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Enzymatic Bioremediation of Polyethylene Terephthalate Contaminated Environment

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Abstract

Enzyme-based bioremediation has emerged as a sustainable and eco-friendly approach to mitigating environmental pollution caused by plastic wastes, pesticides, heavy metals, and industrial effluents. This article explores recent advancements in enzymatic degradation, using polyethylene terephthalate hydrolase (PETases) and mono(2-hydroxyethyl) terephthalate hydrolase (MHETases) for polyethylene terephthalate (PET) breakdown, enzymatic mechanisms for bioleaching. PET is one of the most widely used synthetic plastics, contributing significantly to global plastic pollution due to its persistence in the environment. Conventional methods of PET disposal, such as incineration and landfilling, have proven to be inefficient and hazardous to the environment. In contrast, enzymatic biodegradation offers a promising eco-friendly solution to tackle PET biodegradation through the use of specialized enzymes like PETase and MHETase. We explore the mechanisms of PET biodegradation with focus on key enzymes and their catalytic pathways. Furthermore, the discovery, structure, and activity of PETase, the role of MHETase in processing degradation intermediates, and the

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contributions of corroborating enzymes such as cutinases, lipases, and esterases were also examined. Despite the promising potential of enzymatic PET degradation, several challenges remain, including enzyme, inefficiency in product recovery, and biosafety concerns associated with genetically modified organisms. In the same vein, regulatory hurdles and the need for standardization in bioremediation practices are discussed. Future research should focus on; the integration of enzymatic biodegradation into the circular bioeconomy, the use of artificial intelligence in enzyme design and the importance of global collaborations in advancing bioremediation strategies.

Keywords: Terephthalate Hydrolase, Mono(2-Hydroxyethyl) Terephthalate Hydrolase, Polyethylene Terephthalate, Enzymatic Bioremediation

Introduction

Plastic pollution has become one of the most pressing environmental issues of the 21st century, primarily due to the extensive production and use of synthetic polymers such as polyethylene terephthalate (PET). PET is a widely used thermoplastic polymer found in everyday consumer products including beverage bottles, food packaging materials, and textile fibers as shown in figure 1b. Its desirable properties such as durability, chemical resistance, and transparency have led to a sharp increase in global demand and production, which currently exceeds 82 million metric tons per year (Geyer *et al.,* 2017). Unfortunately, PET's resistance to natural biodegradation has made it a significant contributor to environmental pollution, particularly in marine and terrestrial ecosystems. PET waste accumulates in landfills, water bodies, and natural habitats, where it can persist for centuries, posing threats to biodiversity, food safety, and public health (Thompson *et al.,* 2009).

The persistence of PET in the environment has severe ecological implications. In aquatic ecosystems, PET fragments are frequently ingested by marine organisms, leading to physical and physiological harm. Over time, PET undergoes mechanical and photochemical fragmentation to form microplastics, which are even more pervasive and challenging to remediate. These microplastics can enter the food chain, potentially bioaccumulating in organisms and disrupting hormonal systems in humans and wildlife alike (Andrady, 2011). The global scope of PET pollution necessitates the development of effective, scalable, and sustainable solutions.

Conventional methods of plastic waste management including landfilling, incineration, and chemical recycling have been widely adopted, yet each of these strategies carries considerable limitations. Landfilling, although economically favorable and simple to implement, results in long-term environmental degradation and occupies valuable land resources. Furthermore, the leachate from plastic-containing landfills can contaminate soil and groundwater (Hopewell *et al.,* 2009). Incineration offers the benefit of volume reduction and energy recovery; however, it often results in the release of hazardous pollutants such as dioxins, furans, and heavy metals, contributing to air pollution and public health concerns (Singh and Sharma, 2016). Chemical recycling techniques such as glycolysis, methanolysis, and hydrolysis provide pathways for PET depolymerization but typically require high temperatures, expensive catalysts, and large energy inputs, making them economically and environmentally unsustainable for large-scale application (Shah *et al.,* 2008).

