Sustainable Environmental Insight

Review
Volume 3(1), 2026, 17–43
https://doi.org/10.53623/sein.v3i1.1003

Applications of Synthetic Biology in Microbial and Enzymatic Systems for Microplastic Degradation: A Review

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SUBMITTED: 21 January 2026; REVISED: 6 February 2026; ACCEPTED: 8 February 2026

ABSTRACT: Microplastic pollution poses a persistent environmental challenge due to the chemical recalcitrance, low bioavailability, and environmental stability of synthetic polymers. Synthetic biology has emerged as a powerful, integrative framework for enhancing biological degradation of microplastics by systematically engineering enzymes, microbial chassis, and metabolic pathways. This narrative review examines recent advances in enzyme engineering, whole-cell engineering, and metabolic engineering that collectively enhance the efficiency, robustness, and scalability of microbial and enzymatic systems for plastic degradation. At the enzyme level, rational design, directed evolution, and computationally guided approaches have driven substantial improvements in the catalytic performance of plastic-degrading enzymes, particularly polyester hydrolases such as PETase, MHETase, cutinases, and LCC variants. Structure-guided mutagenesis and machine-learning–assisted workflows have yielded next-generation enzymes with enhanced activity, thermostability, and substrate affinity, enabling the depolymerization of semicrystalline and post-consumer plastics under increasingly mild, industrially relevant conditions. Domain fusion strategies further address mass-transfer limitations by improving enzyme–polymer interactions, especially for highly crystalline substrates. Beyond isolated enzymes, whole-cell engineering integrates enzyme production, localization, and activity within living systems. Surface display platforms, biofilm-based immobilization, secretion systems, and multi-enzyme cascades facilitate sustained enzyme–substrate contact, reduce diffusional losses, and enable sequential depolymerization. Engineered microbial chassis have demonstrated effective microplastic degradation in controlled environments, although catalytic efficiency, intermediate toxicity, and biosafety concerns currently limit deployment in open environments. Metabolic engineering complements depolymerization by enabling microbial assimilation and conversion of plastic-derived monomers into central metabolites or value-added products, supporting closed-loop recycling and upcycling concepts. However, pathway complexity, flux imbalance, and substrate toxicity remain significant constraints. Overall, the review highlights that the most effective synthetic biology strategies for microplastic degradation arise from integrating enzyme engineering with whole-cell and systems-level optimization. While technical and economic challenges persist, continued advances in computational design, process integration,

and systems synthetic biology hold strong promise for developing scalable, environmentally safe solutions aligned with circular economy principles.

KEYWORDS: Directed evolution; enzyme engineering; metabolic engineering; microbial chassis; rational design; whole-cell engineering

1. Introduction

The widespread presence of microplastics, plastic particles smaller than 5 mm, has become one of the most pressing global environmental challenges of the 21st century. Derived from the fragmentation of larger plastic debris or direct industrial sources such as microbeads and fibers, microplastics are now ubiquitous in aquatic, terrestrial, and atmospheric compartments [1−3]. Their persistence, ability to adsorb co-contaminants, and potential to enter food webs raise growing concerns about ecological and human health risks [4, 5]. Recent studies have shown that microplastics can induce oxidative stress, inflammatory responses, and genotoxic effects in exposed organisms [6]. Consequently, there is increasing emphasis on integrating evidence from acute, chronic, and sub-lethal bioassays to capture both immediate toxicity and longer-term, subtle biological effects relevant to population- and ecosystem-level risk assessment. The pervasive detection of microplastics even in remote and pristine environments further underscores their transport resilience and environmental persistence [7]. Despite rising global awareness, efficient and sustainable methods for mitigating microplastics in natural systems remain limited.

Conventional plastic removal and degradation methods, such as mechanical filtration, thermal decomposition, and chemical oxidation, are often energy-intensive, non-selective, and prone to generating secondary pollutants [8]. Biodegradation, mediated by microorganisms and their enzymes, offers a promising, environmentally benign alternative. However, natural microbial and enzymatic systems typically exhibit low catalytic efficiency, narrow substrate specificity, and poor stability under fluctuating environmental conditions [9]. For instance, the hydrolytic enzymes identified from *Ideonella sakaiensis* and other microbes can partially degrade polyethylene terephthalate (PET) and related polymers, but operate optimally only under controlled laboratory conditions, limiting their practical deployment in complex natural matrices [10].

In recent years, the emergence of synthetic biology has opened transformative opportunities to overcome these limitations. Synthetic biology combines the principles of systems biology, metabolic engineering, and molecular design to reprogram living cells and enzymes for desired functions [11]. By integrating advanced tools such as CRISPR/Cas genome editing, directed enzyme evolution, and computational pathway optimization, scientists can now construct customized microbial and enzymatic systems capable of accelerated and targeted pollutant degradation [12]. In the context of microplastics, this approach enables the rational design of microbial chassis and enzymatic networks that not only degrade polymers more efficiently but can also function in environmentally relevant conditions [13].

Synthetic bioremediation represents a paradigm shift from relying on naturally evolved pathways to designing and optimizing biological systems for specific pollutants. Engineered microorganisms can be equipped with enhanced polymer-binding proteins, secretory enzymes, and metabolic circuits that convert plastic-derived intermediates into benign or value-added

compounds [14]. Similarly, synthetic enzymes such as redesigned PETases and cutinases have demonstrated improved thermostability and catalytic turnover, offering feasible routes for scalable microplastic degradation [15]. Moreover, the development of synthetic microbial consortia, i.e., cooperative communities with distributed metabolic functions, mirrors the complexity of natural ecosystems while enabling controlled degradation of mixed or composite plastics [16].

Despite advances in synthetic bioremediation, few reviews have comprehensively presented them. Thakur et al. [13] review a wide range of biotechnological techniques that enhance the capacity of microorganisms to degrade microplastics. The review did not focus on synthetic biology and its application in producing more effective enzymes for this purpose. Similarly, Anand et al. [17] present general biotechnological methods for removing microplastics, with little attention to synthetic biology. Gaur et al. [18] focus primarily on the sources and toxicity of microplastics, with a brief section on the use of genetic and metabolic engineering to address microplastic pollution. Kim et al. [19] review past studies on polystyrene biodegradation, covering analytical techniques, isolation of polystyrene-degrading microorganisms, and discovery of key biodegradative enzymes. Their review includes only a brief discussion of the application of systems biology to identify polystyrene-degrading enzymes. The review by Martin-Gonzalez [20] compiles the key advancements in microbial technologies developed for plastic degradation and recycling generally.

This narrative review aims to provide a comprehensive synthesis of the current progress, feasibility, limitations, and future directions in the use of synthetic biology for microplastic degradation. It provides an overview of whole-cell, metabolic, and enzyme or protein engineering strategies developed to enhance degradation efficiency. Furthermore, it qualitatively discusses the feasibility and limitations of the strategies. It contributes to highlighting the knowledge gaps and emerging opportunities, thus guiding future research toward the design of more efficient, sustainable, and scalable synthetic bioremediation solutions to address microplastic pollution.

2. Review Methodology

A comprehensive literature search was performed across major scientific databases, including Scopus, Web of Science, PubMed, and Google Scholar, covering publications from January 2018 up to December 2025 to present the most recent advances in this area. The following keywords and their combinations were used: *"synthetic biology," "microplastic degradation," "engineered microbes," "biodegradation," "engineered plastic-degrading enzymes," "genetic engineering,"* and *"bioremediation."* Reference lists of key articles were also screened to identify additional relevant studies.

A narrative review approach was adopted to synthesize and interpret findings from the selected studies, allowing for a qualitative integration of diverse experimental strategies, enzyme systems, microbial chassis, and synthetic biology tools. This approach emphasizes thematic discussion and critical evaluation over quantitative meta-analysis, facilitating a comprehensive understanding of trends, technological advances, and current limitations in the field.

Only peer-reviewed articles, reviews, and relevant book chapters written in English were included. Studies were selected if they addressed (i) synthetic genetic tools or pathways for enhancing plastic degradation, (ii) enzymes or microbial consortia engineered with synthetic

biology demonstrating improved degradation capacity, or (iii) feasibility and limitations in applying synthetic biology for environmental remediation. Papers focused solely on natural biodegradation, without synthetic or engineered components, were excluded. Additionally, papers on physical and chemical modifications and immobilization of enzymes were excluded. The article screening and selection process is shown in Figure 1.

