

## Biodegradation of Plastic and the Role of Microbial Enzymes in Plastic Waste Management

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### Abstract

Plastic pollution has emerged as a major environmental problem affecting terrestrial and aquatic ecosystems and posing human health risks through microplastic exposure and chemical leaching. This research reviews the biodegradability of common plastics and examines biological approaches—particularly microbial and enzymatic degradation—as complementary strategies to mechanical and chemical recycling. Drawing on recent experimental and review literature, it describes microbial colonization of plastic surfaces and the enzymatic mechanisms underlying depolymerization, with emphasis on PET hydrolases such as PETase and MHETase, cutinases, and oxidative enzymes including laccases. The study further outlines analytical methods for assessing plastic degradation and evaluates strategies to enhance enzymatic depolymerization, such as protein engineering, enzyme immobilization, and physical or chemical pretreatment of substrates. In addition, it discusses scale-up challenges, biosafety considerations, and pathways for integrating biotechnological solutions into circular economy frameworks, and formulates recommendations for future research and pilot-scale deployment. Overall, the review highlights the potential and limitations of microbial and enzymatic plastic

biodegradation as part of a broader portfolio of interventions needed to mitigate plastic pollution.

**Keywords:** Plastic Biodegradation; PETase; Enzyme Engineering; Bioreactor Design; Circular Economy.

## Introduction

Since their large-scale industrial introduction in the mid-20th century, plastics have transformed modern life due to their durability, versatility, lightweight nature, and cost-effectiveness. Global plastic production has exceeded 400 million metric tons annually, with projections indicating continued growth driven by packaging, construction, automotive, and healthcare sectors (He et al., 2024; UNEP, 2023). Unfortunately, these advantages also underpin plastics' environmental persistence, their resistance to degradation has led to their accumulation in terrestrial and aquatic ecosystems, where they fragment into microplastics and nanoplastics that infiltrate food webs and water systems (Qiu et al., 2024; Geyer et al., 2023).

Conventional waste management systems such as landfilling, incineration, and mechanical recycling remain inadequate for mitigating plastic pollution. Landfills contribute to leachate and greenhouse gas emissions; incineration releases carbon dioxide, dioxins, and heavy metals; and mechanical recycling suffers from downcycling, contamination, and loss of material quality after successive reprocessing cycles (Rillig et al., 2023; Kawai et al., 2024). Globally, less than 10% of all plastics produced have been effectively recycled, while the majority persists in the environment for decades (UNEP, 2023).

The urgent need for sustainable waste management innovations has catalyzed interest in biodegradation, the natural or engineered microbial breakdown of polymers into simpler compounds such as carbon dioxide, water, and biomass. In particular, enzymatic depolymerization offers a promising complement to thermochemical and mechanical recycling. This approach utilizes microbial enzymes, such as PETase and cutinases, to cleave polymer chains under mild conditions, recovering monomeric precursors suitable for repolymerization into virgin-grade plastics (Kawai et al., 2024; Han et al., 2024).

Compared to chemical depolymerization methods, enzymatic biodegradation has distinct advantages: it requires lower temperatures and pressures, produces fewer toxic by-

products, and allows for substrate specificity and recyclability (Richter et al., 2024). Recent advances in protein engineering, directed evolution, and metagenomic discovery have significantly improved enzyme activity, stability, and substrate range, enabling the potential industrialization of biochemical recycling pathways (Jahanshahi et al., 2025).

Beyond global innovation, the African context, particularly in rapidly urbanizing countries such as Nigeria, faces additional challenges in plastic waste management. Limited waste segregation infrastructure, informal recycling systems, and lack of standardized policies hinder efficient collection and recycling (Adebayo et al., 2024). Introducing bio-based and enzymatic approaches in local waste management frameworks could enhance environmental sustainability while generating economic opportunities in biotechnology and waste valorization sectors.

This seminar report aims to provide a comprehensive synthesis of recent developments in microbial and enzymatic plastic degradation. It systematically categorizes major plastic types based on their chemical structure and biodegradability, explores the mechanisms of microbial colonization and enzyme catalysis, outlines analytical techniques used to assess degradation, and discusses scale-up, biosafety, and policy integration. The objective is to highlight biodegradation as a viable pathway toward a circular economy, where plastics can be continuously recycled into high-quality materials with minimal ecological impact.

## **Types of plastics and their biodegradability**

### **1. Polyethylene terephthalate (PET)**

PET is a thermoplastic polyester composed of repeating terephthalic acid and ethylene glycol units linked by ester bonds. These ester linkages are hydrolysable by microbial hydrolases including PETase, MHETase and engineered cutinases. PET's semi-crystalline structure influences biodegradation: amorphous regions are more susceptible to enzymatic attack than crystalline domains, which reduce chain mobility and enzyme accessibility (Kawai et al., 2024). Enzyme engineering efforts target thermostability and activity near PET's glass transition temperature ( $T_g$ ) to improve depolymerization yields.

## **2. Polyethylene (PE) and Polypropylene (PP)**

PE and PP are polyolefins composed of C–C backbones and lack hydrolyzable functional groups, rendering them highly recalcitrant to direct enzymatic hydrolysis. Initial abiotic or enzymatic oxidation (e.g., UV/photooxidation, peroxidase-mediated oxidation) is typically required to introduce carbonyl or hydroxyl functionalities that make the polymer susceptible to further microbial processing. Reports describe bacterial alkane monooxygenases and fungal oxidative enzymes that participate in surface oxidation, but complete biodegradation under environmental conditions remains slow (Sui et al., 2023).

## **3. Polystyrene (PS) and PVC**

PS comprises styrene monomers with aromatic rings and a hydrocarbon backbone; PVC contains halogenated (chlorinated) backbones that complicate biodegradation due to toxic by-products. Aromatic-degrading microbes and highly oxidative fungal systems (white-rot fungi) have shown partial degradation of PS, while PVC degradation is further complicated by dechlorination steps and associated ecotoxicological concerns (Temporiti et al., 2022).