As plastic production continues to outpace the capacity of conventional waste management systems, there is a growing consensus in the scientific community that innovative and biologically inspired approaches are urgently needed. Traditional methods have proven insufficient for addressing the long-term consequences of plastic accumulation, thus opening the door for more sustainable alternatives.

Enzymatic bioremediation has emerged as a promising and environmentally friendly alternative for addressing the challenge of PET degradation. This approach involves the use of specific microbial enzymes such as PETase and MHETase that can catalyze the hydrolysis of PET into its constituent monomers: terephthalic acid (TPA) and ethylene glycol (EG) (Yoshida *et al.,* 2016). Unlike conventional remediation techniques, enzymatic degradation operates under mild conditions, requires fewer hazardous chemicals, and exhibits high substrate specificity, reducing the likelihood of unwanted byproducts. Furthermore, the enzymes involved can be genetically engineered to improve their thermostability, catalytic efficiency, and environmental adaptability (Tanasupawat *et al.,* 2020).

The discovery of *Ideonella sakaiensis*, a bacterium capable of utilizing PET as a carbon source, has propelled research into microbial plastic degradation. Its PETase enzyme has since been characterized structurally and functionally, sparking interest in mining similar enzymes from metagenomic databases and diverse ecological niches (Joo *et al.,* 2018).

Advances in protein engineering, synthetic biology, and metagenomics now offer exciting opportunities to design next-generation enzymes tailored for industrial-scale PET bioconversion. As enzymatic bioremediation gains traction, it holds great potential for transforming waste management practices and supporting a circular economy.

By integrating bioinformatic and biochemical data, this seminar contributes to the growing body of knowledge necessary for developing efficient enzymatic platforms for PET degradation. The findings are expected to inform future efforts in environmental biotechnology, particularly in the design of robust microbial systems for sustainable plastic wastes remediation. Ultimately, the seminar report underscores the relevance of enzyme-based strategies in addressing the global plastic crisis and highlights promising avenues for industrial and ecological applications.

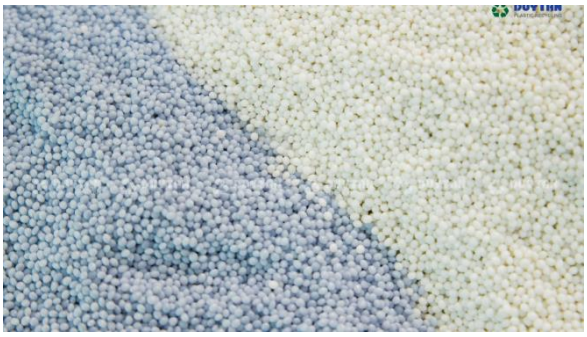


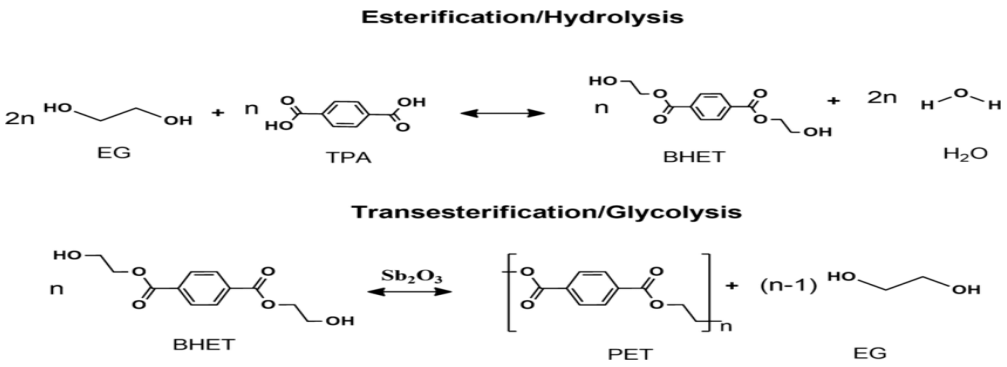
Figure 1a: Polyethylene Terephthalate Pet Resin Bottle Grade: Polyethylene Terephthalate Pet Pellet (Benyathiar *et al.*, 2022).