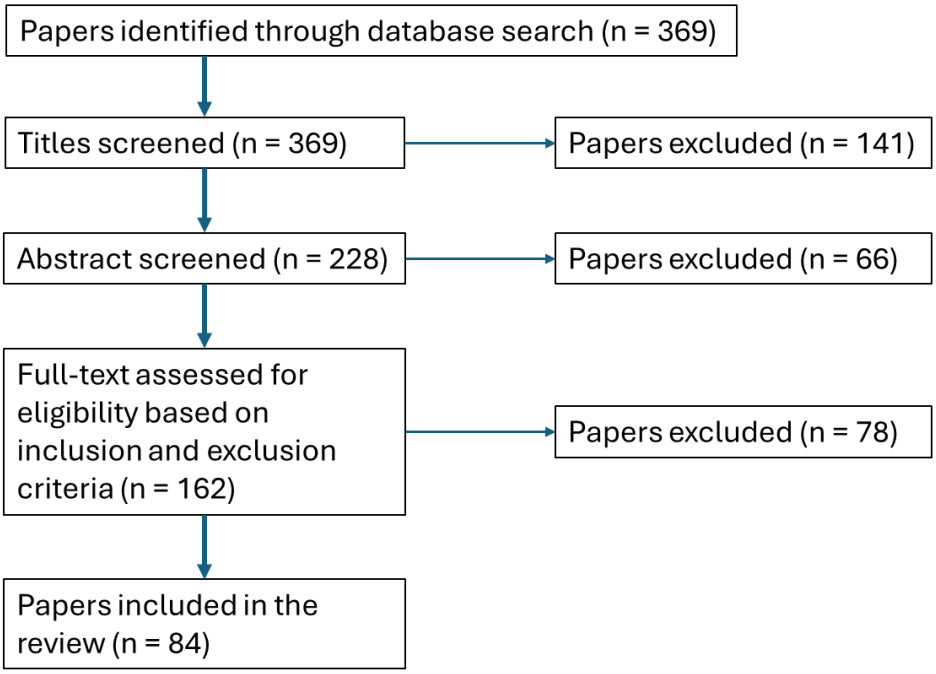

Figure 1. Flowchart showing the screening and selection of literature.

Selected studies were analyzed to extract data on microbial chassis or enzymes, synthetic biology strategies, substrates, degradation performance, and key advantages. The information was categorized into two main themes: whole-cell engineering (including metabolic engineering as a subset) and enzyme engineering. Given the heterogeneity of experimental designs in synthetic biology–based microplastic degradation studies, including differences in polymer type and formulation, exposure duration, reaction conditions, and performance metrics, results were synthesized using a qualitative, narrative weight-of-evidence approach. Rather than directly comparing absolute degradation efficiencies, findings were interpreted in relation to the specific synthetic biology strategies employed, such as enzyme engineering, surface display, biofilm integration, and multi-enzyme coordination, as well as the characteristics of the tested substrates (e.g., crystallinity, particle size, aging state, and presence of additives). Conflicting outcomes across studies were evaluated by identifying recurring mechanistic patterns and design principles that consistently enhanced degradation performance, including improved enzyme–substrate proximity, enhanced thermostability, and mitigation of product inhibition.

3. Synthetic Biology Approaches for Enhanced Microplastic Degradation

Synthetic biology has emerged as a transformative discipline for addressing persistent environmental contaminants such as microplastics. It provides a powerful toolkit for improving the efficiency, specificity, and robustness of microbial microplastic degradation. Given the intrinsic recalcitrance of most synthetic polymers, natural enzymes often exhibit low catalytic

efficiency, limited substrate accessibility, or poor environmental stability. To overcome these constraints, several complementary synthetic biology strategies have been employed.

Rational design leverages structural, biochemical, and computational insights to improve enzyme–polymer interactions. By analyzing crystal structures and molecular docking models, targeted amino acid substitutions can be introduced to enhance substrate binding, catalytic turnover, or thermal stability of plastic-degrading enzymes such as PETase, mono(2-hydroxyethyl) terephthalate hydrolase (MHETase), and polyurethane esterases (Figure 2) [21]. Rational design has been particularly effective in enlarging active-site clefts, increasing surface hydrophobicity, and optimizing charge distributions to better accommodate polymer chains [22].

Directed evolution offers a complementary, data-driven approach that does not require detailed mechanistic understanding. Iterative cycles of random mutagenesis, high-throughput screening, and selection have been used to evolve enzymes with higher activity, improved thermostability, and broader substrate ranges (Figure 2) [23]. This strategy is especially valuable for optimizing plastic-degrading enzymes under industrially or environmentally relevant conditions, such as elevated temperatures, variable pH, or high salinity [24].

Semi-rational and machine learning–assisted approaches integrate rational design with directed evolution by targeting mutagenesis to structurally or functionally important regions (Figure 2). Recent advances in protein language models and machine learning algorithms enable the prediction of beneficial mutations and epistatic interactions, substantially reducing experimental search space while accelerating enzyme optimization [25].

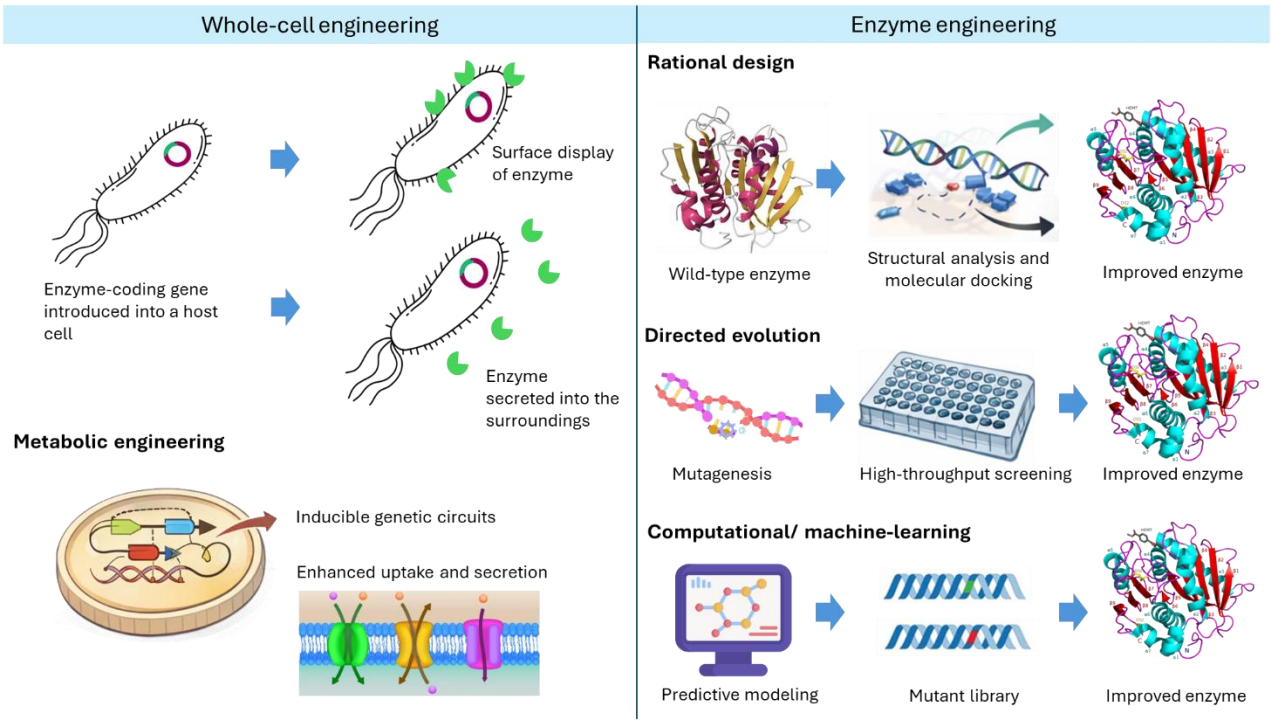

Figure 2. Synthetic biology approaches commonly employed in plastic bioremediation. (Images in the figure were derived from open sources, including Wikimedia Commons, Freepik, Pngtree, and Shutterstock)

Beyond enzyme engineering, pathway and host engineering strategies aim to enhance whole-cell degradation performance. These include assembling multi-enzyme degradation pathways, optimizing gene expression and secretion systems, and engineering membrane transporters to facilitate uptake of plastic-derived oligomers and monomers (Figure 2) [26].