## **4. Bioplastics (PLA, PHA)**

Bioplastics such as polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) possess ester or polyester linkages similar to natural biopolymers, making them more readily biodegradable under composting or specific environmental conditions. Enzymes such as cutinases, lipases, and PHA depolymerases efficiently hydrolyze these polymers, and industrial composting standards (e.g., ASTM D6400, EN 13432) set conditions for their degradation.

## **Environmental fate of plastics and the plastisphere**

Once released into the environment, plastics are subjected to a range of abiotic weathering processes that gradually alter their physical and chemical characteristics. Factors such as ultraviolet (UV) radiation, thermal oxidation, hydrolysis, photodegradation, and mechanical abrasion contribute to polymer embrittlement, cracking, and the generation of secondary microplastics (Song et al., 2023; Gigault et al., 2024). These transformations not only fragment plastics into smaller particles but also increase their surface area and hydrophilicity, making them more amenable to microbial colonization (Rillig et al., 2023).

In aquatic systems, floating and submerged plastics are continuously exposed to solar radiation and oxidative stress, leading to chain scission and carbonyl group formation on the polymer surface (Zettler et al., 2023). Similarly, in terrestrial environments, soil microbial enzymes and fluctuating temperature–moisture cycles accelerate surface oxidation and leaching of additives such as plasticizers and stabilizers. These processes contribute to the accumulation of microplastics (1  $\mu\text{m}$ –5 mm) and nanoplastics (<1  $\mu\text{m}$ ), which can migrate across trophic levels and enter food chains (Geyer et al., 2023).

The physicochemical modifications resulting from abiotic weathering facilitate the establishment of microbial biofilms, forming a distinct ecological niche known as the "plastisphere", a term introduced to describe the microbial assemblages that colonize plastic surfaces in aquatic and terrestrial environments (Zettler et al., 2013; Amaral-Zettler et al., 2024). The plastisphere differs from surrounding microbial communities in both composition and function, often hosting taxa capable of hydrocarbon degradation, biofilm formation, and enzymatic oxidation of polymeric substrates (Richter et al., 2024).

Bacteria, archaea, and fungi dominate plastisphere consortia, with genera such as *Pseudomonas*, *Rhodococcus*, *Vibrio*, *Bacillus*, *Alcanivorax*, and *Aspergillus* frequently reported on marine and freshwater plastic debris (Han et al., 2024; Jahanshahi et al., 2025). These microorganisms interact synergistically to modify polymer surfaces, secrete extracellular polymeric substances (EPS), and initiate oxidative and hydrolytic reactions that lead to partial depolymerization (Kettner et al., 2023). The EPS matrix plays a dual role by enhancing cell adhesion and protecting the microbial community from environmental stress, thereby maintaining stable biofilm microenvironments (Amaral-Zettler et al., 2024).

Moreover, the plastisphere can act as a vector for pathogenic organisms and gene exchange hotspots, facilitating the spread of antibiotic resistance and virulence genes (Wu et al., 2024). This ecological dimension of plastic pollution adds further complexity to environmental risk assessment. Studies have demonstrated that plastisphere-associated microbes may metabolize not only polymer fragments but also additives and sorbed pollutants, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs) (Rillig et al., 2023; Gigault et al., 2024).

Importantly, the long-term fate of plastics and plastisphere communities depends on the interaction between abiotic and biotic degradation processes. Abiotic weathering increases polymer accessibility, while microbial colonization accelerates degradation through

enzymatic pathways. However, most environmental degradation remains partial, producing micro- and nano-scale residues that can persist for decades and pose ecological and health risks. Hence, understanding the plastisphere's composition, function, and metabolic capabilities is critical to designing bioaugmentation or bioremediation strategies that promote complete biodegradation of plastic pollutants.

### **Microbial colonization and initial steps**

Microbial colonization of plastic surfaces represents the initial and critical stage of biodegradation. It begins with the formation of a conditioning film, which occurs within hours of plastic exposure to natural environments. This film consists of dissolved organic matter (DOM), including proteins, polysaccharides, humic substances, and lipids, that adsorb onto the plastic surface through hydrophobic, electrostatic, and van der Waals interactions (Song et al., 2023; Zettler et al., 2023). The conditioning film alters the surface energy and hydrophilicity of the plastic, creating biochemical cues that facilitate microbial adhesion and subsequent biofilm development (Amaral-Zettler et al., 2024).

Once attached, pioneer microorganisms secrete extracellular polymeric substances (EPS) composed primarily of polysaccharides, proteins, nucleic acids, and lipids, which form a hydrated matrix that anchors cells to the substrate (Rummel et al., 2023). This EPS not only enhances adhesion and cohesion but also provides a microenvironment that retains water, nutrients, and enzymes, thereby supporting stable biofilm growth even under fluctuating environmental conditions (Kettner et al., 2023). The EPS also serves as a diffusion barrier, maintaining locally elevated concentrations of extracellular enzymes that participate in the oxidation or hydrolysis of the polymer surface (Richter et al., 2024).

Microbial colonization typically follows successional dynamics, initial colonizers condition the surface and alter its chemistry, allowing secondary colonizers with more specialized metabolic capabilities to establish. The resulting multi-species biofilms, known as plastisphere communities, are often dominated by taxa capable of degrading hydrocarbons and other recalcitrant compounds (Zettler et al., 2023). Bacterial genera commonly detected on plastic debris include *Pseudomonas*, *Rhodococcus*, *Bacillus*, *Vibrio*, *Alcanivorax*, and *Sphingomonas*, which are known producers of oxygenases, lipases, and dehydrogenases implicated in polymer oxidation and fragmentation (Han et al., 2024; Wu et al., 2024).

