent advancements in synthetic biology enable the utilization of plastic waste as alternative carbon sources after depolymerization by enzymes (Magnin et al., 2020). In alignment with the global sustainability efforts, recent progress has focused on developing sustainable PUs (Mouren and Avérous 2023). Traditionally a product of polyols and polyisocyanates, PUs offer versatile properties due to diverse chemical structures of their building blocks. Thermoplastic PUs (TPUs), derived from bifunctional chemicals, exhibit characteristics of thermoplastic elastomers and thermosets through phase segregation of hard segments (HS) and soft segments (SS). Typically, TPUs are prepared in a two-step process: first reaction of excess diisocyanate with a long-chain linear polyol to form a prepolymer with isocyanate end-groups, followed by chain extension via a short diol or small diamine to achieve the final high molar mass polymer.

Recent studies have explored the synthesis of aromatic TPUs using chemicals derived from waste materials (Mouren and Avérous 2023; Mouren et al., 2024). For instance, sustainable aromatic diols 4-hydroxybenzoic acid and syringic acid and conventional 1,4-benzenedimethanol and 1,4-butanediol were evaluated as chain extenders. The aromatic rings enhanced phase segregation, improving thermomechanical and mechanical performance of TPUs while symmetrical structures and ether bonds promote organized HS, while methoxy groups and asymmetry hinder HS organization, impacting overall TPU properties. These insights underscore the potential to tailor sustainable TPU architectures to meet specific industrial needs across applications like construction, automotive, footwear, and medical sectors.

6.6 Innovative additives for different polymer-based systems

Novel additives for plastic formulations are crucial in enhancing properties of polymers such as mechanical strength, thermal stability, electrical properties, and resistance to environmental factors like fire and UV degradation. Functional additives include antioxidants, plasticizers, anti-UV agents, fire retardants, colorants, fillers, and reinforcements. Selecting appropriate additives depends on factors like polymer type and chemical structure, ensuring compatibility and effective dispersion within the material.

Antioxidants inhibit oxidative degradation, preserving polymer integrity and longevity, while plasticizers enhance flexibility and workability by reducing rigidity and increasing elasticity. Biobased phenolic acids like 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid are emerging as promising antioxidants (Mouren et al., 2024) and demonstrated comparable performance to petroleum-based resveratrol and Irganox 1076 in polyolefin blends (PP/PE) and TPUs with some variability (Mouren and Avérous, 2023).

Plasticizers, particularly the PHA poly(hydroxy-octanoate) (PHO) are crucial for enhancing polymers' processability, mechanical, and thermal properties. PHO is promising due to its ability to plasticize rigid polymers such as polyvinyl chloride (PVC). In a preliminary study, PHO derivatives including PHO-hexyl were synthesized via controlled transesterification. PHO-hexyl significantly reduced polymer glass transition temperatures, exhibited plasticizing effects comparable to conventional hazardous phthalates. Conversely, PHO-diol showed a more limited plasticizing effect.

7. TEA analysis enzymatic plastic degradation and plastic monomer upcycling

Techno-economic analysis (TEA) is important for assessing the economic feasibility of research in sustainable manufacturing and bioprocessing. TEA provides insights into

profitability, cost-effectiveness, and scalability, informs decisions on investments, process optimization, and resource allocation. TEA guides the development of environmentally friendly and economically sustainable technologies.

With a TEA we evaluated the economic viability of enzymatic hydrolysis of a mixed plastic/water slurry containing PP, PET, PLA, and PHB upcycled to the bioplastic PHB. (**Figure 8**) (Reifsteck et al., 2023). PET, PLA, and PHB undergo stoichiometric hydrolysis, while PP is a proxy for inert plastics. Monomers generated are used as substrates for engineered microbes, which produce PHB at theoretical yields (Tiso et al., 2022). Downstream, cell disruption with sodium dodecyl sulfate (SDS), centrifugation, and countercurrent washing produce pure PHB (Fernández-Dacosta et al., 2015).

<Figure 8>

An Aspen Plus® model was established to simulate this process for 50 kt annual production. Capital expenses (CAPEX) and operational expenses (OPEX) were calculated assuming 150 €/kg for mixed plastic waste and 300 g/L mixed plastic/water feed slurry. Using 25% PHB, PET, PLA, and PHB, the PHB cost is 4.25 €/kg, lower than commercially available PHB (8 $/kg; 7.3 €/kg) (Alvarez Chavez, Raghavan, and Tartakovsky 2022). (**Figure 9**). Notably, equipment for fermentation and hydrolysis, which are essential for converting mixed plastic waste into valuable products like PHB, constitutes 70% of the CAPEX. This significant investment in equipment underscores the capital-intensive nature of the process, highlighting the importance of optimizing these assets for cost-effective production.

<Figure 9>

The mixed plastic waste composition has a large impact on the model. A sensitivity study varying the proportion of inert polypropylene (PP) was performed while holding CAPEX constant. **Figure 10** indicates lower PP fractions varied COM based on predominant degradable plastics, with PET yielding the lowest COM and PLA the highest, aligning with assumed theoretical yields. As PP fraction increases, differences diminish (**Figure 10).** The COM falls within the 4-5 $/kg range (depicted by the blue box) for all analyzed compositions, up to a 48% PP fraction in PLA-dominated feeds and 56% in PET-dominated feeds. Beyond a PP fraction of 60-70%, COM increases exponentially, making the plant economically unfeasible. However, with an assumed high price of 150 €/kg for mixed plastic waste, these figures could increase in scenarios with lower or even negative substrate costs, potentially widening the process's viability across various mixed plastic waste compositions.

In summary, our TEA highlights the economic viability of using mixed plastics as substrate for PHB. Key factors influencing COM include the mixed plastic stream's composition, especially PP content. Optimizing process parameters is crucial for achieving cost-effective PHB production. Although COM aligns with commercially available PHB and production costs, uncertainties in CAPEX persist. Therefore, efforts to enhance microbial titer, rate, and yield (TRY) remain essential.