Surface display systems and synthetic scaffolds have also been employed to spatially organize enzymes, improving substrate channeling and reducing diffusional losses [27].

Finally, regulatory circuit or metabolic engineering enables dynamic control of enzyme expression in response to plastic-derived inducers or environmental cues, improving metabolic efficiency and minimizing cellular burden (Figure 2) [27]. Collectively, these synthetic biology approaches offer a modular and scalable framework for developing next-generation biological systems capable of addressing microplastic pollution more effectively. The following recent advances of synthetic biology applications in addressing microplastic pollution integrate these approaches.

4. Whole-cell Engineering

Recent advances in whole-cell engineering have significantly expanded the biological toolkit for microplastic degradation by integrating enzyme production, localization, and catalytic activity within living microbial systems. These approaches move beyond purified enzymes and instead exploit engineered microbial chassis to enhance enzyme–substrate interactions, stabilize catalytic activity, and enable multi-step depolymerization processes in complex environments [28].

One major development is the use of biofilm-forming bacteria as whole-cell degradation platforms. Huang et al. engineered the robust biofilm-forming bacterium *Stenotrophomonas pavanii* JWG-G1 to overexpress DuraPETase, achieving sustained degradation of high-crystallinity PET microplastics at 30 °C with a total product release of 38.04 μM after 30 days. Genome sequencing of *S. pavanii* revealed nine endogenous PET hydrolases, which were heterologously expressed in *Escherichia coli*, leading to the identification of Est_B as a novel Bis(2-hydroxyethyl) terephthalate hydrolase (BHETase) capable of complete BHET degradation within 4 h at 30 °C. Although endogenous hydrolases exhibited lower PET activity than DuraPETase, biofilm-mediated aggregation of the overexpressed enzyme on PET surfaces contributed to enhanced degradation performance. The engineered strain also maintained activity across multiple aquatic environments and exhibited the ability to degrade other polyester plastics [29].

Cell surface display strategies represent another major advance, enabling direct enzyme–plastic contact and minimizing enzyme diffusion losses. Li et al. engineered the fast-growing halophile *Vibrio natriegens* to surface-display PET-degrading enzymes from *Ideonella sakaiensis*. By anchoring IsPETase and PETase–MHETase chimeras to the outer membrane using Lpp'OmpA homologs, the engineered cells achieved rapid BHET hydrolysis (>95% conversion within 3 h) and effective PET particle depolymerization under seawater-like conditions. Surface display was essential for PET degradation, as intracellularly expressed enzymes and anchor-only controls showed negligible activity. Enzyme choice, chimera configuration, and anchoring proteins were shown to influence depolymerization efficiency and monomer release [30].

Curli-based surface engineering has further enabled biofilm-integrated enzyme immobilization. Wang et al. developed a bacterial enzyme cascade reaction system in *E. coli*, displaying PETases and carbohydrate-binding module CBM3 on CsgA curli fibers to enhance PET adsorption and degradation. This system achieved a PET film degradation rate of 3437 ± 148 μg $(d\ cm^2)^{-1}$ and converted crystalline PET microplastics entirely into terephthalic acid (TPA). Molecular dynamics simulations confirmed that CsgA fusion did not interfere with

enzymatic activity [31]. Similarly, Zhu et al. introduced the biofilm-integrated nanofiber display (BIND) platform, in which PETase was genetically fused to CsgA, enabling autonomous immobilization on curli fibers. BIND-PETase demonstrated sustained degradation of PET films, microplastics, wastewater-borne PET, and postconsumer PET waste under a wide range of environmental conditions [32].

Whole-cell engineering has also been extended beyond PET to more recalcitrant polymers such as polyethylene (PE). Xiong et al. [33] applied atmospheric and room-temperature plasma (ARTP) mutagenesis to enhance PE microplastic degradation by bacterium XZ-A. The mutagenized strain XZ-60S induced substantial changes in PE morphology and molecular weight after 50 days, with transcriptomic analyses revealing upregulated laccase-related genes. ARTP mutagenesis has been highlighted as an effective non-targeted genetic modification approach that induces diverse mutations without introducing foreign DNA [34-36].

Yeast has emerged as a complementary chassis for whole-cell catalysis due to its robustness and versatility in surface display. Loll-Krippleber et al. [37] engineered *Saccharomyces cerevisiae* to display MHETase on the cell surface using multiple anchoring proteins, enabling efficient conversion of MHET into TPA and ethylene glycol. Subsequent studies expanded yeast-based systems to multi-enzyme surface display, including co-display of PETase and MHETase on *S. cerevisiae* [38] and the assembly of scaffoldin-based multi-enzyme clusters using cohesin–dockerin interactions [39]. These architectures enabled coordinated depolymerization of PET without intermediate accumulation and substantially increased TPA yields through spatial organization of enzymatic cascades.

Whole-cell strategies based on enzyme secretion and downstream assimilation further integrate depolymerization with metabolism. Cao et al. engineered *Comamonas testosteroni* CNB-1, a dominant activated sludge bacterium, to secrete DuraPETase extracellularly, enabling ambient-temperature degradation of PET microplastics. The engineered strain was also capable of utilizing PET degradation intermediates such as TPA, ethylene glycol, and BHET as sole carbon sources, supporting complete transformation within a single microbial system [40].

Finally, whole-cell surface display has been applied to oxidative enzymes involved in non-hydrolytic plastic degradation. Zhang et al. [41] developed an InaKN (truncated ice nucleation protein anchoring motif)-mediated surface display platform in *E. coli* for a cold-active laccase (PsLAC), achieving efficient PE degradation at low temperatures and sustained catalytic activity across multiple reaction cycles.

Collectively, these studies illustrate that recent whole-cell engineering efforts for microplastic degradation emphasize enzyme localization (biofilm integration, surface display, secretion), multi-enzyme coordination, chassis selection, and environmental adaptability. The convergence of synthetic biology, protein engineering, and microbial ecology continues to drive the development of increasingly sophisticated whole-cell systems for microplastic transformation. A summary of whole-cell engineering strategies is presented in Table 1.

Table 1. Representative whole-cell engineering strategies for microplastic degradation.

Reference	Host organism/ chassis	Whole-cell engineering strategy	Target plastic /substrate	Key performance metrics	Key advantages
[29]	*Stenotrophomonas pavanii* JWG-G1	Overexpression of DuraPETase; exploitation of strong biofilm formation	PET microplastics (high crystallinity)	38.04 μM total products after 30 d at 30 °C	Biofilm-enhanced enzyme aggregation on PET surface; effective in diverse aquatic environments; suitable for bioreactor deployment
[30]	*Vibrio natriegens*	Surface display of IsPETase, PETase–MHETase chimeras, and FAST-PETase via Lpp'OmpA anchors	PET particles; BHET	>95% BHET conversion in 3 h; up to 4.0 μM TPA in 7 d	High activity in seawater-like conditions; rapid growth, halotolerance; surface display essential for solid PET depolymerization
[31]	*Escherichia coli* (ΔcsgA)	Curli fiber (CsgA)-based surface display with PETase and CBM3	PET films and crystalline PET microplastics	3437 ± 148 μg $(d \cdot cm^2)^{-1}$ degradation; 21.4% PET microplastics degraded	Enhanced PET adsorption; high stability and reusability; complete conversion to TPA
[32]	*Escherichia coli*	Biofilm-integrated nanofiber display (BIND-PETase) via curli–PETase fusion	PET films and microplastics	>3000 μM products; 9.1% degradation of postconsumer PET in 7 d	Autonomous enzyme immobilization; reusable and stable; effective in wastewater matrices
[33]	Bacterium XZ-A (ARTP mutant XZ-60S)	Atmospheric and room-temperature plasma (ARTP) mutagenesis	Polyethylene microplastics	Up to 53.65% degradation; number-average and weight-average molecular weights reduced by 15.21% and 4.80%	Non-genetically modified organism approach; enhanced laccase expression; applicable to recalcitrant polyolefins
[37]	*Saccharomyces cerevisiae*	Surface display of MHETase using cell wall anchors	MHET	k_{cat} (catalytic efficiency) comparable to purified/secreted enzyme; improved long-term stability	Yeast robustness; enzyme reuse; higher K_m (lower substrate affinity) reflects surface-display trade-offs
[40]	*Comamonas testosteroni* CNB-1	Secretion of DuraPETase by activated-sludge bacterium	PET micro- and nanoplastics	~9% PET microplastic mass loss in 7 d at ambient temperature	In situ degradation potential; assimilation of TPA, ethylene glycol, and BHET
[41]	*Escherichia coli* BL21	InaKN-mediated surface display of cold-active laccase PsLAC	Polyethylene	48% degradation in 48 h at 15 °C; 66% after 144 h	High display efficiency; low-temperature activity; excellent reusability
[38]	*Saccharomyces cerevisiae* EBY100	Yeast surface display of PETase and MHETase	PET films; postconsumer bottles	>20-fold higher rate than free enzymes; 30% activity after 4 cycles	Reusable whole-cell catalyst; effective for high-crystallinity PET
[39]	*Saccharomyces cerevisiae* EBY100	Scaffoldin-based co-display of FAST-PETase and MHETase	PET films	4.95 mM TPA in 7 d; 124.7 μg d^{-1} cm^{-2}	Complete depolymerization without MHET accumulation; highly efficient multi-enzyme clustering