In terrestrial environments, Actinobacteria such as *Streptomyces* and *Nocardia* exhibit strong oxidative capabilities through the secretion of monooxygenases and laccases, enabling the partial oxidation of polyethylene and polypropylene surfaces (Sui et al., 2023). In contrast, fungal colonizers such as *Aspergillus*, *Penicillium*, *Cladosporium*, and white-rot fungi like *Phanerochaete chrysosporium* produce an array of oxidative enzymes, laccases, peroxidases, and manganese peroxidases (MnP), that can depolymerize aromatic polymers and oxidize polyolefin films (Temporiti et al., 2022; Gao et al., 2023).

These microbial consortia do not necessarily achieve complete mineralization; instead, they perform partial oxidation or depolymerization, generating smaller oxygenated oligomers and monomers that can be assimilated by heterotrophic members of the community (Jahanshahi et al., 2025). Such metabolic complementarity, where oxidative microbes initiate polymer weathering and other microbes catabolize the by-products, enhances the overall biodegradation potential of the plastisphere (Amaral-Zettler et al., 2024).

Environmental parameters such as temperature, salinity, nutrient availability, and surface roughness influence colonization efficiency and biofilm structure (Rillig et al., 2023). For example, roughened or pre-oxidized surfaces enhance microbial attachment by providing micro-pits and hydrophilic functional groups, while nutrient limitation can induce biofilm formation as a stress response (Gigault et al., 2024). The resulting biofilms act as dynamic biochemical microreactors, concentrating extracellular enzymes and facilitating the local degradation of polymer substrates.

In summary, microbial colonization and biofilm formation on plastics constitute essential preliminary steps in biodegradation. Through cooperative metabolism and extracellular enzymatic activity, plastisphere communities transform inert polymer surfaces into biochemically active interfaces capable of initiating the depolymerization and assimilation of synthetic polymers.

## **Microbial enzymes involved in plastic degradation**

### **1. PET-specific enzymes: PETase, MHETase, cutinases and others**

PETases (e.g., IsPETase-like enzymes) hydrolyze ester bonds in PET, producing MHET and BHET oligomers; MHETase further hydrolyzes MHET to terephthalic acid

(TPA) and ethylene glycol (EG) (Qiu et al., 2024). Cutinases, originally plant-cutin-degrading enzymes, also display PET hydrolase activity and have been engineered for improved thermostability (e.g., LCC variants). Combining PETase and MHETase activities, or engineering bifunctional constructs, enhances depolymerization efficiency (Kawai et al., 2024).

## **2. Hydrolases and esterases (cutinases, lipases, carboxylesterases)**

Cutinases and lipases hydrolyze ester linkages in aliphatic polyesters and, to varying extents, PET. Their activity is influenced by substrate accessibility, surface hydrophobicity, and the presence of inhibitors. Rational design and directed evolution approaches have improved catalytic efficiency and substrate affinity for several hydrolases (Fritzsche et al., 2023).

## **3. Oxidative enzymes: laccases, manganese peroxidases, peroxidases, and monooxygenases**

Laccases and peroxidases from fungi generate reactive radicals that can oxidize polymer surfaces, introducing oxygen-containing functional groups (carbonyls, alcohols) that increase hydrophilicity and susceptibility to further enzymatic attack. Bacterial alkane monooxygenases catalyze initial C–H activation in aliphatic chains, producing alcohols and ketones that enter downstream metabolic pathways (Sui et al., 2023; Temporiti et al., 2022).

## **4. Alkane monooxygenases and oxygenases for PE/PP initial oxidation**

Alkane hydroxylases (e.g., AlkB family) and cytochrome P450 systems catalyze terminal and subterminal hydroxylation of aliphatic chains. These reactions can initiate a sequence of oxidations leading to beta-oxidation-like metabolism of oxidized fragments. However, rates are slow and require surface oxidation and increased accessibility (Sui et al., 2023).

## **5. Metagenomic discovery and computational prediction**

Metagenomic sequencing, functional metagenomics, and structure-guided computational screens have greatly expanded the list of candidate plastic-degrading enzymes derived from landfill, compost, and marine plastispheres (Jahanshahi et al., 2025; Richter et al., 2023). These approaches enable the identification of distant homologs and novel catalytic folds that may be optimized for industrial applications.

## **Factors influencing enzymatic biodegradation**

The efficiency of enzymatic plastic biodegradation is governed by a complex interplay between polymer characteristics, enzyme properties, environmental conditions, and pretreatment methods. These factors collectively determine the rate and extent of depolymerization, influencing whether a polymer is only superficially eroded or completely mineralized to carbon dioxide, water, and biomass (Kawai et al., 2024; Qiu et al., 2024).

### **1. Polymer Structure and Physicochemical Properties**

Intrinsic polymer properties play a decisive role in enzyme accessibility. Crystallinity is one of the most critical parameters, highly crystalline regions exhibit tightly packed polymer chains with limited amorphous domains, reducing enzyme penetration and catalytic efficiency (Wei & Zimmermann, 2023). Amorphous regions, in contrast, provide more space for enzyme adsorption and cleavage. Similarly, molecular weight and degree of branching affect the polymer's mechanical rigidity and surface roughness, influencing enzyme binding affinity and substrate diffusion (Sui et al., 2023). Polymers such as polyethylene terephthalate (PET) and polyethylene (PE) exhibit variable susceptibility based on crystallinity; amorphous PET is more readily degraded by PETase and cutinase variants than its crystalline counterparts (Han et al., 2024).

Additives used in plastic manufacturing, such as plasticizers, stabilizers, flame retardants, and pigments, can either enhance or inhibit enzymatic activity. For instance, stabilizers and anti-oxidants inhibit oxidative degradation, while plasticizers may increase polymer flexibility and enzyme access (Rillig et al., 2023). Moreover, the hydrophobicity and surface charge of the polymer influence enzyme adsorption kinetics, as most hydrolases interact through hydrophobic binding domains (Zheng et al., 2023).