Figure 1b: Sample of PET materials. Source: compactor-runi.com (Benyathiar *et al.*, 2022).

Polyethylene Terephthalate (PET): Structure and Environmental Persistence

Polyethylene terephthalate (PET) is a semi-crystalline thermoplastic polymer synthesized through the polycondensation reaction between terephthalic acid (TPA) and ethylene glycol (EG) to form bis(2-hydroxyethyl) terephthalate (BHET) as shown in equation 1. Its chemical structure is characterized by repeating units of ester linkages (-COO-) that contribute to its rigidity and high tensile strength. PET exhibits a high glass transition temperature (Tg~75°C) and melting temperature (Tm~250°C), properties that make it suitable for high-temperature processing and long-term storage (George and Kurian, 2014). The polymer's resistance to hydrolysis and most chemical agents, along with its excellent barrier properties against gases and moisture, accounts for its widespread use in the packaging and textile industries. However, these same chemical and physical characteristics render PET highly resistant to natural degradation processes, making it one of the most persistent forms of plastic waste in the environment.



Equation 1: Main reactions in the esterification step of PET synthesis (Ana. *et al.*, 2019)

The global prevalence of PET stems from its extensive applications across various sectors. It is predominantly used in the production of beverage and food containers, accounting for nearly 30% of all plastic packaging worldwide (Real, 2022). Additionally, PET is a primary material in the manufacturing of synthetic fibers, particularly in the textile industry, where it is commonly known as polyester. Its lightweight nature, cost-effectiveness, and recyclability have driven its popularity as a substitute for traditional materials such as glass and aluminum. Nevertheless, the increasing consumption of PET products has resulted in a proportional rise in post-consumer PET waste, much of which escapes proper waste management systems and accumulates in terrestrial and aquatic environments.

Degradation of PET is a major challenge due to its high crystallinity and strong ester bonds. While amorphous regions of PET may be more susceptible to hydrolytic cleavage,

the crystalline regions remain highly resistant to enzymatic and microbial attack (Wei and Zimmermann, 2024). Traditional physical and chemical degradation methods such as thermal degradation, glycolysis, and hydrolysis require harsh conditions and are energy-intensive, limiting their sustainability and scalability. In natural environments, abiotic factors such as ultraviolet (UV) radiation and mechanical abrasion lead to surface fragmentation but fail to fully mineralize the polymer, resulting in the formation of microplastics and nanoplastics that are more difficult to detect and remove (Andrady, 2011).

The environmental fate of PET is closely tied to its persistence and interaction with ecosystems. Once discarded, PET can persist for decades, especially in anaerobic environments like landfills or cold marine waters. Its slow degradation contributes to the accumulation of macro- and microplastic particles that affect soil quality, water purity, and biological health. In aquatic environments, PET debris is commonly ingested by marine organisms, leading to internal injuries, malnutrition, and bioaccumulation of toxic substances adsorbed on plastic surfaces (Wright *et al.,* 2013). Moreover, the presence of PET in the food web raises concerns about human exposure to microplastics through seafood consumption. These ecological and health risks highlight the urgent need for environmentally friendly and efficient degradation strategies that can mitigate the long-term impacts of PET pollution as shown in figure 2a ans 2b below.



Figure 2a: Images of plastic pollution on both terrestrial and aquatic (Aneto *et al.,* 2022).



Figure 2b: Picture Showing Polluted land with plastics

Health Implications of PET Pollution

Endocrine Disruption, while PET itself is considered relatively inert, certain additives and degradation products can leach into contents, potentially disrupting the endocrine system. Phthalates, used as plasticizers in some PET products, and antimony, a catalyst residue, have been identified as endocrine disruptors. Studies indicate that these substances can mimic hormones, leading to potential reproductive and metabolic health issues (Sax, 2010). **Ingestion of Microplastics** from PET containers can enter the human body through ingestion of contaminated food and beverages. A study by (Muncke, 2009) highlighted that PET bottles might leach phthalates and antimony, substances known for their endocrine-disrupting effects. Furthermore, microplastics have been detected in human tissues, including the placenta and testicles, raising concerns about potential health impacts (Campen *et al.,* 2023; Pearlman *et al.,* 2023).