7. Future prospects

Research in plastic biodegradation and valorization is rapidly evolving to address plastic pollution and develop sustainable waste management solutions. The integration of enzyme identification, enzyme and metabolic engineering, plastic monomer metabolism, biopolymer valorization, product diversification, separation technologies, and life cycle assessment will shape the future of plastic biotechnology. Future research aims to engineer novel enzyme variants with improved specificity and efficiency, enhance enzyme production, and optimize microbial depolymerization strategies for industrial-scale applications. Concurrently, efforts will focus on elucidating metabolic pathways of plastic monomer metabolism to design tailored microbial strains that suits biorefinery platforms for efficient conversion of plastics into bioproducts.

The future of plastic biotechnology hinges on universal biodegradation tools like mixed enzymes and microbial consortia, enabling the breakdown of diverse plastic polymers and producing chemicals of interest from these monomer mixtures. This will be aided by using physical and chemical technologies that can open up the polymer structure or alter the chemical nature of the polymer (e.g., oxidation) for easier enzymatic degradation, much like pre-treatments used to open up the structure of wood to remove the lignin, which protects the cellulose/hemi-cellulose and give enzymes greater access to the sugar polymers. It is important to recognize that enzyme catalyzed depolymerization of plastics, without chemical interventions, will see greater success with polyesters compared to polymers with carbon-carbon bonds, supporting PHA bioplastic development. Polyolefins need chemical and/or physical interventions in order to allow enzymes to have a role in their depolymerization.

Genetic technologies will drive innovation across plastic biotechnology, from enzyme engineering to optimizing microbial strains. Synthetic biology will aid in designing genetic constructs to enhance bioprocess efficiency and scalability, fostering sustainable solutions for plastic waste valorization and the shift toward a circular economy. Collaborative interdisciplinary efforts across continents promise significant strides in addressing global plastic pollution challenges and advancing environmental sustainability.

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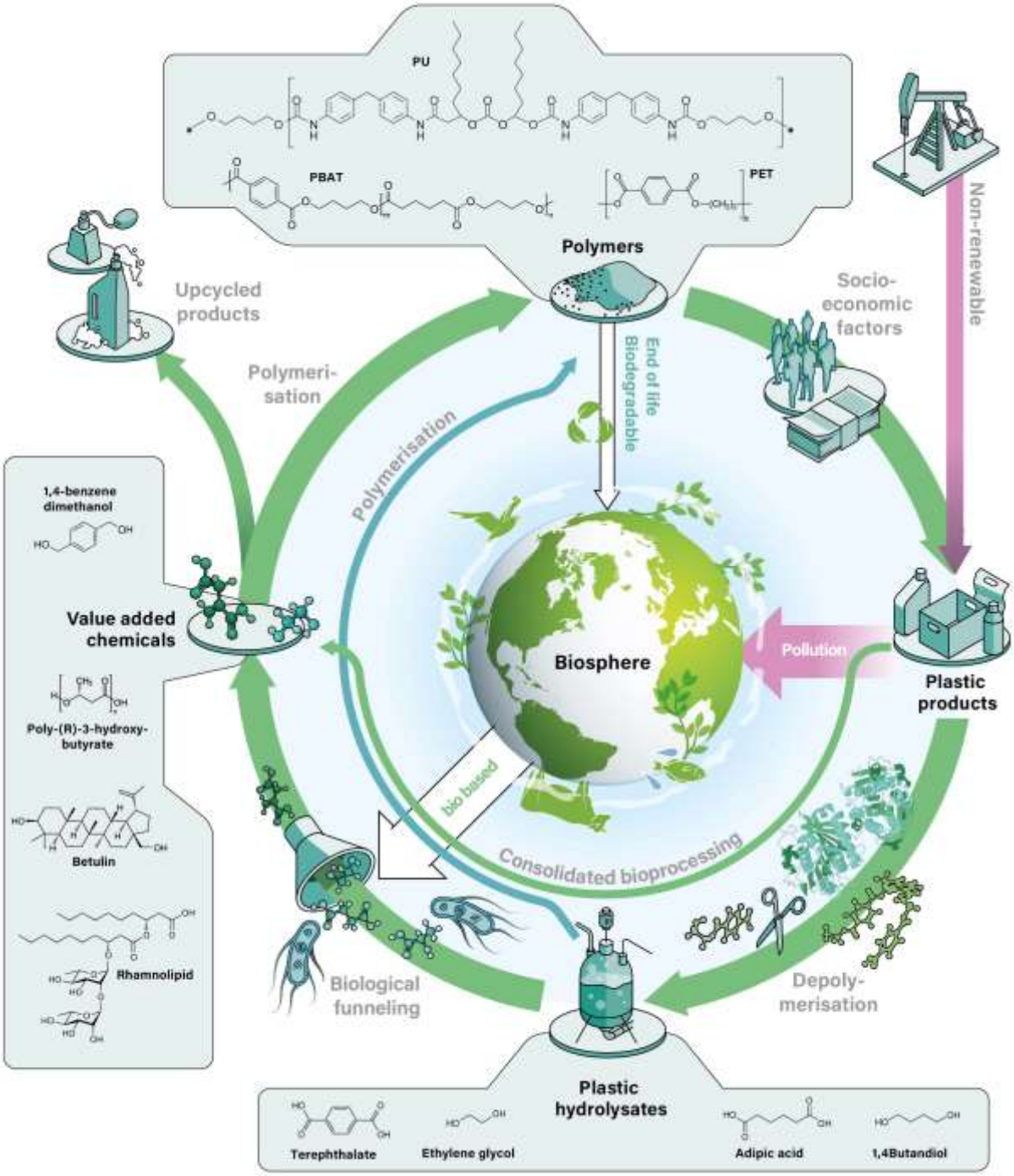


Figure 1: Transitioning plastics into the circular economy through biotechnology.