5. Metabolic Engineering

Whole-cell engineering and metabolic engineering are closely related but conceptually distinct approaches within synthetic biology. Metabolic engineering primarily focuses on the rational modification of intracellular metabolic pathways to redirect carbon, energy, and redox fluxes toward desired products through gene insertion, deletion, or regulation. This approach typically emphasizes pathway reconstruction, flux balancing, and product formation from defined substrates [42]. In contrast, whole-cell engineering adopts a broader systems-level perspective, integrating not only intracellular metabolism but also extracellular processes such as polymer depolymerization, enzyme secretion or surface display, substrate uptake, stress tolerance, and interactions with complex feedstocks [26]. As such, whole-cell engineering often encompasses metabolic engineering as a core component, while additionally addressing enzyme localization, substrate accessibility, and coupling between depolymerization and assimilation [12].

This distinction is evident in plastic bioprocessing. Metabolic engineering has been widely applied to convert plastic-derived monomers into value-added products in the valorization or upcycling of plastic materials, whereas whole-cell engineering integrates depolymerization, uptake, metabolism, and biosynthesis within a single or cascaded biological system [43]. Since upcycling of plastics via metabolic engineering is outside the scope of this review, only studies focusing on plastic degradation were included, with selected examples illustrating how the resulting degradation products can be further utilized to synthesize value-added materials.

Whole-cell engineering advances extend beyond intracellular pathway optimization to integrate polymer depolymerization, uptake, and metabolism. Diao et al. identified *Rhodococcus jostii* strain PET (RPET) as a microbial chassis capable of directly utilizing PET hydrolysate as a sole carbon source. Whole-genome sequencing revealed close relatedness to *R. jostii* RHA1 and variants in regulatory genes such as *lsr2*. Through rational metabolic engineering, RPET was further engineered to produce lycopene, achieving approximately 1,300 μg/l lycopene from PET hydrolysate via cascading with alkaline PET hydrolysis [44].

Integrated metabolic engineering has also been developed to link chemical depolymerization with biological conversion. Kim et al. [45] established a one-pot process combining betaine-catalyzed PET glycolysis with whole-cell bioconversion of PET-derived monomers. In this system, *E. coli* PCA-1 converted TPA into protocatechuic acid via heterologous *tph* genes, while *Gluconobacter oxydans* oxidized ethylene glycol into glycolic acid with a molar yield exceeding 90%. This work built upon earlier demonstrations of PET valorization via whole-cell conversion of TPA into diverse aromatic compounds, including gallic acid, catechol, muconic acid, and vanillic acid [45, 46].

Seminal work by Werner et al. exemplifies a stepwise metabolic engineering strategy in *Pseudomonas putida* KT2440 to convert PET-derived intermediates into the platform chemical β-ketoadipic acid (βKA). Through four sequential engineering stages, i.e., enabling ethylene glycol utilization, introducing terephthalate catabolism genes from *Comamonas* and *Rhodococcus*, expressing PETase and MHETase from *Ideonella sakaiensis*, and deleting *pcaIJ*, the engineered strain achieved 15.1 g L^{-1} βKA from BHET at a 76% molar yield and enabled conversion of depolymerized PET into βKA in bioreactors [47].

Similarly, Ackermann et al. expanded the substrate spectrum of *P. putida* KT2440 to medium-chain dicarboxylates, including adipate, through heterologous expression of *dcaAKIJP* genes from *Acinetobacter baylyi*, followed by adaptive laboratory evolution.

Genome resequencing revealed the involvement of the *paa* gene cluster and regulatory modifications such as *psrA* disruption, enabling efficient growth on adipate and production of polyhydroxyalkanoates from dicarboxylates [48].

In parallel, Zhao et al. reconstructed a five-step reverse adipate degradation pathway from *Thermobifida fusca* in *E. coli*. By identifying and overexpressing the rate-limiting enzyme Tfu_1647, deleting competing pathways via CRISPR/Cas9, and increasing succinyl-CoA availability, the final strain achieved up to 68.0 g L^{-1} adipic acid with 93.1% of the theoretical yield in fed-batch fermentation [49]. These studies demonstrate how metabolic engineering enables efficient assimilation and conversion of plastic-derived monomers once depolymerization has occurred.

Recent advances have extended metabolic engineering concepts to polyolefin-derived substrates. Connor et al. engineered *Pseudomonas aeruginosa* to convert depolymerized polyethylene, using hexadecane as a proxy substrate, into recombinant proteins such as green fluorescent protein and spider silk–inspired proteins. Genomic integration of recombinant genes enabled protein titers exceeding 10 mg L^{-1} using chemically depolymerized polyethylene as the sole carbon source [50].

At the level of nitrogen-containing plastic additives and intermediates, Arab et al. employed CRISPR-assisted directed evolution to engineer *P. putida* KT2440 for the utilization of 1,6-hexamethylenediamine (HD) as the sole nitrogen source. Through CRISPRi-guided screening and transcriptomic analysis, key enzymes involved in HD uptake and conversion were identified, including the KgtP transporter, AlaC transaminase, and FrmA dehydrogenase. This work highlights how whole-cell engineering can couple degradation of plastic-derived compounds directly to microbial growth and metabolism [51].

6. Enzyme Engineering

Enzyme engineering has emerged as a central strategy for enhancing the degradation of microplastics, particularly synthetic polyesters and polyamides, by improving catalytic efficiency, thermostability, substrate binding, and resistance to product inhibition. Recent studies demonstrate how rational design, directed evolution, structure-guided mutagenesis, and high-throughput screening platforms have been applied to tailor plastic-degrading enzymes for improved performance under environmentally and industrially relevant conditions [25].

Early advances in enzyme engineering for PET degradation were driven by structure–function analyses of cutinases and related polyester hydrolases. Wei et al. investigated the *Thermobifida fusca* cutinase TfCut2 and leaf-branch compost cutinase (LCC), two structurally similar enzymes with exposed active sites located in surface-accessible substrate-binding grooves. By exchanging amino acid residues involved in substrate binding between TfCut2 and LCC, key positions such as G62 and I213 were identified as critical determinants of catalytic performance. Rationally designed TfCut2 variants G62A and G62A/I213S exhibited markedly enhanced PET hydrolysis at 65 °C, achieving more than 42% PET film weight loss after 50 h and a 2.7-fold increase relative to the wild-type enzyme. Kinetic analyses showed a fourfold increase in hydrolysis rate constant and reduced substrate binding constant, which was linked to alleviated product inhibition by MHET [52].

More recent efforts integrate rational protein engineering with directed evolution to achieve synergistic improvements. Groseclose et al. engineered the natural PET hydrolase Polyester Hydrolase Leipzig #7 (PHL7) using salt-bridge engineering and site-saturation

mutagenesis followed by multiple rounds of directed evolution. Rational modifications such as Q175E/R205K enhanced intramolecular electrostatic interactions and thermostability, while active-site mutations, including Q95Y, significantly improved catalytic activity. Four evolved variants, namely PHL7-Jemez, PHL7-Santa Fe, PHL7-Taos, and PHL7-Tusas, displayed more than twofold higher PET hydrolytic activity than wild-type PHL7 in reactions with amorphous PET substrates. In bioreactor experiments, PHL7-Jemez demonstrated substantially increased depolymerization of PET films at elevated substrate loadings [53].