Toxicity of Additives such as antimony trioxide, used as a catalyst in PET production, is classified as a potential carcinogen. Chronic exposure, such as from consuming bottled water stored in PET containers over extended periods, may lead to health issues including skin irritation, lung problems, and gastrointestinal disturbances (Choe *et al.,* 2003). **Infant and Child Risk, c**hildren and infants are particularly vulnerable to low-dose chemical exposure due to their developing systems. Research has shown that infants can ingest significant amounts of microplastics daily, primarily from plastic bottles and toys. These exposures may pose risks to their developing endocrine and immune systems (Wired, 2020).

Enzymes Involved in PET Biodegradation

PETase

The discovery of enzymes capable of catalyzing the biodegradation of polyethylene terephthalate (PET) marked a significant breakthrough in addressing the environmental challenge posed by plastic pollution. One of the most promising enzymes identified to date is PETase, a novel hydrolase that demonstrates the capacity to depolymerize PET under mild environmental conditions. PETase was first isolated in 2016 from the bacterium *Ideonella sakaiensis* 201-F6, discovered in sediment samples collected from a PET-contaminated recycling facility in Japan (Yoshida *et al.,* 2016). This bacterium was found to utilize PET as its primary carbon and energy source, with PETase initiating the degradation process by cleaving the ester bonds within the polymer chain to yield mono(2-hydroxyethyl) terephthalic acid (MHET), which is subsequently hydrolyzed by MHETase into terephthalic acid (TPA) and ethylene glycol (EG).

Structurally, PETase shares homology with cutinases and lipases, belonging to the α/β-hydrolase fold family of enzymes. Its active site features a catalytic triad composed of serine, histidine, and aspartate residues, which are essential for ester bond hydrolysis (Joo *et al.,* 2018). However, PETase exhibits distinct structural adaptations that enhance its activity against PET. Notably, it possesses a more open and flexible active site cleft compared to classical cutinases, which allows it to accommodate and bind to the rigid PET polymer more effectively. High-resolution crystallographic studies have revealed the presence of an extended loop region and an enlarged substrate-binding pocket that facilitate the binding of PET chains and improve substrate accessibility (Han *et al.,* 2017). These features contribute to PETase's superior performance in degrading PET at ambient temperatures, making it a highly attractive candidate for biotechnological applications in plastic recycling and environmental remediation.

The catalytic mechanism of PETase involves nucleophilic attack on the ester carbon by the active-site serine, leading to the formation of a tetrahedral intermediate that is stabilized by an oxyanion hole. The intermediate subsequently collapses, releasing one end of the polymer chain and forming an acyl-enzyme intermediate. This intermediate is then hydrolyzed by a water molecule activated by the histidine residue, releasing the degradation product and regenerating the enzyme for further catalytic cycles (Chen *et al.,* 2018). Understanding this mechanism has enabled protein engineers to develop PETase variants

with improved thermal stability, higher catalytic efficiency, and enhanced degradation activity against high-crystallinity PET substrates. These advances underscore the significance of PETase in the emerging field of enzymatic plastic degradation and highlight its potential as a cornerstone in sustainable waste management strategies.

MHETase

In the PET degradation pathway facilitated by *Ideonella sakaiensis* enzymem (PETase), MHETase plays a crucial complementary role to PETase. While PETase initiates the hydrolysis of polyethylene terephthalate (PET) by cleaving its polymeric chains into smaller intermediates, the primary product generated from this reaction is mono(2-hydroxyethyl) terephthalic acid (MHET). MHETase, a serine hydrolase also secreted by *I. sakaiensis*, is responsible for further catalyzing the hydrolysis of MHET into its monomeric constituents: terephthalic acid (TPA) and ethylene glycol (EG) (Palm *et al.,* 2019). This step is vital because it ensures the complete conversion of PET into environmentally benign and reusable building blocks, thereby closing the loop in the PET biodegradation cycle.