To avoid the linear conversion of fossil resources into pollution (purple arrows), biotechnology can be used to foster a more circular use of plastic products. This concept is based on the depolymerization of plastics, with enzymes or through thermo/chemical conversion. When done with mixed waste streams or complex materials, this will yield hydrolysates containing a variety of plastic building blocks. In some cases, individual monomers can be purified and re-polymerized (blue arrow), but often this purification from a mixture will not be economical. In such cases we propose to use biological funneling, establishing plastic hydrolysates as carbon source for biotechnology by engineering synthetic metabolism of plastic monomers.

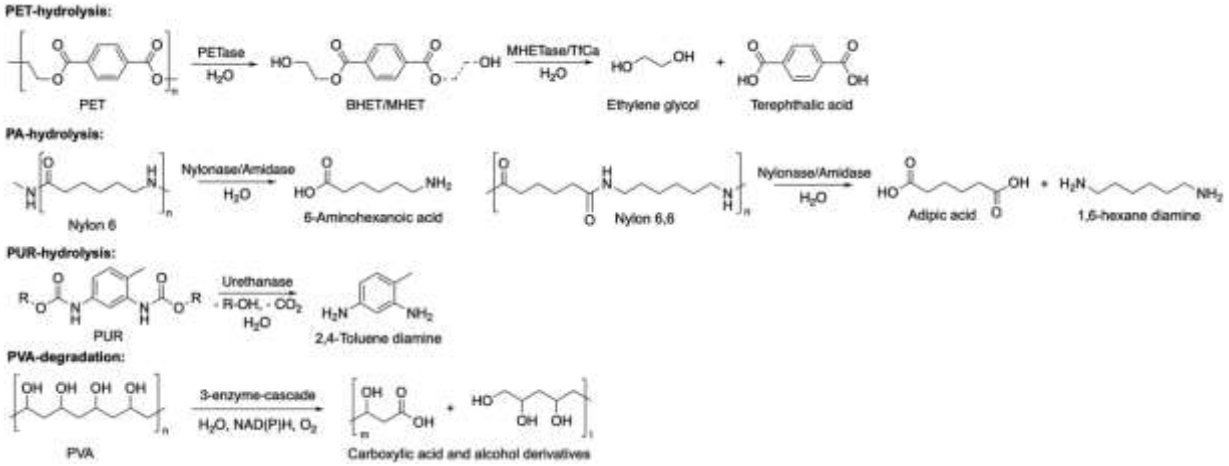


Figure 2. Ester hydrolases (IsPETase, LCC, PES-H1, etc.) catalyse the hydrolysis of PET, producing the building blocks terephthalic acid and ethylene glycol.

The degradation intermediates mono-(2-hydroxyethyl)-terephthalate (MHET) and bis(2-hydroxyethyl) terephthalate (BHET) may inhibit PET depolymerizing enzymes but might be quickly hydrolysed by a secondary enzyme. The hydrolysis of nylons is exemplified for nylon 6 and nylon 6,6 using a nylonase or an amidase. The hydrolysis of the carbamate bond in polyurethanes by a urethanase is demonstrated by a polymer containing 2,4-toluene diamine as a building block. The carbon-to-carbon backbone in PVA and derivatives can be converted by a three-enzyme cascade (alcohol dehydrogenase, Baeyer-Villiger monooxygenase, esterase/lipase) into functionalized products like carboxylic acids and alcohols (doi: 10.1002/anie.202216962).