Thermostability has been a major focus in enzyme engineering to enable PET degradation near or above the glass transition temperature of the polymer. Zeng et al. [54] reported the crystal structure of the highly thermostable leaf-branch compost cutinase ICCG in complex with a PET analogue, providing insights into the enzyme–substrate interaction network. Structure-based engineering yielded ICCG variants with melting temperatures approaching 99 °C and optimal PET hydrolytic activity at 74–85 °C. These variants produced higher concentrations of hydrolytic products than the parental enzyme when acting on PET films, containers, and reinforced PET, with structural analyses indicating enhanced local stabilization of flexible loop regions and improved core packing [54].

Directed evolution has also been extensively applied to PETases derived from *Ideonella sakaiensis*. Brott et al. employed error-prone PCR to generate mutant libraries based on thermostable IsPETase variants, including DuraPETase. Screening against polyester substrates identified mutations such as K95N/F201I and N233C/S282C that enhanced thermostability and high-temperature activity. Introduction of engineered disulfide bonds increased melting temperatures (T_m) by more than 36 °C relative to wild-type IsPETase, while selected variants exhibited substantially improved PET hydrolysis at temperatures up to 60 °C [55].

Metagenomic mining has provided access to novel PET hydrolases with intrinsic thermostability. Nakamura et al. engineered PET2, a thermostable enzyme identified from a metagenome library, using homology modeling based on IsPETase. Mutations targeting surface charge, backbone stabilization, and disulfide bond formation yielded PET2-7M, which showed higher melting temperature, increased PET binding rate constants, and a 6.8-fold increase in hydrolytic activity at its optimal temperature. Structural and single-molecule fluorescence analyses demonstrated that positively charged surface residues enhanced enzyme–PET surface interactions, contributing to improved catalytic efficiency [56].

Advances in enzyme engineering for microplastic degradation are not limited to polyesters. Puetz et al. developed a validated high-throughput screening platform (AMIDE) for the directed evolution of polyamidases and polyurethanases using a colorimetric assay to detect polymer-derived amines. Application of this system to the nylon-degrading enzyme NylCTS enabled the identification of improved variants such as NylCTSP27Q/F301L with enhanced catalytic turnover toward polyamide-6. Furthermore, combining evolved NylC variants with downstream oligomer hydrolases enabled multi-step enzymatic depolymerization of polyamide films into monomers, demonstrating the extensibility of enzyme engineering approaches beyond PET [57]. Table 2 summarizes the enzyme engineering approaches for microplastic degradation.

Table 2. Representative enzyme engineering strategies for plastic depolymerization.

Reference	Enzyme (origin)	Engineering strategy	Key mutations/ design features	Target polymer	Key performance improvements
[52]	TfCut2 (*Thermobifida fusca* KW3); LCC (metagenome)	Rational design guided by structural comparison	G62A; G62A/I213S substitutions to relieve MHET inhibition	PET films and fibers	>42% PET film weight loss in 50 h; 2.7-fold activity increase; G62A showed 4-fold higher rate constant and 5.5-fold lower inhibitor binding
[53]	PHL7 (Leipzig #7)	Rational design + site-saturation mutagenesis + directed evolution	Salt bridge Q175E/R205K; active-site mutation Q95Y; evolved variants (Jemez, Santa Fe, Taos, Tusas)	Amorphous PET films and powders	>2-fold higher activity than wild type; outperformed LCC-ICCG at 70 °C on amorphous PET; up to 270% higher hydrolysis in bioreactors
[54]	ICCG cutinase (compost metagenome)	Structure-based engineering informed by crystal structure	Multiple stabilizing mutations targeting β8–α6 loop and β-sheet–α-helix interfaces	PET films; reinforced PET	Melting temperature approaching 99 °C; 6–8 mM products from PET films; triple mutants released up to ~600 μM products from reinforced PET
[55]	IsPETase and DuraPETase variants (*Ideonella sakaiensis*)	Error-prone PCR + site-directed mutagenesis	K95N/F201I; N233C/S282C disulfide; multi-site combinations	PET nanoparticles and amorphous PET films	T_m increased up to 81.1 °C; up to 8-fold higher activity at 60 °C; enhanced high-temperature PET degradation
[56]	PET2 (metagenome-derived)	Homology-guided rational design	Surface charge modification; backbone stabilization; added disulfide bonds (PET2 7M)	Amorphous PET films	T_m increased from 69.0 to 75.7 °C; 6.8-fold higher activity; 2.7-fold higher PET binding rate; optimal temperature shifted to 68 °C
[57]	NylC (NylCTS)	Directed evolution enabled by high-throughput MAFC (amine-reactive chromogen) assay	P27Q/F301L	Polyamide 6 (PA 6)	1.9–2.3-fold higher turnover rate; improved catalytic efficiency without loss of substrate affinity
[57]	NylCTS + NylB	Enzyme cascade design	Bienzymatic conversion of oligomers	Polyamide 6	93% conversion of dimers to monomers within 24 h; complete depolymerization at low dimer concentrations
[58]	PETase (*Ideonella sakaiensis*)	Semi-rational design + stability-focused screening under sludge-simulating conditions	T51A/S54W/N173R ("sludge-PETase"); T51A/S54W/A180M	PET under sludge-like chemical conditions	Sustained activity for ≥6 days at 40 °C; 7.7-fold higher peak activity, 17.4-fold higher overall activity, and 3.4-fold higher depolymerase velocity than wild-type PETase

6.1. Computational design.

Recent developments in enzyme engineering have increasingly leveraged computational strategies to optimize plastic-degrading enzymes, combining structural modeling, molecular dynamics (MD), machine learning, and evolutionary analysis to guide rational mutagenesis and high-throughput screening [59]. These approaches have accelerated the identification of key residues, enhanced thermostability, and improved substrate binding to maximize polymer depolymerization efficiency.

6.1.1. Structure-guided and docking-based design.

Molecular docking and contact-surface analyses have been applied to identify active-site residues critical for PET binding and catalysis (Figure 3). Tournier et al. [60] used these methods to analyze wild-type LCC interacting with a PET model substrate (2-HE(MHET)$_3$). Eleven residues within a hydrophobic binding groove were subjected to site-saturation mutagenesis, resulting in 209 variants. Variants at position F243 enhanced catalytic performance, whereas additional mutations improved thermal stability. A disulfide bridge (D238C/S283C) replaced a divalent metal-binding site, increasing the melting temperature by ~10 °C. Combining stabilizing and activity-enhancing mutations led to optimized variants (ICCG, ICCM, WCCG, WCCM) with melting temperatures up to ~13 °C higher than wild-type LCC, achieving 82–85% PET conversion within 15–20 h at 72 °C and ultimately 90% depolymerization over 10 h in bioreactor studies [60]. Austin et al. [61] used high-resolution X-ray crystallography (0.92 Å) to analyze PETase, revealing an open active-site cleft relative to homologous cutinases. Computationally guided mutations (S238F/W159H and W185A) narrowed the binding cleft and improved PET degradation, demonstrating that ancestral PETase folds could be re-engineered to optimize interaction with crystalline PET substrates. These studies also revealed activity toward polyethylene-2,5-furandicarboxylate, highlighting the ability of computationally informed structural modifications to extend substrate specificity [61].