### **2. Environmental and Operational Conditions**

Environmental parameters such as temperature, pH, oxygen availability, and moisture significantly impact enzymatic degradation. Most known plastic-degrading enzymes, including PETase and MHETase, exhibit optimum activity between 30–70°C and pH 7–9, depending on their microbial origin (Kawai et al., 2024). Elevated temperatures close to the polymer's glass transition temperature ( $T_g$ ) soften the material, increasing enzyme accessibility to the polymer chains (Wei & Zimmermann, 2023). For PET, this typically occurs around 65–75°C, where enzymatic hydrolysis rates dramatically increase (Jahanshahi et al., 2025).

Oxygen availability affects oxidation-dependent degradation pathways, particularly for polyolefins such as polyethylene and polypropylene, where oxidative pre-functionalization via photo-oxidation or peroxidase activity is required before depolymerization (Temporiti et al., 2022). Humidity and nutrient availability also influence microbial metabolism and enzyme secretion in biofilm-based degradation systems (Rummel et al., 2023).

### **3. Pretreatment and Surface Modification**

Because most plastics are chemically inert and hydrophobic, pretreatment methods are often necessary to initiate biodegradation. Techniques such as mechanical milling, UV irradiation, heat treatment, plasma treatment, and chemical oxidation introduce polar functional groups (carbonyl, hydroxyl, or carboxyl) onto the polymer surface, increasing its hydrophilicity and enzyme-binding potential (Sui et al., 2023; Gigault et al., 2024). For instance, UV-oxidized polyethylene exhibits enhanced microbial adhesion and peroxidase activity due to increased surface roughness and oxygenated moieties (Rillig et al., 2023).

Biological pretreatments are also gaining attention, including exposure to oxidative microorganisms such as *Rhodococcus ruber* or *Aspergillus niger* prior to enzymatic degradation. Such approaches partially oxidize polymers, thereby improving subsequent enzyme performance (Gao et al., 2023). In addition, enzyme immobilization on nanoparticles, hydrogels, or polymer matrices has been shown to enhance enzyme stability and reusability while maintaining catalytic activity over prolonged degradation cycles (Qiu et al., 2024).

### **4. Enzyme Properties and Engineering**

The catalytic performance of plastic-degrading enzymes is determined by their substrate affinity ( $K_m$ ), turnover rate ( $k_{cat}$ ), and thermal stability. Advances in protein engineering have produced thermostable and more active variants of PETase and cutinase that function efficiently under industrially relevant conditions (Kawai et al., 2024). Directed evolution and computational design approaches have been employed to optimize enzyme-substrate interactions, expand substrate specificity, and improve hydrolysis rates of crystalline plastics (Han et al., 2024; Jahanshahi et al., 2025). For instance, engineered PETases from *Ideonella sakaiensis* and Leaf Branch Compost Cutinase (LCC) variants now exhibit over tenfold increases in catalytic efficiency on high-crystallinity PET films (Richter et al., 2024).

In summary, enzymatic plastic biodegradation is controlled by multiple synergistic parameters, polymer structure, environmental context, pretreatment strategy, and enzyme adaptability. Effective biodegradation requires optimized environmental conditions and surface modification strategies that enhance enzyme binding and catalytic efficiency. Ongoing research integrating enzyme engineering, material science, and process optimization aims to enable the industrial-scale deployment of biochemical recycling technologies within a sustainable circular economy framework.

## **Assays and methods to measure biodegradation**

Accurate quantification of plastic biodegradation requires multidimensional analytical approaches that integrate physical, chemical, thermal, and biological assessments. No single method can unambiguously demonstrate complete biodegradation, as plastics often undergo simultaneous fragmentation, oxidation, and partial depolymerization. Therefore, researchers employ complementary techniques to evaluate mass loss, surface modifications, chemical transformations, and mineralization (Qiu et al., 2024; Wei & Zimmermann, 2023).

### **1. Gravimetric and Mass Loss Measurements**

The most straightforward indicator of biodegradation is weight loss, reflecting bulk polymer decomposition due to microbial or enzymatic activity. Plastic films or pellets are incubated under controlled conditions, periodically washed, dried, and reweighed to determine percentage mass loss (Sui et al., 2023). However, this method alone may be misleading because mass reduction can also result from fragmentation or additive leaching rather than true depolymerization (Rillig et al., 2023). Therefore, gravimetric data are usually complemented by chemical and structural analyses.

### **2. Respirometric and CO<sub>2</sub> Evolution Assays**

Respirometric tests provide direct evidence of polymer mineralization, the ultimate confirmation of biodegradation. These assays quantify CO<sub>2</sub> evolution in aerobic systems or CH<sub>4</sub> production in anaerobic systems, often following standards such as ISO 14855 or ASTM D5338 (Han et al., 2024). The CO<sub>2</sub> produced is measured via infrared gas analysis or gas chromatography, and the amount is compared against theoretical maximum carbon conversion (Qiu et al., 2024). Recent improvements in closed respirometric chambers and

isotopic tracing ( $^{13}\text{C}$ -labeled polymers) have enabled more precise differentiation between biogenic and abiotic  $\text{CO}_2$  sources (Gigault et al., 2024).

### 3. Chromatographic Detection of Monomers and Oligomers

To evaluate enzymatic depolymerization, researchers employ high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC–MS) to identify and quantify soluble degradation products such as terephthalic acid (TPA), ethylene glycol (EG), mono-(2-hydroxyethyl) terephthalate (MHET), and bis-(2-hydroxyethyl) terephthalate (BHET) (Kawai et al., 2024). These assays provide molecular-level confirmation of bond cleavage and are essential for characterizing enzyme kinetics and substrate specificity (Wei & Zimmermann, 2023). For instance, the rate of MHET and TPA formation from PET films correlates directly with PETase catalytic efficiency and can be quantified using UV detection at 240–260 nm or coupled enzymatic assays (Han et al., 2024).