MHETase operates with high substrate specificity, preferentially hydrolyzing MHET over similar compounds. Structurally, the enzyme adopts the α/β-hydrolase fold typical of carboxylic ester hydrolases and contains a catalytic triad similar to PETase comprising serine, histidine, and aspartate residues (Pinto *et al.,* 2021). Crystallographic and computational studies have shown that MHETase possesses a relatively narrow active site pocket tailored to the geometry of MHET, which contributes to its substrate discrimination and efficient turnover (Wei *et al.,* 2022). Unlike PETase, MHETase exhibits limited activity on larger PET fragments or structurally dissimilar intermediates, emphasizing its specialized role in the enzymatic cascade.

Functionally, MHETase's activity is indispensable for achieving complete PET mineralization. Without MHETase, the intermediate MHET would accumulate and impede the full recovery of TPA and EG, thus reducing the efficacy and environmental sustainability of the enzymatic degradation pathway. Furthermore, engineered chimeric constructs that physically link PETase and MHETase or spatially co-localize them have demonstrated enhanced PET degradation efficiencies, underscoring the synergistic interplay between the two enzymes (Feng *et al.,* 2021). As research into enzyme engineering and synthetic biology progresses, optimizing MHETase's catalytic performance and

thermal stability will be pivotal in advancing scalable, enzyme-based solutions for plastic wastes recycling figure 3.

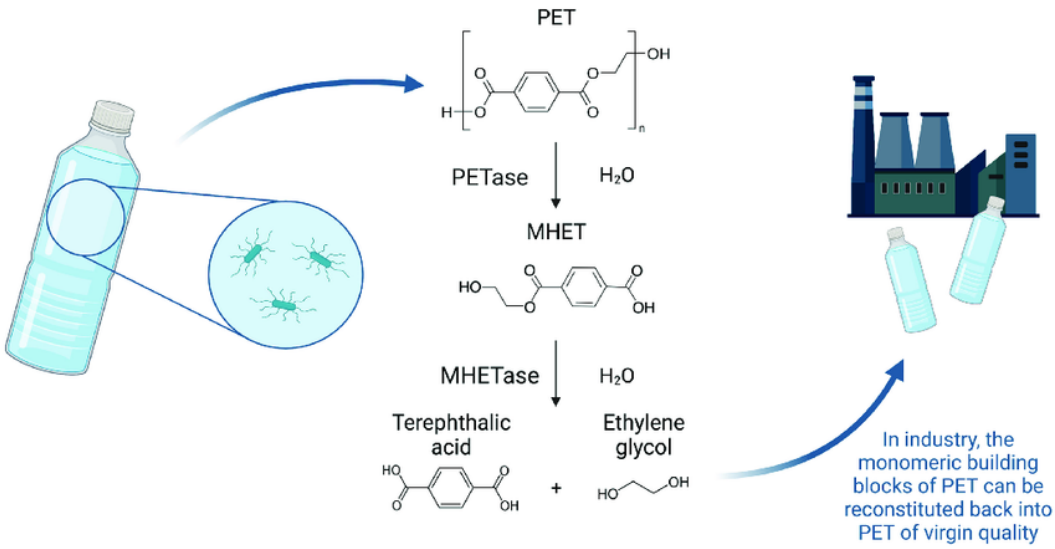


Figure 3: Enzymatic degradation of polyethylene terephthalate (PET) by PETase and MHETase enzymes into terephthalic acid (TPA) and ethylene glycol (EG) (Josephine *et al.*, 2022).