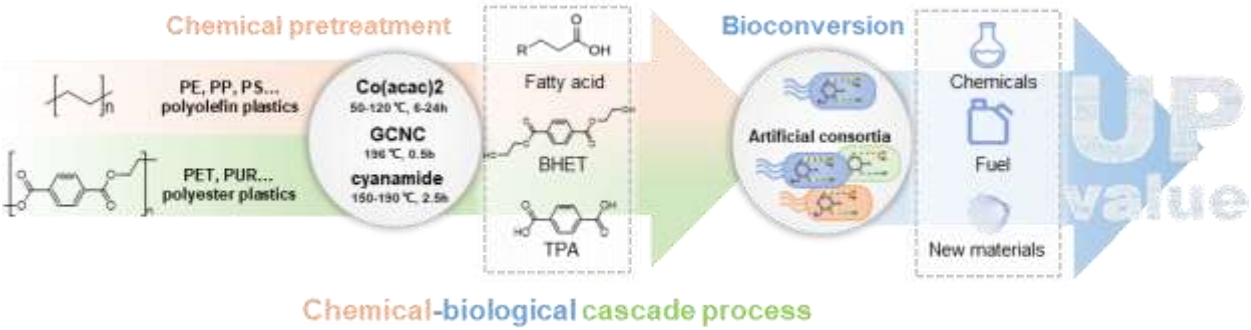


Figure 3 Concept for chemical-biological cascade degradation of mixed plastics

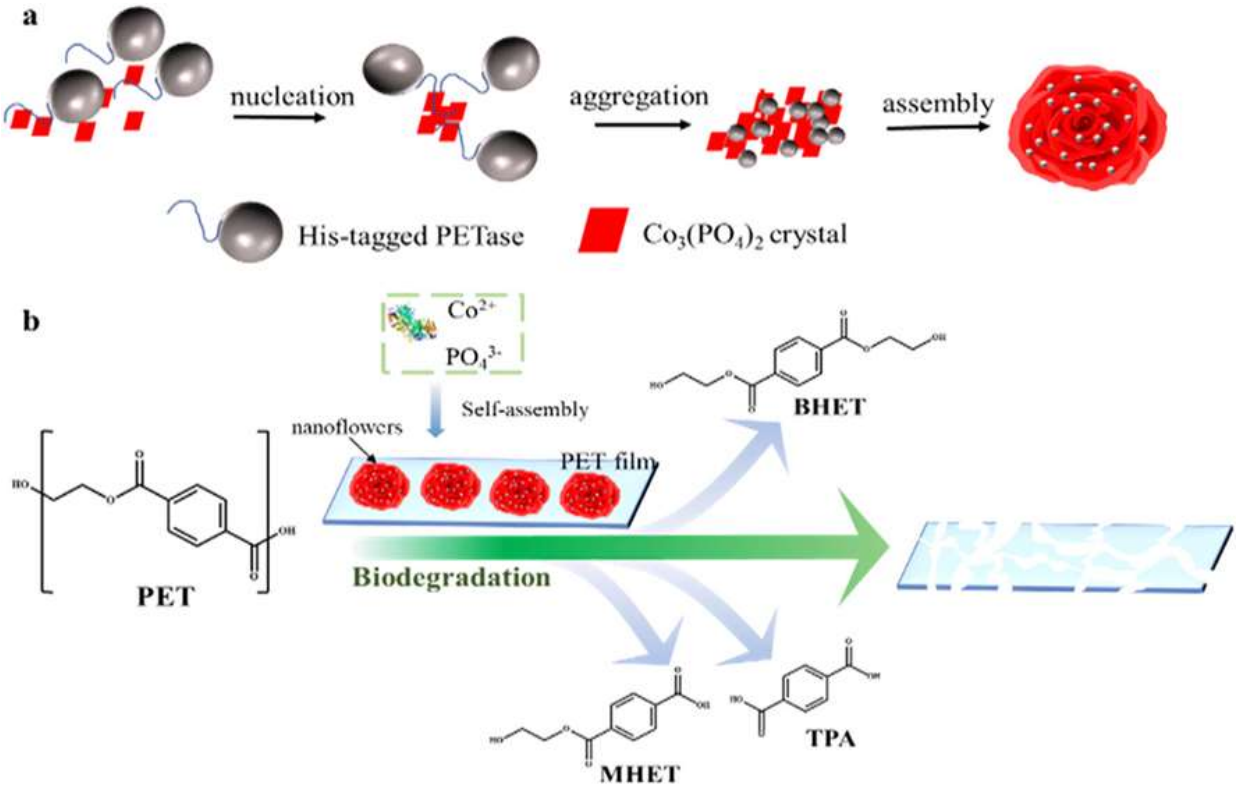


Figure 4: Schematic illustration of immobilization of (a) PETase enzyme by biomimetic mineralization and (b) PET film degradation using the immobilized PETase

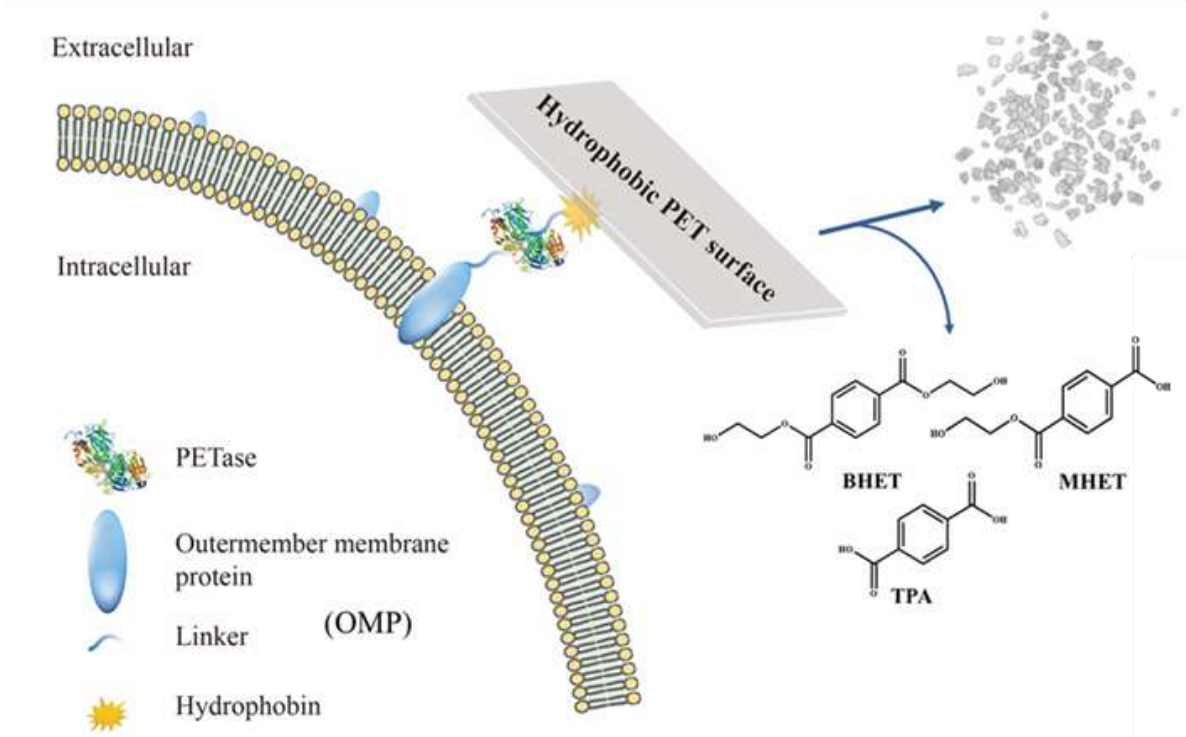


Figure 5. The cell surface display system for a PETase is immobilized on the outer membrane of *E. coli* for degradation of PET

The system introduced hydrophobic proteins that reduced the contact angle with PET surfaces, enhancing PETase affinity for PET. The system retained 73% activity after 7 days at 40°C and maintained 70% activity after 7 catalytic cycles, demonstrating process robustness (Jia et al., 2022).