### 4. Spectroscopic Characterization

Fourier-transform infrared (FTIR) and Raman spectroscopy are powerful tools for monitoring chemical bond transformations during biodegradation. The carbonyl index (ratio of carbonyl to methylene absorbance peaks) is often used as a diagnostic measure of oxidation in polyolefins and polyesters (Rummel et al., 2023). FTIR spectra reveal the formation of hydroxyl, carbonyl, or ester groups that result from enzymatic or photo-oxidative activity (Temporiti et al., 2022). Raman spectroscopy complements FTIR by mapping chemical heterogeneity at micro- and nanoscale resolutions, enabling visualization of oxidation zones within partially degraded films (Gigault et al., 2024).

### 5. Thermal and Crystallinity Analyses

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are used to monitor thermal transitions (e.g., melting point, glass transition temperature,  $T_g$ ) and crystallinity changes associated with enzymatic degradation (Wei & Zimmermann, 2023). Enzymatic hydrolysis often preferentially attacks amorphous regions, resulting in an increase in crystallinity as the amorphous fraction is consumed (Kawai et al., 2024). These thermal shifts provide indirect evidence of structural reorganization and degradation progression.

### 6. Microscopic and Morphological Observations

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) are used to visualize surface erosion, pitting, and biofilm formation on polymer substrates. SEM

imaging provides high-resolution evidence of enzymatic etching, surface roughening, and microbial colonization, while AFM captures nanometer-scale topographical changes and mechanical property alterations (Song et al., 2023). Combined with energy-dispersive X-ray spectroscopy (EDS), these techniques can also detect oxygen incorporation during oxidation.

## **7. Enzyme Kinetic and Surrogate Substrate Assays**

Because enzymes act on insoluble and heterogeneous substrates, traditional kinetic models are difficult to apply. Researchers therefore use soluble model substrates such as p-nitrophenyl esters or fluorescent oligomers to assess catalytic parameters ( $K_m$ ,  $V_{max}$ ) (Qiu et al., 2024). Alternatively, product-release assays, such as MHET or TPA quantification over time, serve as reliable proxies for enzymatic activity on polymer films (Han et al., 2024). The development of colorimetric and fluorescence-based microplate assays has improved throughput and enabled rapid screening of enzyme libraries (Jahanshahi et al., 2025).

## **8. Integrated Assessment Approaches**

Comprehensive biodegradation evaluation increasingly involves multi-parameter integration, combining gravimetric loss, spectroscopic changes, thermal transitions, and molecular product quantification. Recent computational models integrate these datasets to estimate reaction rate constants, activation energies, and enzyme-substrate affinity landscapes (Wei & Zimmermann, 2023; Jahanshahi et al., 2025). Such integrated analyses provide a more accurate depiction of true biodegradation rather than simple physical deterioration.

## **Strategies to enhance biodegradation**

### **1. Pretreatment approaches**

Pretreatments, UV/photooxidation, chemical oxidation (ozone, peroxides), thermal or thermo-oxidative treatment, and mechanical milling, increase surface area and introduce oxygenated functionalities that make polymers more amenable to enzymatic attack. Synergistic combinations of pretreatment and enzyme catalysis often yield higher depolymerization rates.

### **2. Enzyme engineering & directed evolution**

Protein engineering (rational design, directed evolution, and computational redesign) has produced PET hydrolases with higher thermostability, improved substrate binding, and

elevated catalytic rates. Example successes include LCC variants and engineered PETases with improved activity near PET Tg (Kawai et al., 2024).

### **3. Microbial consortia and co-cultures**

Consortia that combine oxidative and hydrolytic microbes can fragment polymers and subsequently metabolize the resulting oligomers and monomers, enhancing overall mineralization. Designing stable consortia for industrial use requires attention to metabolic compatibility, resource competition, and containment.

### **4. Enzyme immobilization & surface display**

Immobilization improves enzyme stability, enables reuse, and can create high local enzyme concentrations on solid supports or within reactors. Cell-surface display strategies localize catalytic activity at cell–substrate interfaces, potentially improving reaction rates on solid plastics (Han et al., 2024).

### **5. Bioreactor design**

Bioreactor selection depends on substrate form and process economics. Powdered feedstocks are compatible with stirred-tank reactors, while film- or pellet-based feedstocks may be processed in packed-bed or rotary reactors. Continuous processes with product removal reduce product inhibition; heat integration and enzyme recycling are important design considerations.

## **Scale-up and industrial considerations**

The transition from laboratory-scale enzymatic plastic biodegradation to industrial-scale recycling presents substantial scientific, economic, and engineering challenges. While numerous microbial and enzymatic systems, particularly PETases, cutinases, and esterases, have demonstrated impressive performance under controlled conditions, their application to heterogeneous post-consumer waste streams demands robust process optimization, cost-effective enzyme production, and environmentally sound operational strategies (He et al., 2024; Wei & Zimmermann, 2023).

### **1. Enzyme Production and Cost Optimization**

One of the foremost challenges in scaling up enzymatic recycling lies in reducing enzyme production costs. Industrially viable enzymes must be produced in high yields using

cost-efficient expression systems such as *Escherichia coli*, *Pichia pastoris*, *Bacillus subtilis*, or filamentous fungi (Kawai et al., 2024). Advances in metabolic engineering and synthetic biology have enabled the overexpression of thermostable PETases and cutinases with minimal post-translational modification requirements (Jahanshahi et al., 2025). Moreover, fermentation optimization, including fed-batch and continuous bioreactor systems, has been shown to enhance volumetric productivity while lowering downstream purification costs (Zheng et al., 2023).

Enzyme immobilization and cell-surface display systems are additional strategies to improve enzyme recovery and reusability. For instance, immobilized PETases on silica, chitosan, or magnetic nanoparticles have retained over 80% of their catalytic activity after multiple degradation cycles (Han et al., 2024). Immobilization also facilitates enzyme recycling, a critical factor in reducing operational expenditures for large-scale processes.