Terephthalic Acid Dioxygenase and E*thylene Glycol Dehydrogenase*

Subsequent degradation of TPA and EG by microorganisms leads to the production of CO_2. TPA is metabolized through a series of enzymatic reactions by enzymes like *TPA dioxygenase*, which introduce oxygen into the TPA molecule, eventually breaking it down into smaller organic acids that are further degraded to CO_2 (Zhu *et al.,* 2020). Similarly, ethylene glycol is oxidized by enzymes such as *ethylene glycol dehydrogenase* to produce CO_2 and H_2O (Kawai *et al.,* 2025). The final mineralization of these compounds results as shown in figure 4.

Thus, the key enzymes involved in the degradation of PET include PETase, MHETase, TPA dioxygenase, and ethylene glycol dehydrogenase, which together facilitate the breakdown of PET into CO_2 and H_2O, contributing to its mineralization as shown in figure 4.

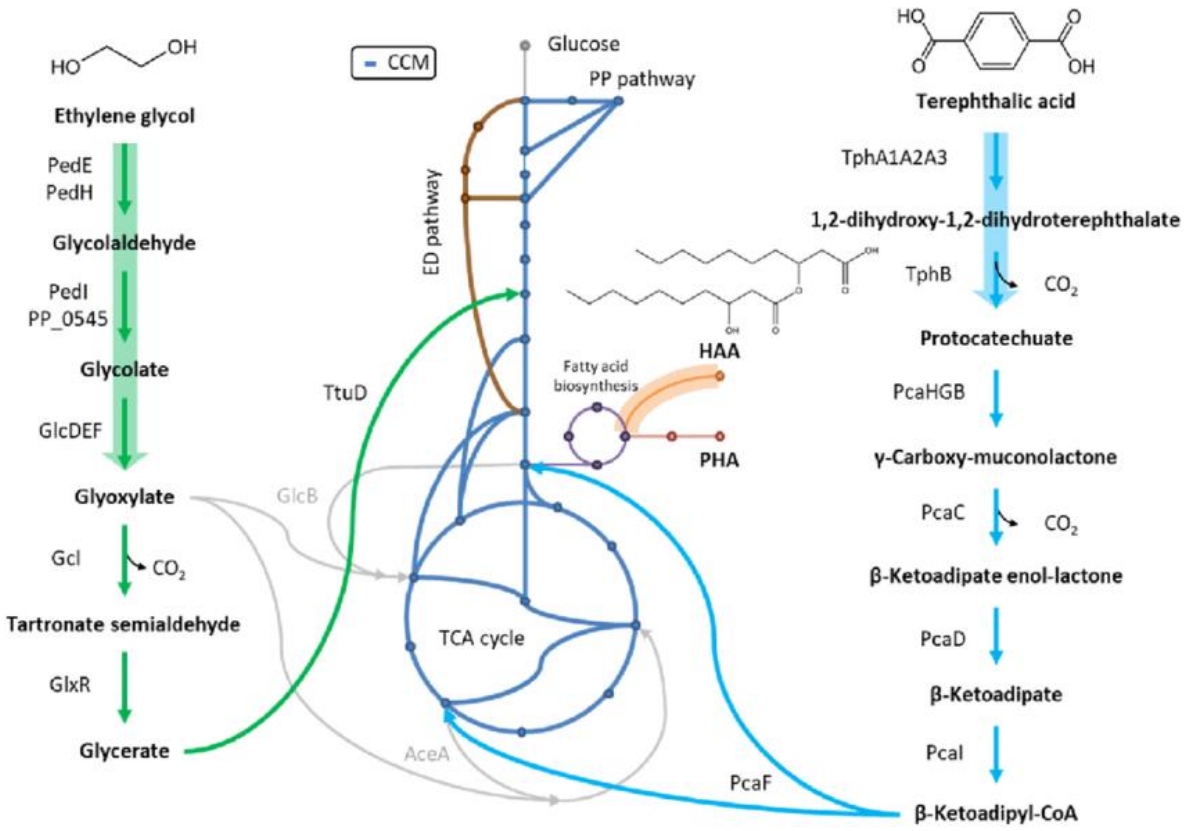


Figure 4: Metabolic pathways for the metabolization of ethylene glycol (EG) and terephthalic acid (TA) (Till *et al.*, 2021).