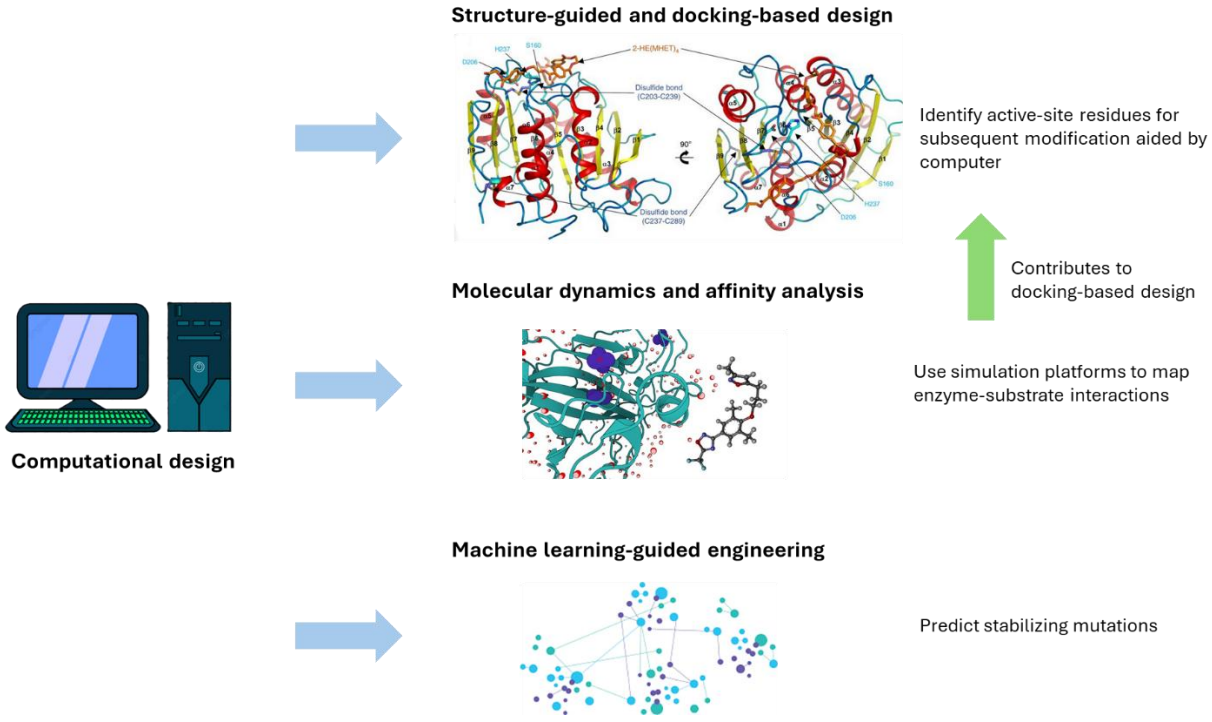

Figure 3. Computational design approaches commonly reported in the literature. These approaches are interrelated; for instance, structure-guided and docking-based design can be operationalized through molecular dynamics and affinity analysis. (Images in the figure were derived from open sources, including Wikimedia Commons, Pngtree, HiClipart and Shutterstock).

6.1.2. Molecular dynamics and affinity analysis.

Dynamic docking and MD simulations have become essential for mapping enzyme–substrate interactions (Fig. 2). Zheng et al. developed an affinity analysis of dynamic docking (ADD) approach to strengthen PET binding by identifying hotspot residues through MD simulations. Iterative rounds of ADD-guided mutagenesis combined with high-throughput screening produced LCC variants with single, double, and triple mutations (e.g., H218Y/N248D/S247A, LCC-A3; H218Y/N248D/I243Q, LCC-A3-2), which released up to ~26 mM PET hydrolysis products within 6 h at 72 °C and depolymerized >90% of pretreated post-consumer PET within 3.3 h at 78 °C, representing the fastest PET depolymerization rates reported to date [62].

MD simulations have also guided thermostabilization. Then et al. [63] designed a disulfide bridge in TfCut2 to replace a calcium-binding site, resulting in a variant with a melting point of 94.7 °C and a half-inactivation temperature of 84.6 °C. Subsequent mutations (D204C-E253C-D174R) further increased the optimum temperature to 75–80 °C and improved PET film depolymerization at 70 °C [63].

MD simulations of PES-H1 bound to a PET trimer (3PET) revealed dominant binding poses maintained over 100 ns, with hydrophobic interactions (F62, W155, I178) stabilizing the complex and contributing to PET hydrolysis [64]. Structure-guided design yielded the L92F/Q94Y variant, which increased hydrolytic activity 2.3- to 3.4-fold against amorphous PET films and real-world PET waste, while the R204C/S250C disulfide bridge increased thermostability by 6.4 °C without reducing activity. Combining both modifications provided insights into balancing stability and catalytic efficiency through computational design [64].

6.1.3. Machine Learning-guided Engineering

Machine learning has been applied to predict stabilizing mutations and streamline enzyme optimization (Fig. 2). Cui et al. implemented the GRAPE strategy (greedy accumulated strategy for protein engineering) for PETase from *Ideonella sakaiensis*, which combined computational predictions of stabilizing mutations, clustering, and greedy accumulation of beneficial variants. This approach produced DuraPETase, with a 31 °C higher apparent melting temperature and over 300-fold enhanced PET degradation toward semicrystalline films under mild conditions, achieving complete biodegradation of microplastics into water-soluble products [65].

Lu et al. used a 3D self-supervised convolutional neural network (MutCompute) to predict poorly optimized residues in PETase and identify stabilizing substitutions. Variants including S121E, T140D, R224Q, and N233K were validated experimentally, yielding FAST-PETase, which combined functional, active, stable, and tolerant features. FAST-PETase effectively depolymerized untreated post-consumer PET across 51 thermoformed products within one week and fully degraded amorphous portions of commercial water bottles at 50 °C [66].

Ding et al. applied deep learning–guided rational redesign to thermophilic LCC_ICCG. Using Preoptem and coevolutionary analysis, six single-point mutants (S32L, D18T, S98R, T157P, E173Q, N213P) were combined to create LCC_ICCG_I6M, which exhibited higher catalytic efficiency and increased optimal temperatures (75–80 °C) for PET degradation. Enzymatic digestion of bottle-grade PET powders by LCC_ICCG_I6M yielded 3.64-fold higher soluble product concentrations compared with wild-type LCC_ICCG [67].

Li et al. integrated MD and machine learning to predict protein thermostability for TfCut2. The designed variant S121P/D174S/D204P increased ΔTm by 9.3 °C, retained 14% residual activity after heat treatment at 70 °C, and improved PET hydrolysis by 46.4-fold. MD analysis indicated enhanced structural stability due to proline-induced rigidity and reduced solvent-accessible cavities [68].

6.2. Domain fusion.

Domain fusion has emerged as a powerful strategy to enhance the catalytic performance of plastic-degrading enzymes, particularly against highly crystalline PET. By covalently linking auxiliary domains, such as zwitterionic polypeptides, carbohydrate-binding modules (CBMs), chitin-binding domains (ChBDs), hydrophobins (HFBs), and antimicrobial peptides, to PET hydrolases, researchers have increased substrate affinity, adsorption efficiency, and hydrolytic activity [69].

Chen et al. [70] explored the fusion of zwitterionic polypeptides, consisting of alternating glutamic acid (E) and lysine (K) residues, to the C-terminus of PETase. Variants with longer fusion peptides, such as PETase-EK30, demonstrated over 11-fold increased hydrolytic activity against highly crystalline PET films (45.2% crystallinity) compared with wild-type PETase. Structural analyses and MD simulations revealed that EKylation strengthens overall protein stability, enlarges the substrate-binding pocket, exposes hydrophobic residues (W185, I208, W159), and optimizes the orientation of Y87, all of which contribute to enhanced substrate binding and shorter catalytic distances. These modifications collectively improved substrate affinity and reduced binding free energy, thereby accelerating PET hydrolysis [70].

Fusion of PET-binding modules has been widely applied to enhance enzyme adsorption and hydrolysis. Xue et al. [71] engineered C-terminal fusions of LCC_ICCG with ChBD from *Chitinolyticbacter meiyuanensis* SYBC-H1, CBMs from *Hypocrea jecorina*, polyhydroxyalkanoate-binding modules (PBMs) from *Alcaligenes faecalis*, and hydrophobins HFB4. Among these, LCC_ICCG-ChBD exhibited superior hydrolysis of highly crystalline PET (Hc-PET, 40%), releasing 0.335 mM hydrolysis products, which represents an 11.6-fold increase over the parent enzyme. Across PET substrates of varying crystallinity (6.7%, 16%, and 40%), the ChBD and CBM fusions consistently enhanced depolymerization performance, with ChBD outperforming other modules due to its hydrophobic nature mediated by tryptophan residues, which promoted enzyme adsorption to PET surfaces [71].

Similarly, Dai et al. fused CBM, PBM, and HFB4 domains to a previously engineered IsPETase variant (IsPETaseEHA, S121E/D186H/R280A). The IsPETaseEHA_CBM fusion displayed 71.5% and 44.5% higher hydrolytic activity at 30 °C and 40 °C, respectively, and an 86% increase in catalytic activity with higher protein loading, illustrating the significant effect of binding domain fusion on substrate accessibility and turnover [72].