## **2. Process Engineering and Feedstock Management**

The heterogeneity of post-consumer plastic waste poses a major barrier to consistent enzyme performance. Contaminants such as dyes, fillers, multilayer laminates, and residual food or soil can inhibit enzyme access and catalytic efficiency (Rillig et al., 2023). Thus, upstream sorting, washing, and pretreatment steps are essential. Industrial plants employ mechanical shredding, thermal softening, or chemical oxidation to produce flakes or pellets with optimized surface area and hydrophilicity prior to enzymatic hydrolysis (Qiu et al., 2024).

For polyethylene terephthalate (PET), enzymatic hydrolysis reactors are designed to operate near the polymer's glass transition temperature (65–75°C), where chain mobility enhances accessibility to ester bonds. Continuous stirred-tank reactors (CSTRs) or packed-bed bioreactors equipped with immobilized enzymes are preferred configurations for maintaining high throughput and controlled residence time (Wei & Zimmermann, 2023).

## **3. Monomer Recovery and Repolymerization**

Downstream processing focuses on the separation and purification of monomers, typically terephthalic acid (TPA) and ethylene glycol (EG) in the case of PET recycling. These monomers can be recovered through filtration, crystallization, and distillation steps and subsequently repolymerized into virgin-grade PET using conventional polymerization techniques (Kawai et al., 2024). High-purity recovery is essential to maintain polymer quality comparable to petroleum-derived resins. Integrating biocatalytic depolymerization with

chemical repolymerization forms a closed-loop process consistent with circular economy principles.

#### **4. Techno-Economic and Life-Cycle Assessments (TEA & LCA)**

Scaling enzymatic recycling also requires a rigorous evaluation of economic feasibility and environmental sustainability. Techno-economic assessment (TEA) models evaluate the balance between enzyme cost, conversion efficiency, energy input, and product yield. Life-cycle assessment (LCA) quantifies greenhouse gas emissions, water consumption, and waste generation compared to mechanical or chemical recycling (He et al., 2024; Gigault et al., 2024).

Recent analyses indicate that, when integrated with renewable energy systems and high enzyme turnover, enzymatic recycling can reduce CO<sub>2</sub> emissions by up to 40–60% relative to incineration and virgin plastic synthesis (Geyer et al., 2023). However, large-scale adoption will require reducing enzyme production costs by at least an order of magnitude and improving process intensification to meet industrial throughput demands.

#### **5. Policy, Regulatory, and Industrial Partnerships**

Successful scale-up is contingent upon supportive policy frameworks and industrial collaboration. Governments can incentivize biocatalytic recycling facilities through tax credits, green procurement mandates, or extended producer responsibility (EPR) schemes (UNEP, 2023). Meanwhile, industrial partnerships, such as Carbios' PET enzymatic recycling plant in France and ongoing academic–industry consortia, demonstrate the commercial potential of enzyme-driven circularity models (Kawai et al., 2024). The integration of artificial intelligence (AI) and machine learning tools to optimize enzyme design and process parameters is also accelerating industrial readiness (Jahanshahi et al., 2025).

Overall, the scale-up of enzymatic plastic recycling requires a multidisciplinary approach that unites enzyme engineering, bioprocess optimization, material preprocessing, and sustainable economics. Continuous innovations in enzyme stability, bioreactor design, and process integration, coupled with life-cycle validation, are paving the way toward commercially viable and environmentally benign enzymatic recycling technologies that can complement and, in some cases, replace traditional mechanical and chemical methods.

## **Environmental and biosafety considerations**

While enzymatic and microbial biodegradation offers sustainable solutions to plastic pollution, it also introduces environmental and biosafety challenges that must be critically managed to prevent unintended ecological impacts. Plastic biodegradation is rarely complete in natural or engineered systems; instead, it often results in partial depolymerization, producing microfragments, oxidized oligomers, and soluble degradation intermediates (Wei & Zimmermann, 2023). These by-products may persist in the environment and exhibit unknown or potentially harmful ecotoxicological effects, particularly toward aquatic organisms, soil microbiota, and trophic interactions (Gigault et al., 2024).

### **1. Ecotoxicological Risks of Partial Degradation Products**

Partial degradation of polymers, such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), can yield oxygenated fragments that remain bioactive and surface-reactive. These fragments may adsorb heavy metals, persistent organic pollutants (POPs), and antibiotics, acting as carriers that amplify environmental toxicity (Rillig et al., 2023; Wu et al., 2024). Studies have shown that micro- and nanoplastic fragments can induce oxidative stress, inflammation, and genotoxicity in aquatic organisms such as *Daphnia magna*, *Danio rerio*, and benthic invertebrates (Song et al., 2023; Geyer et al., 2023). Furthermore, degraded plastics can release additives like bisphenol A (BPA), phthalates, and brominated flame retardants, which disrupt endocrine systems in both wildlife and humans (Temporiti et al., 2022).

To minimize these risks, biodegradation processes must target complete mineralization to CO<sub>2</sub>, water, and biomass or the quantitative recovery of monomers suitable for repolymerization. Integrated bioprocesses combining enzymatic depolymerization and chemical purification can ensure the removal of toxic intermediates while maintaining material circularity (Kawai et al., 2024).

### **2. Containment and Biosafety in Engineered Systems**

The use of genetically engineered microorganisms (GEMs) for enhanced biodegradation introduces additional biosafety concerns. Engineered strains often express optimized enzymes (e.g., PETase variants) with higher catalytic activity or thermal stability (Jahanshahi et al., 2025). However, if released unintentionally, these strains could transfer genetic elements or disrupt native microbial communities (Richter et al., 2024). Therefore,

stringent biocontainment strategies, including auxotrophy design, kill-switch mechanisms, and genetic firewall systems, are essential for laboratory and industrial applications (He et al., 2024).

Regulatory agencies such as the U.S. Environmental Protection Agency (EPA), European Food Safety Authority (EFSA), and Nigeria's National Biosafety Management Agency (NBMA) emphasize the need for risk assessment, containment protocols, and post-release monitoring of any biotechnological process involving live engineered microbes. In many cases, the enzyme-only approach, where purified or immobilized enzymes are used instead of whole cells, represents a safer and more controllable alternative (Wei & Zimmermann, 2023).