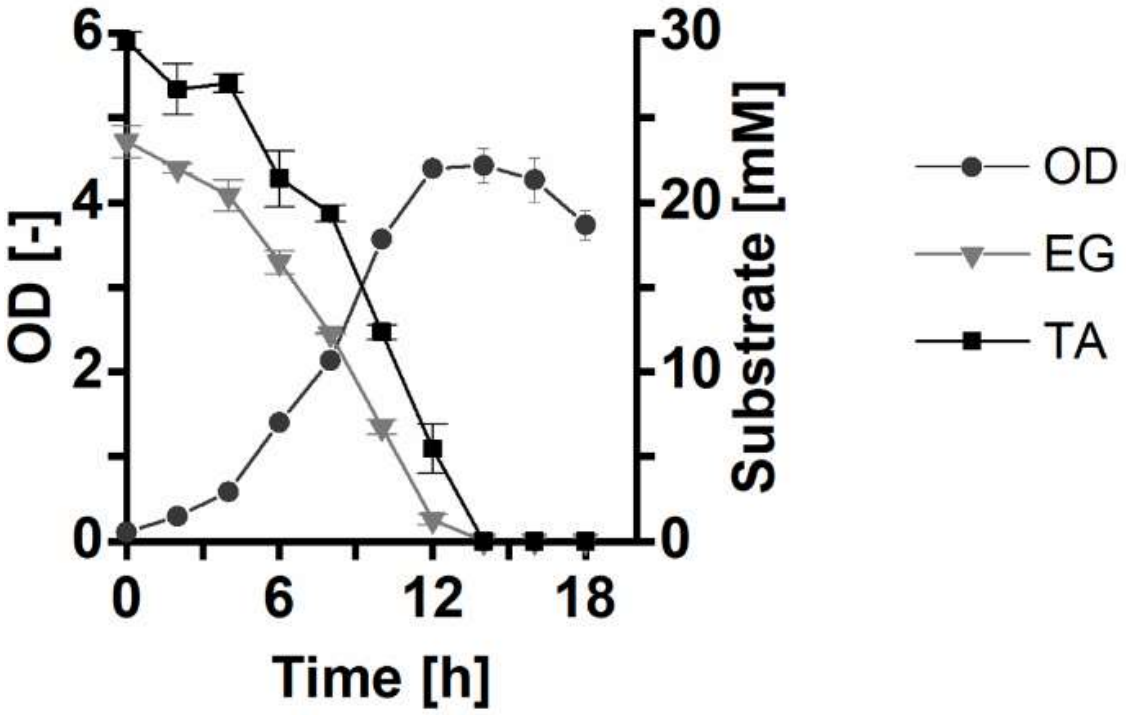


Figure 6: Simultaneous metabolization of EG and TA by *P. putida* PET2bio.
Shake flask in mineral salt media (MSM) supplemented with an average of 24 mM EG and 29 mM TA. Cultivations were conducted at 300 rpm with a shaking diameter of 50 mm and 10 % filling volume. MSM cultures were inoculated to an OD of 0.1. The error bars show the deviation from the mean (n=3). OD: optical density at 600 nm; EG: ethylene glycol; TA: terephthalic acid.

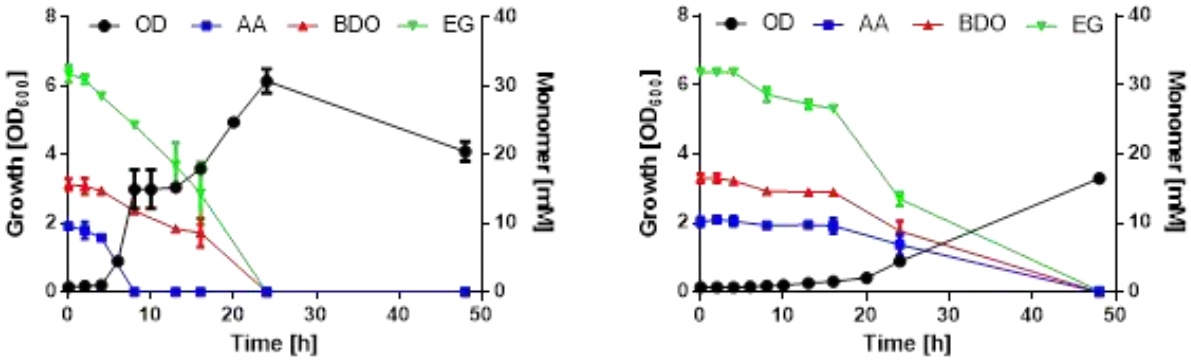


Figure 7: Growth and substrate concentration of a defined mixed culture of four *Pseudomonas* strains in MSM with PU monomer mixtures consisting of A: 10 mM adipic acid (AA), 15 mM 1,4-butanediol (BDO), and 30 mM ethylene glycol (EG) and B: the same mixture with additionally 8.5 mM 2,4-toluenediamine. Error bars depict the deviation of the mean (n = 2).