Beyond CBMs and ChBDs, fusion of amphipathic or hydrophobic peptides has also been demonstrated to enhance PET hydrolysis. Liu et al. fused Dermaseptin SI (DSI), a water-soluble, thermostable peptide, to the N-terminus of *Thermobifida fusca* cutinase mutant D204C/E253C (Tfuc2). DSI-Tfuc2 degraded PET amorphous membrane particles 22.7-fold faster than Tfuc2 at 70 °C. Fluorescence binding assays confirmed that DSI enhanced the enzyme's adsorption to PET surfaces, likely due to its high content of hydrophobic nonpolar amino acids (68.9% in DSI versus 36–61% in other binding domains). Hydrophobin fusions,

such as HFB4 and HFB7, also improved PET surface binding, supporting the role of hydrophobic interactions in domain fusion strategies [73].

Graham et al. assessed CBM fusions to thermostable LCC variants at industrially relevant PET solids loadings. While CBM fusions improved aromatic monomer release at low solids (<10 wt%), conversion reached up to 97% at higher substrate concentrations with or without CBMs, indicating that the effectiveness of accessory binding domains can be context-dependent and highlights their utility in modulating enzyme–substrate interactions at varying scales [74].

7. Feasibility and Limitations

7.1. Whole-cell and metabolic engineering.

Whole-cell and metabolic engineering have emerged as promising strategies to address the global challenge of microplastic pollution, offering pathways not only for polymer depolymerization but also for conversion into value-added products. Whole-cell engineering leverages microbial chassis to express, secrete, or display plastic-degrading enzymes directly on their surface or within biofilms (Figure 4) [75]. This approach allows enzymes to act in close proximity to polymer surfaces, enhancing substrate accessibility and reducing enzyme loss during depolymerization. Surface display systems and biofilm-mediated enzyme immobilization, for instance, have demonstrated enhanced degradation efficiency and operational stability in PET microplastic systems, facilitating multi-cycle reuse and tolerance to varying environmental conditions [29, 30, 32, 38]. Multi-enzyme constructs, including combinations of PETase and MHETase, can catalyze the sequential breakdown of PET into monomers, overcoming the bottleneck of intermediate accumulation and enabling near-complete polymer hydrolysis. These strategies have been shown to function effectively in controlled bioreactor environments and may be adapted to complex aqueous matrices, including seawater or wastewater effluents [76, 77].

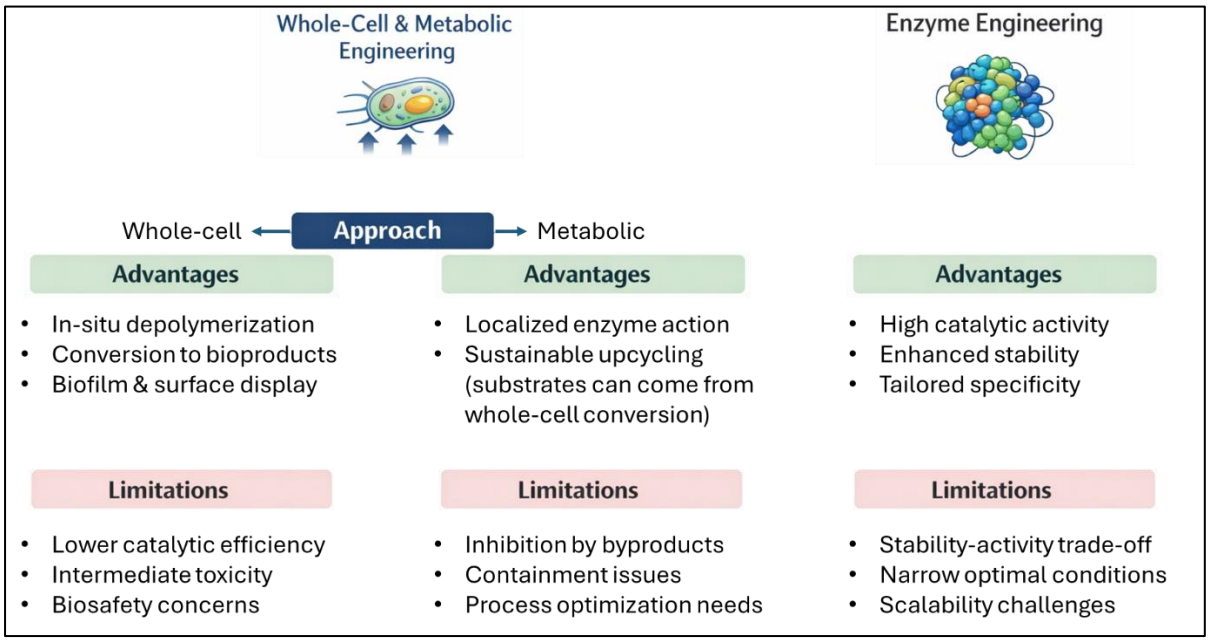

Figure 4. Summary of advantages and limitations of the two overarching synthetic biology approaches for microplastic degradation. (Images in the figure were generated by Chatgpt).

Despite these advantages, whole-cell approaches face intrinsic limitations. The catalytic efficiency of surface-displayed enzymes is often lower than that of purified enzymes due to factors such as steric hindrance, altered substrate accessibility, and the impact of the cellular microenvironment on enzyme kinetics (Figure 4) [37]. Additionally, microbial growth and activity can be inhibited by high concentrations of plastic degradation intermediates, such as TPA or ethylene glycol, which may accumulate in localized microenvironments (Figure 4). Environmental deployment of genetically modified organisms introduces biosafety concerns, requiring containment strategies such as auxotrophy, kill switches, or deployment within controlled reactors to minimize ecological risk [12,79]. Enzyme stability is further influenced by physicochemical conditions, including pH, temperature, and the hydrophobicity or crystallinity of the polymer substrate, which must be optimized to maintain consistent degradation rates [78].

Metabolic engineering complements whole-cell depolymerization by enabling the assimilation of plastic-derived monomers into cellular metabolism and their conversion into value-added chemicals (Figure 4). Engineered strains can metabolize PET hydrolysates into a variety of products, ranging from βKA and adipic acid to lycopene and other bioproducts [44, 47, 79]. This approach not only promotes sustainable upcycling but also mitigates the accumulation of potentially inhibitory monomers. However, metabolic engineering presents its own challenges. Designing effective catabolic pathways requires careful integration of heterologous genes, balancing carbon flux, and providing adequate cofactors. Substrate toxicity, osmotic stress, and the complex regulation of native metabolic networks can limit growth and product formation [80]. Adaptive laboratory evolution and pathway optimization are often necessary to overcome these hurdles, yet achieving high titers and product yields in industrially relevant conditions remains difficult (Figure 4) [81]. Pretreatment of highly crystalline or recalcitrant plastics is also required to facilitate microbial uptake and efficient downstream metabolism [45].

Integrating whole-cell depolymerization and metabolic engineering represents a synergistic strategy for microplastic management. Whole-cell catalysts can achieve initial polymer breakdown, while metabolic pathways enable conversion of released monomers into high-value chemicals or complete mineralization. Such integrated systems offer the potential for scalable and sustainable bioprocesses, particularly when deployed in controlled reactors or bio-upcycling platforms. While technical challenges remain, including substrate limitations, enzyme stability, microbial tolerance, and regulatory concerns, advances in synthetic biology, surface display systems, and metabolic pathway engineering provide a robust framework for future applications. With careful design, whole-cell and metabolic engineering approaches could contribute substantially to environmentally sustainable microplastic degradation and valorization, addressing both ecological and economic dimensions of plastic pollution.

7.2. Enzyme engineering.

Enzyme engineering has become a central strategy for enhancing microplastic biodegradation, particularly for recalcitrant polymers such as PET and polyamides. Through rational design, directed evolution, computational modeling, and domain fusion, engineered enzymes have achieved dramatic improvements in activity, thermostability, and substrate affinity, demonstrating clear feasibility for biotechnological applications (Figure 4). However, these

advances also reveal intrinsic limitations related to polymer heterogeneity, stability–activity trade-offs, and process scalability.

Rational design and directed evolution have proven highly effective in optimizing polyester hydrolases by targeting residues involved in substrate binding, product inhibition, and thermal robustness [22]. Early work on TfCut2 showed that rational substitution of substrate-interacting residues (e.g., G62A, I213S) based on structural similarity to LCC significantly enhanced PET hydrolysis while alleviating inhibition by MHET, achieving multi-fold increases in reaction rates and film weight loss [52]. Similar principles underpin later studies on PHL7, where salt-bridge engineering and site-saturation mutagenesis generated variants with superior performance to benchmark enzymes under high-temperature and high-substrate-load conditions [53]. Directed evolution using error-prone PCR has further enabled incremental gains in thermostability and activity in IsPETase and related enzymes, particularly through disulfide bond engineering and surface charge optimization [55].