### **3. Environmental Process Integration and Life-Cycle Safety**

Biodegradation systems must be evaluated using life-cycle assessment (LCA) frameworks that incorporate not only carbon footprint and energy consumption but also toxicological endpoints, such as bioaccumulation potential and ecotoxicity indices (Gigault et al., 2024; UNEP, 2023). For example, enzymatic recycling integrated with renewable energy sources demonstrates a lower overall environmental burden than incineration or mechanical recycling, provided that intermediate by-products are efficiently captured and treated (Kawai et al., 2024).

Moreover, biosafety extends beyond process design to post-treatment management. Residual biomass, enzymes, or degraded oligomers must be handled according to hazardous waste regulations to prevent secondary contamination. Closed-loop bioreactors and filtration systems are recommended for preventing release of engineered enzymes or residual microplastics into natural ecosystems (Richter et al., 2024).

### **4. Ethical and Societal Implications**

The deployment of synthetic biology and enzyme engineering in plastic waste management raises ethical questions regarding ecosystem alteration, biotechnological equity, and public acceptance. Transparent communication, environmental education, and stakeholder engagement are critical to ensure societal trust in enzymatic recycling initiatives (He et al., 2024). The prioritization of enzyme-based systems that are non-pathogenic, biodegradable, and free of gene-transfer risks aligns with biosafety level 1 (BSL-1) containment practices and global sustainability goals.

In summary, ensuring environmental safety and biosafety in enzymatic plastic biodegradation demands an integrated framework that emphasizes complete mineralization, biocontainment, and compliance with international biosafety regulations. By focusing on enzyme-only processing, controlled closed-system bioreactors, and comprehensive LCA-based risk assessment, researchers and industries can advance biodegradation technologies that are not only effective but also ecologically and socially responsible.

## **Circular economy and policy integration**

The concept of the circular economy provides a transformative framework for rethinking plastic production, consumption, and disposal. Unlike the conventional linear model of “take–make–dispose,” circularity aims to retain materials in use for as long as possible through reuse, recycling, and recovery processes that regenerate value and minimize waste (UNEP, 2023). Within this paradigm, enzymatic and microbial recycling technologies represent innovative tools for closing the material loop by converting plastic waste into high-purity monomers suitable for repolymerization into virgin-grade plastics (Kawai et al., 2024; Qiu et al., 2024). These biochemical pathways operate under milder conditions than chemical recycling, use renewable biological catalysts, and have the potential to significantly reduce energy inputs and greenhouse gas emissions (Wei & Zimmermann, 2023).

### **1. Role of Enzymatic Recycling in the Circular Economy**

Biocatalytic recycling supports circular economy objectives by allowing closed-loop recovery of monomers such as terephthalic acid (TPA) and ethylene glycol (EG) from polyethylene terephthalate (PET). These recovered monomers can be repolymerized into plastics of comparable or superior quality to virgin materials (Han et al., 2024). Such processes exemplify “upcycling within circularity,” where waste becomes a feedstock for high-value products. Moreover, enzyme-based recycling can be integrated with renewable energy systems, further improving the environmental footprint compared to mechanical or pyrolytic methods (Gigault et al., 2024).

Globally, several pilot projects are demonstrating how enzyme-enabled recycling aligns with UN Sustainable Development Goals (SDGs), notably SDG 12 (Responsible Consumption and Production) and SDG 13 (Climate Action). Industrial collaborations, such as Carbios (France) and Loop Industries (Canada), have successfully scaled up enzymatic

depolymerization of PET bottles and fibers, showcasing the commercial feasibility of this circular approach (UNEP, 2023).

## **2. Policy Instruments for Circular Plastic Management**

The successful implementation of circular recycling systems depends on robust policy frameworks. Extended Producer Responsibility (EPR) is a cornerstone mechanism that mandates producers to finance or manage the post-consumer phase of their products, incentivizing eco-design and waste reduction (OECD, 2024). Countries such as France, Germany, and South Korea have demonstrated that EPR schemes can significantly increase recycling rates and feedstock quality.

In Nigeria and other African nations, national plastic action plans and emerging EPR frameworks, supported by the UNEP Global Partnership on Plastic Pollution and Marine Litter (GPML), are being piloted to integrate the informal sector, improve collection logistics, and build local recycling capacity (Adebayo et al., 2024). Fiscal incentives, including tax credits for recycling plants, reduced import duties for recycling equipment, and subsidies for biodegradable or recyclable materials, can further stimulate investment in biotechnological recycling (He et al., 2024).

Standardization and certification schemes are equally vital. Establishing quality standards for recycled monomers and feedstocks ensures compatibility with industrial polymerization processes and builds market trust. National and regional standards bodies (such as the Standards Organisation of Nigeria (SON) and the European Committee for Standardization, CEN) can support these frameworks by adopting clear definitions of “recycled content” and “circular products.”

## **3. Social Inclusion and the Informal Waste Sector**

In many low- and middle-income countries, informal waste pickers contribute significantly to plastic collection and sorting. Integrating these actors into formal circular economy systems not only improves feedstock consistency but also enhances social inclusion and poverty reduction (Adebayo et al., 2024). Inclusive strategies include:

Recognition of informal collectors in policy documents and EPR frameworks;

Provision of fair compensation, training, and occupational safety measures; and

Formation of cooperatives and micro-enterprises linked to producer responsibility organizations (PROs).

Such inclusive models have been successful in South Africa, Ghana, and Kenya, where social enterprises and partnerships with multinational companies have increased recycling rates while improving livelihoods (UNEP, 2023). In Nigeria, integrating informal collectors through structured partnerships under EPR schemes can secure a steady supply of clean, sorted plastics essential for enzymatic processing.