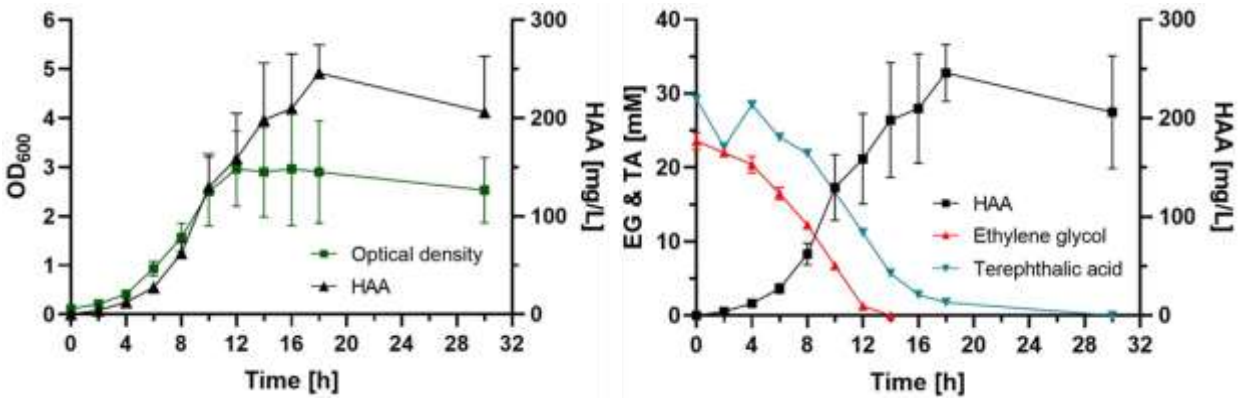


Figure 8: HAA production from the PET monomers EG and TA in shake flasks.

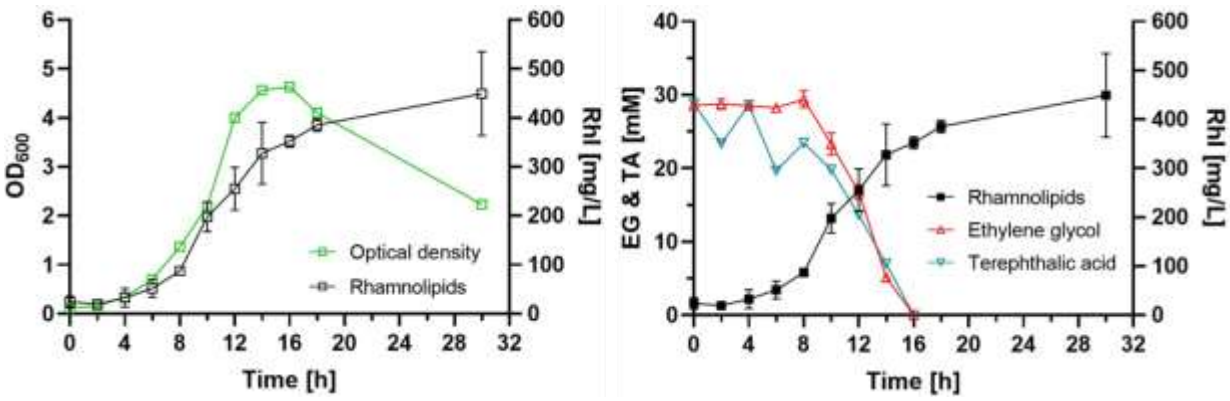


Figure 9: RL production from the PET monomers EG and TA in shake flasks.

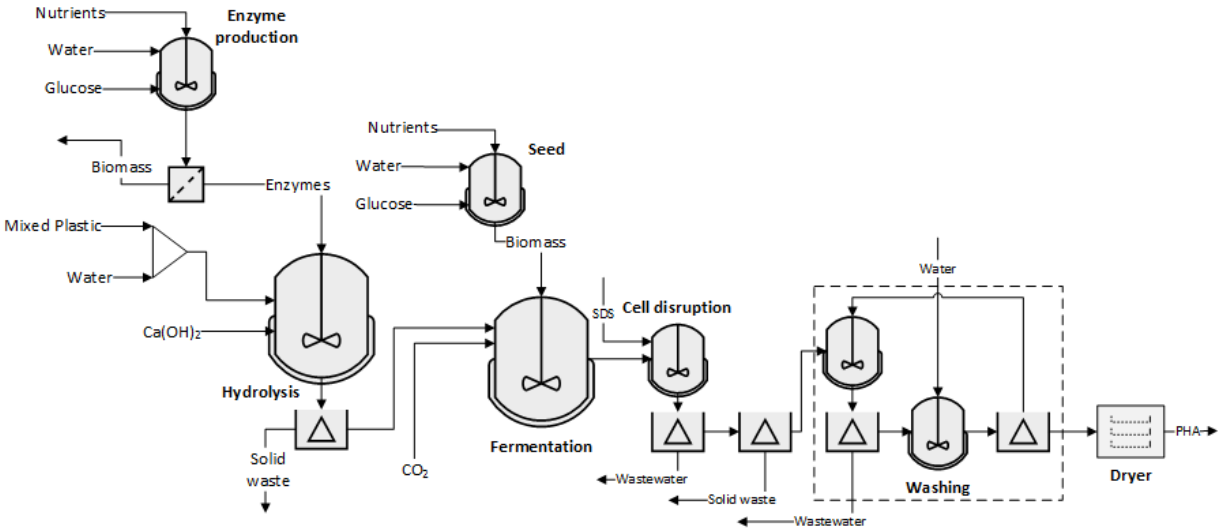


Figure 10. Considered process for the enzymatic valorization of mixed plastic waste, comprising four distinct plastic types (PET, PHB, PLA, PP), and subsequential upcycling to PHB by a microbial mixture. While PP is considered inert, the other plastics are metabolized by the microbial mixture in accordance with their respective theoretical yields to PHB.