Computational design has substantially expanded the scope and efficiency of enzyme engineering by enabling systematic exploration of sequence–structure–function relationships. Structure-guided docking and molecular dynamics simulations have identified binding "hotspots" and stability determinants in LCC, PETase, TfCut2, and metagenome-derived enzymes, facilitating targeted mutagenesis with high success rates [56,60,61]. Advanced machine learning–assisted frameworks, such as GRAPE, MutCompute, Preoptem, and MD-based learning models, have further accelerated the identification of stabilizing and activity-enhancing mutations, yielding enzymes such as DuraPETase, FAST-PETase, and LCCICCG_I6M that can depolymerize semicrystalline or real-world PET under milder or industrially relevant conditions [65-68]. These approaches demonstrate that computationally informed design can overcome epistatic constraints and guide navigation of complex fitness landscapes that are difficult to traverse experimentally alone.

Despite these successes, several limitations persist. A recurring challenge is the trade-off between thermostability and catalytic efficiency (Figure 4). Mutations that rigidify enzyme structures or enhance thermal tolerance often reduce the flexibility required for productive substrate binding, particularly for heterogeneous and highly crystalline plastics [55,64]. Moreover, many engineered enzymes achieve optimal performance only under narrowly defined conditions, such as elevated temperatures, high buffer molarity, or pretreated substrates, limiting their direct applicability to environmental microplastics or low-energy processes [53,63]. Computational predictions, while increasingly powerful, remain imperfect and require extensive experimental validation, especially when extrapolating from model substrates to complex, weathered plastics.

Domain fusion represents a complementary enzyme engineering strategy aimed at overcoming mass-transfer and adsorption limitations by enhancing enzyme–polymer interactions. Fusion of zwitterionic peptides, CBMs, ChBDs, hydrophobins, or amphipathic peptides to PET hydrolases has consistently improved substrate adsorption and depolymerization efficiency, particularly for high-crystallinity PET [70−73]. These results highlight the feasibility of modular designs that decouple catalytic function from binding efficiency. However, benefits from binding modules diminish at high solids loadings relevant to industrial recycling, and fusion constructs can introduce expression, folding, or stability challenges [74].

Overall, enzyme engineering, integrating rational design, directed evolution, computational modeling, and domain fusion, has clearly demonstrated feasibility for enhancing microplastic biodegradation, achieving record-breaking depolymerization rates and enabling closed-loop recycling concepts [60, 62]. Nevertheless, limitations related to substrate diversity, operational robustness, and economic scalability remain significant. Future progress will depend on integrating enzyme engineering with process engineering, pretreatment strategies, and systems-level optimization to translate laboratory successes into environmentally and industrially viable solutions.

8. Research Gaps and Recommendations

Despite rapid progress, several critical research gaps must be addressed to translate synthetic biology–enabled microplastic degradation from laboratory demonstrations to environmentally and industrially relevant applications. One major gap lies in substrate realism. Most engineered enzymes and whole-cell systems are still evaluated using pristine PET films, powders, or model microplastics, which fail to capture the physicochemical complexity of environmental plastics [28, 38, 40, 54]. Weathering, additive leaching, oxidation, biofouling, and mixed-polymer compositions fundamentally alter surface chemistry, crystallinity, and enzyme accessibility [82, 83]. Future research should prioritize standardized testing against aged, additive-rich, and mixed plastic matrices, ideally derived from real environmental or waste-stream sources. Developing reference substrates and benchmarking protocols would greatly improve cross-study comparability and accelerate technology maturation.

Another key opportunity lies in systems-level integration. Current studies often optimize enzyme engineering, surface display, or metabolic pathways in isolation, yet real-world performance depends on coordinated interactions among enzyme kinetics, localization, microbial physiology, and reactor conditions [41, 67, 76]. Synthetic biology offers powerful tools for modular system design, including programmable gene circuits, tunable promoters, dynamic pathway regulation, and synthetic scaffolds [26, 27]. Future efforts should focus on integrated platforms that combine enzyme engineering with controlled expression, multi-enzyme coordination, and adaptive regulation in response to substrate availability or inhibitory intermediates. Such approaches could mitigate trade-offs between stability and activity, reduce intermediate toxicity, and improve overall process robustness.

From a whole-cell perspective, biosafety and deployment strategies remain underexplored. While surface display and biofilm-based systems show promise in contained reactors, their application in open or semi-open environments is constrained by regulatory and ecological concerns [79]. Research opportunities exist in developing biocontainment-by-design strategies, such as multi-layer kill switches, metabolic auxotrophy, and self-limiting genetic circuits, alongside non-replicative or cell-free hybrid systems. These advances could enable safer deployment in wastewater treatment plants, stormwater systems, or coastal remediation contexts without uncontrolled persistence of engineered organisms.

In enzyme engineering, a persistent gap is the limited understanding of structure–function relationships under heterogeneous reaction conditions [84]. Most computational models are trained on soluble substrates or idealized polymer surfaces, limiting predictive accuracy for real microplastics [22, 59]. Future work should integrate multiscale modeling, combining molecular simulations with mesoscale descriptions of polymer morphology and enzyme crowding effects. Machine learning frameworks trained on datasets generated under realistic

solids loadings, fluctuating temperatures, and complex matrices could substantially improve predictive power and guide more transferable designs.

Finally, economic and environmental feasibility remains a decisive bottleneck. Few studies incorporate life cycle assessment or techno-economic analysis early in the design phase, leading to solutions that perform well biologically but poorly at scale. Future research should explicitly couple synthetic biology innovation with process engineering, pretreatment optimization, and systems analysis, ensuring that gains in enzymatic performance translate into meaningful reductions in energy use, emissions, and cost.

9. Conclusions

Among synthetic biology approaches, enzyme engineering currently demonstrates the highest technological readiness. Rational design, directed evolution, and, especially, computationally guided engineering have enabled dramatic improvements in the catalytic efficiency, thermostability, and substrate affinity of PET hydrolases and related depolymerases. Machine learning–assisted workflows have proven particularly powerful for navigating complex fitness landscapes and mitigating epistatic constraints, yielding enzymes capable of depolymerizing semicrystalline and post-consumer plastics under increasingly mild, industrially relevant conditions. In parallel, domain fusion strategies that enhance enzyme–polymer interactions address a key bottleneck in heterogeneous catalysis, namely limited substrate accessibility, and are especially effective for high-crystallinity plastics. Collectively, these approaches support enzymatic recycling and upcycling platforms that align well with circular economy principles. Whole-cell engineering is most feasible when applied in contained or semi-contained systems, such as bioreactors, wastewater treatment units, or biofilm-based platforms. Surface display, enzyme immobilization, and multi-enzyme cascades offer operational advantages, including enzyme reuse and localized substrate conversion, while reducing the need for repeated enzyme purification. However, reduced catalytic efficiency relative to purified enzymes, sensitivity to intermediate toxicity, and biosafety concerns currently limit their deployment in open environments. Metabolic engineering is best positioned as a downstream or complementary strategy, enabling assimilation of plastic-derived monomers into value-added products and preventing inhibitory accumulation of degradation intermediates. Nevertheless, its feasibility depends strongly on effective upstream depolymerization and careful balancing of cellular metabolism. Future studies should focus on several priorities. First, bridging laboratory performance with real-world substrates is critical; enzymes must be evaluated against aged, additive-rich, and mixed plastic waste streams rather than idealized model materials. Second, integrating enzyme engineering with process engineering, including pretreatment, reactor design, and product recovery, will be essential for economic viability. Third, systems-level synthetic biology approaches that combine enzyme engineering, whole-cell catalysis, and metabolic pathways within modular, controllable platforms should be further developed. Finally, advances in computational modeling, high-throughput screening, and data-driven design should be coupled with life cycle and techno-economic assessments to guide research toward solutions with genuine environmental and societal impact.

Acknowledgments

The author wishes to thank the University of Arizona for the administrative support provided.

Author Contribution

KHDT conceptualized the review and wrote the manuscript.

Competing Interest

The author declares no conflict of interest.

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