#### **4. Policy Harmonization and International Collaboration**

At the international level, the emerging UN Global Plastics Treaty, currently under negotiation by the Intergovernmental Negotiating Committee (INC), aims to establish a legally binding framework for plastic life-cycle management, including circularity and recycling innovation (UNEP, 2024). Aligning national strategies with such agreements will enable countries to access international technical assistance, climate finance, and technology transfer for deploying advanced recycling infrastructure.

Harmonization across national borders, particularly within regional economic communities like the African Continental Free Trade Area (AfCFTA), can promote cross-border trade in recycled materials and shared R&D investment. In addition, collaborative public–private partnerships (PPPs) and academic–industry consortia are essential to drive knowledge exchange and accelerate technology transfer to developing regions.

Enzymatic and microbial recycling can play a pivotal role in transitioning toward a sustainable, inclusive circular plastic economy. Achieving this vision requires an integrated policy environment that combines:

- a. EPR and fiscal incentives to promote investment and responsibility;
- b. Technical standards and quality control for feedstock and recycled products;
- c. Social inclusion of informal waste collectors; and
- d. Alignment with global treaties to access funding and technical cooperation.

Such multi-level strategies will ensure that biochemical recycling not only reduces environmental pollution but also contributes to economic development, green job creation, and social equity.

#### **Conclusion**

The pursuit of sustainable solutions to the global plastic pollution crisis has positioned biocatalytic and microbial recycling at the forefront of circular economy

innovation. Among the existing approaches, enzymatic depolymerization of polyethylene terephthalate (PET) represents the most technologically mature and commercially viable biochemical recycling pathway. Breakthroughs in enzyme discovery, structural elucidation, and protein engineering have transformed PET biodegradation from a laboratory curiosity into a rapidly advancing industrial technology (Kawai et al., 2024; Qiu et al., 2024).

Since the discovery of *Ideonella sakaiensis* PETase and its synergistic partner MHETase, numerous research groups have developed engineered enzyme variants with enhanced thermostability, substrate affinity, and turnover rates. These advances have enabled efficient depolymerization of semi-crystalline PET at process temperatures near its glass transition range (65–75 °C), achieving nearly complete conversion into terephthalic acid (TPA) and ethylene glycol (EG) within hours under optimized conditions (Han et al., 2024). The integration of such enzymatic processes into industrial recycling plants, such as the Carbios demonstration facility in France, marks a milestone toward closed-loop PET recycling that yields virgin-grade materials suitable for continuous reuse (UNEP, 2023).

However, extending similar progress to other major polymers remains a formidable challenge. Polyolefins (e.g., polyethylene and polypropylene) and polystyrene resist enzymatic attack due to their saturated C–C backbones, hydrophobicity, and crystallinity (Wei & Zimmermann, 2023). Their degradation likely requires combined abiotic–biotic strategies, wherein preliminary oxidation (via UV exposure, plasma treatment, or photo-catalysis) introduces functional groups that microbial enzymes can subsequently act upon (Gigault et al., 2024). Advancing this field will depend on the discovery of potent oxidative enzymes, including laccases, peroxidases, and alkane hydroxylases, capable of catalyzing initial polymer oxidation and chain scission (Gao et al., 2023; Temporiti et al., 2022).

## **Research and Development Priorities**

### **1. Expanded Metagenomic and Functional Screening**

Novel enzyme discovery remains critical. Leveraging metagenomic libraries, machine learning, and computational enzyme mining can uncover new biocatalysts from diverse ecosystems such as landfills, marine sediments, and industrial wastewaters (Jahanshahi et al., 2025). High-throughput screening using fluorescent or chromogenic substrates can rapidly identify enzymes with promising activity toward synthetic polymers. Global collaborations and open-access databases for sequence sharing will accelerate innovation and reproducibility.

## 2. Protein Engineering for Enhanced Thermostability and Activity

Industrial-scale recycling demands enzymes that maintain high catalytic efficiency under harsh operational conditions. Directed evolution, rational design, and computational modeling have successfully generated thermostable PETase and cutinase variants with 10–50× higher activity than wild-type enzymes (Kawai et al., 2024). Future research should focus on multi-enzyme complexes, fusion constructs (e.g., PETase–MHETase hybrids), and immobilized systems that enhance durability, substrate binding, and reusability (Han et al., 2024).

## 3. Pilot-Scale Demonstrations and Life-Cycle Assessment (LCA)

Translating laboratory results into real-world applications requires pilot-scale process validation coupled with techno-economic assessment (TEA) and life-cycle assessment (LCA). These tools quantify the environmental and financial viability of enzymatic recycling compared with mechanical or chemical routes, considering parameters such as enzyme cost, energy demand, and CO<sub>2</sub> footprint (He et al., 2024; Wei & Zimmermann, 2023). Transparent and standardized reporting of these data will build confidence among policymakers and investors.

## 4. Standardization of Analytical Assays and Interlaboratory Benchmarking

A persistent challenge in biodegradation research is the lack of standardized testing protocols. Studies often differ in substrate preparation, incubation conditions, and analytical techniques, leading to inconsistent results. Establishing ISO-aligned standards for biodegradation measurement, including gravimetric analysis, FTIR spectroscopy, CO<sub>2</sub> evolution, and monomer quantification, will ensure reproducibility and comparability across laboratories (Rummel et al., 2023). International benchmarking initiatives and shared reference materials can help unify the field.

## Outlook and Broader Implications

The convergence of enzyme engineering, synthetic biology, and bioprocess optimization is driving enzymatic recycling toward commercialization. Yet, realizing its full potential requires systems-level integration, linking molecular biology with environmental policy, circular economy frameworks, and industrial engineering. Emerging global agreements, such as the UN Global Plastics Treaty, provide an enabling policy environment for these technologies (UNEP, 2024).

Ultimately, enzymatic recycling represents not only a scientific achievement but also an opportunity to reimagine waste as a renewable resource. By aligning technological innovation with sustainability goals, biosafety, and social inclusivity, this approach can help transition societies from linear consumption models toward resilient, low-carbon circular economies.

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