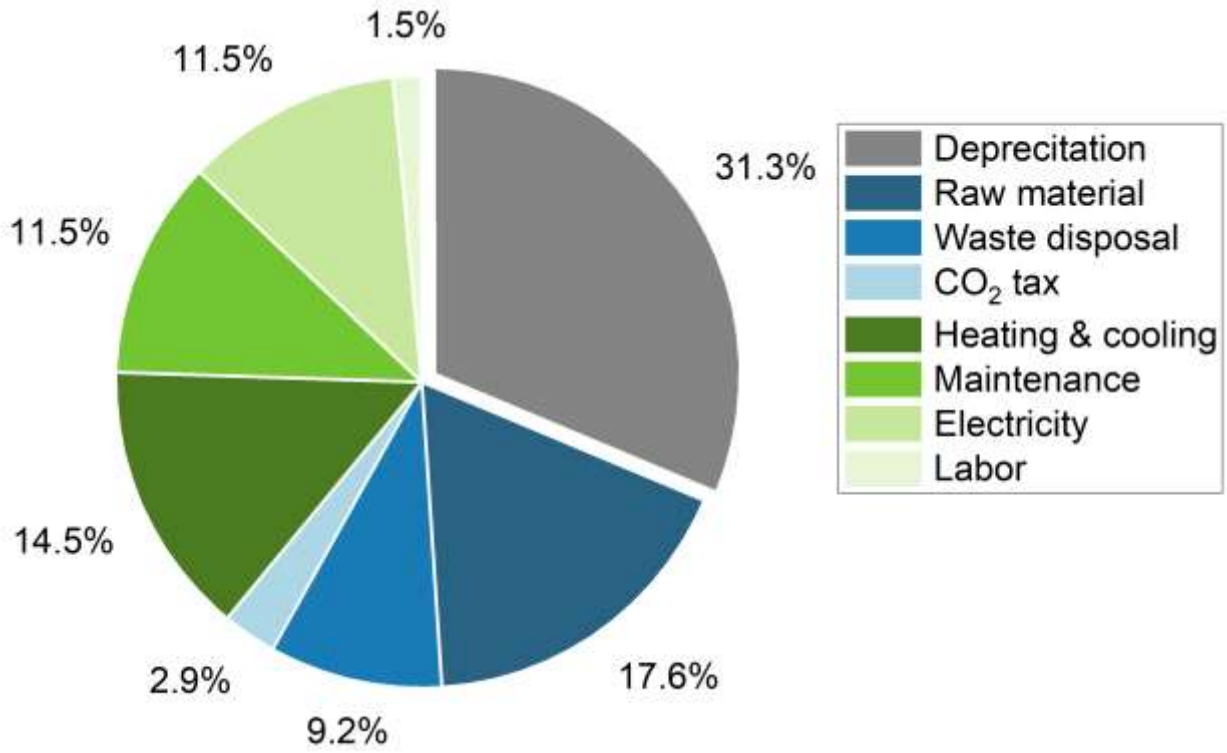
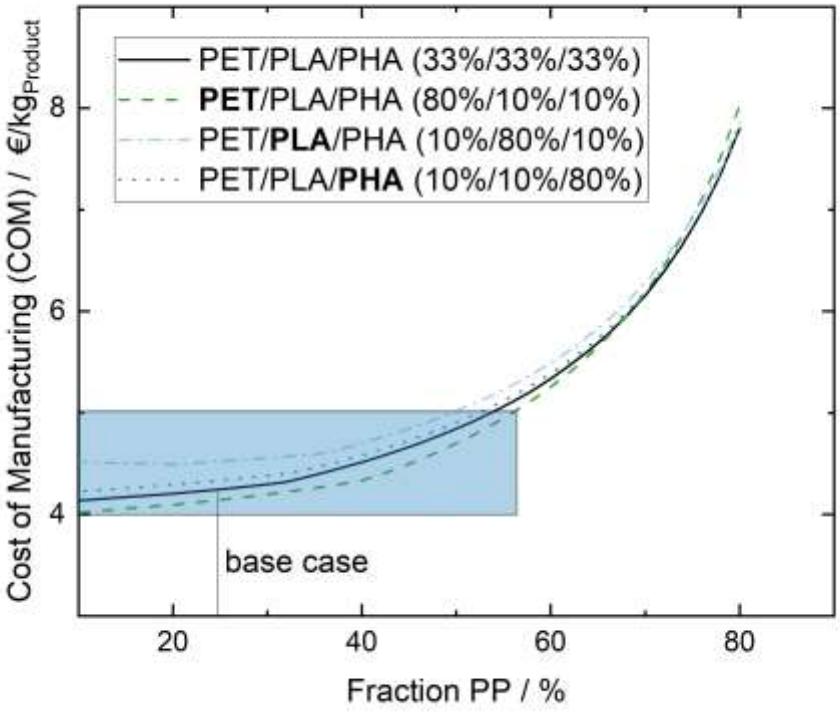


Figure 11 Cost distribution for the considered process. CAPEX in grey and OPEX in blue and green



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1493 Figure 12 The cost of manufacturing PHB using the process illustrated in Figure 11, with varying
1494 proportions of PP as non-degradable plastic. Calculated for four different composition of degradable
1495 plastic shares.

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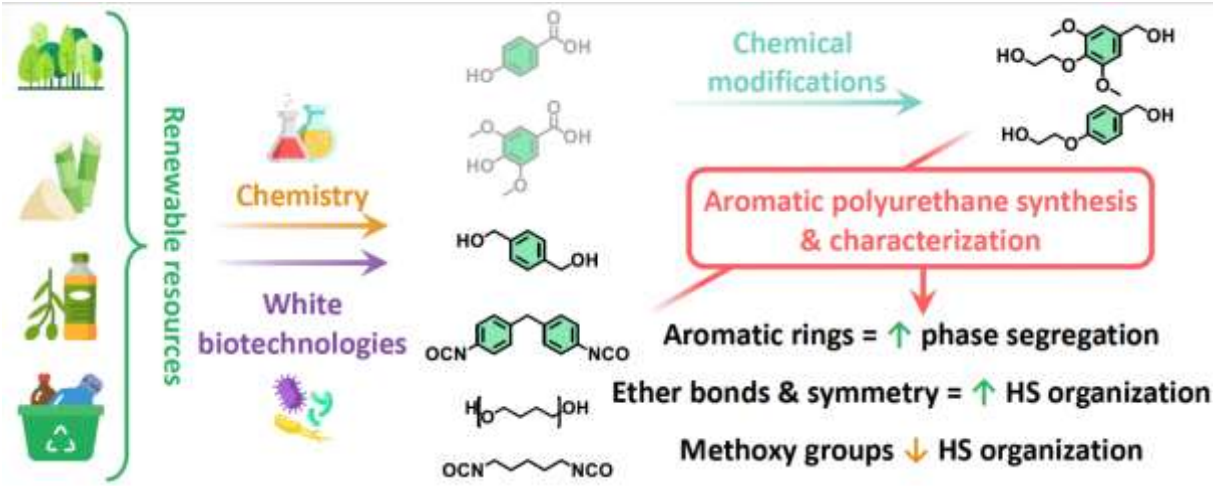

Figure 13. Aromatic polyurethane synthesis and characterization from renewable resources

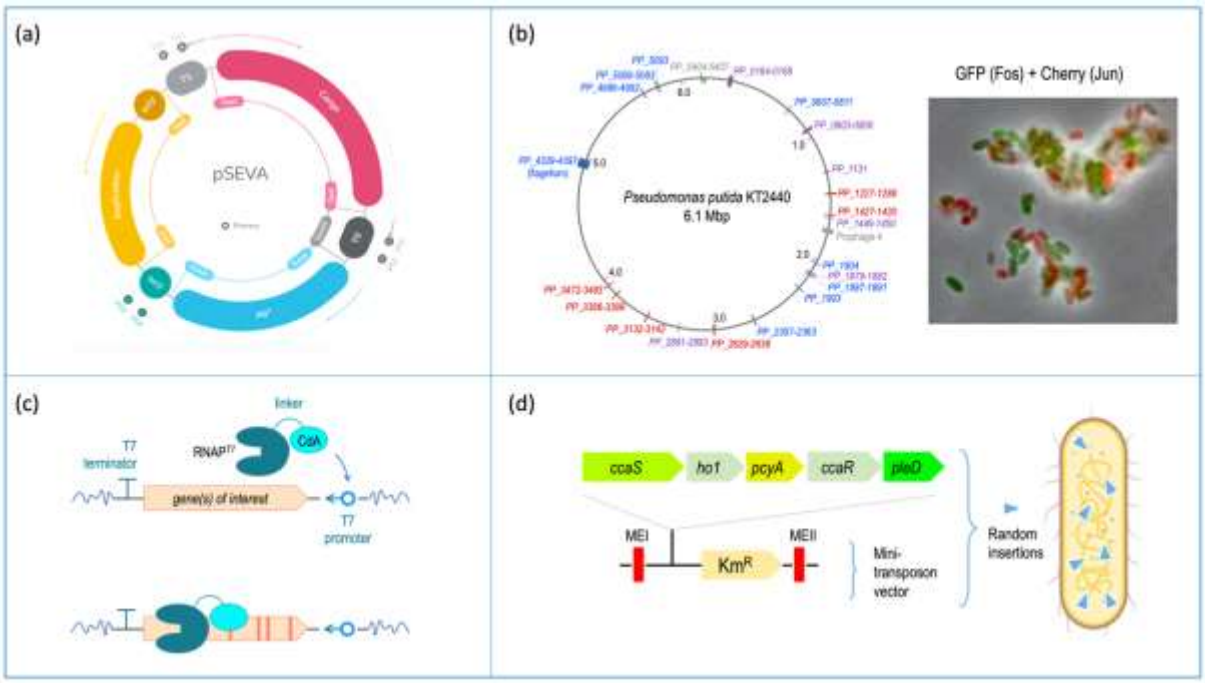

Figure 14. Optimization of optogenetic control of expression of a cdGMP cyclase which in turn determined biofilm formation on the material of interest.

1507 **Table 1** Crystal structures of selected plastic degrading enzymes deposited in the Protein Data Bank.

Enzyme	**Substrate/Ligand**	**PDB code**	**Reference**
*Is*PETase R103G/S131A	*p*-nitrophenol (*p*NP)	5XH2	(Han et al. 2017)
	1-(2-hydroxyethyl) 4-methyl terephthalate (HEMT)	5XH3	(Han et al. 2017)
MHETase	4-[(2-hydroxyethyl) carbamoyl] benzoic acid (MHETA)	6QGA	(Palm et al. 2019)
TfCa	MHETA	7W1J	(von Haugwitz et al. 2022)
TfCa E319L	bis(2-hydroxyethyl) terephthalate (BHET)	7W1L	(von Haugwitz et al. 2022)
PES-H1	MHETA	7W6C, 7W6O, 7W6Q	(Pfaff et al. 2022)
PES-H2	BHET	7W66	(Pfaff et al. 2022)
LCC ICCG	mono-(2-hydroxyethyl)-terephthalate (MHET)	7VVE	(Zheng et al. 2024)
Ple629	MHETA	7VPB	(Z. Li et al. 2022)
TfCut	MHET	7XTV	(Yang et al. 2023)
TfCut	MHET	7XTT	(Yang et al. 2023)

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1510 **Table 2. Material binding peptides for accelerating polymer degradation**

Polymer	Material binding peptide	Polymer degradation improvement	Ref.
Polyethylene terephthalate(PET)	Dermaseptin SI (DSI)	22.7-fold of product (MHET and TPA) release	(Z. Liu, Zhang, and Wu 2022)
Polyurethane (Impranil® DLN-SD)	Tachystatin A2(TA2)	6.6-fold of weight loss	
Polylactic acid (PLA)	Cg-Def	2.0-fold of lactic acid release	
Poly(ethyl acrylate) (PEA)	OMP25	2.13-fold of product release	(Z. Liu et al. 2022)
Poly(vinyl acetate) (PVAc)	OMP25	4.86-fold of product release	(Z. Liu et al. 2022)

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