Mechanistic Insights into PET Enzymatic Biodegradation

The enzymatic biodegradation of polyethylene terephthalate (PET) follows a stepwise mechanism involving the sequential hydrolysis of ester bonds in the polymer backbone. The process begins with the adsorption of PET-degrading enzymes such as PETase onto the PET surface, followed by the catalytic cleavage of the polymer into smaller intermediates like mono(2-hydroxyethyl) terephthalate (MHET), bis(2-hydroxyethyl) terephthalate (BHET), and ultimately to its monomeric components terephthalic acid (TPA) and ethylene glycol (EG) (Joo *et al.,* 2018). PETase initiates the depolymerization by hydrolyzing the outer amorphous regions of PET, producing MHET as a major product, which is then further broken down by MHETase into TPA and EG (Yoshida *et al.,* 2016). The cooperative action of these enzymes is crucial for complete mineralization and efficient recycling of PET waste.

Substrate binding and catalysis are driven by precise molecular interactions between the enzyme and the PET polymer. Enzymes such as PETase possess a canonical α/β-hydrolase fold with a conserved catalytic triad (Ser–His–Asp) located within a hydrophobic active site cleft that accommodates the PET chain (Austin *et al.,* 2018). Binding is enhanced by aromatic residues near the active site that stabilize the terephthalate rings via π–π interactions. Structural adaptations such as a widened substrate-binding groove, observed in engineered PETase variants, facilitate better access to the polymer chain and promote efficient catalysis (Chen *et al.,* 2018). The formation of a tetrahedral intermediate during ester bond cleavage is a hallmark of the catalytic mechanism, with water molecules acting as nucleophiles to aid in hydrolysis.

The efficiency of PET biodegradation is highly influenced by reaction conditions including pH, temperature, and the presence of inhibitors. Optimal activity for many PET-hydrolyzing enzymes occurs at neutral to slightly alkaline pH and at temperatures close to or above the glass transition temperature of PET (~70 °C), which enhances polymer chain mobility and enzyme accessibility (Tournier *et al.,* 2020). However, most native enzymes denature at these elevated temperatures, highlighting the need for thermostable variants. Moreover, inhibitors such as degradation products (e.g., TPA) can accumulate and competitively inhibit enzyme activity, necessitating process optimization or the inclusion of product-removal strategies to maintain reaction efficiency (Wei *et al.,* 2022).

Comparative studies between natural and engineered enzymes reveal significant improvements in performance metrics through protein engineering. Natural PETase exhibits moderate activity and limited thermostability, whereas engineered variants demonstrate enhanced hydrolytic activity, broader substrate tolerance, and increased resistance to thermal and chemical stress (Son *et al.,* 2019; Lu *et al.,* 2022). For instance, structure-guided engineering of cutinases has produced variants capable of complete PET depolymerization in hours rather than days, outperforming their wild-type counterparts in both speed and efficiency (Tournier *et al.,* 2020). These advancements underscore the transformative potential of enzyme engineering in optimizing PET biodegradation for real-world applications.

Other Supporting Enzymes

Beyond PETase and MHETase, several other enzymes, including cutinases, lipases, and esterases, have demonstrated potential in the enzymatic degradation of polyethylene terephthalate (PET). These enzymes, though not as substrate-specific as PETase, contribute to the hydrolytic cleavage of ester bonds within PET polymers or its oligomeric intermediates, often under varying environmental and industrial conditions. Among them, cutinases have attracted substantial interest due to their ability to hydrolyze a broad range of polyesters, including PET, with relatively high efficiency, especially under mild reaction conditions (Wei and Zimmermann, 2024).

Cutinases, originally isolated from phytopathogenic fungi such as *Fusarium solani* and *Humicola insolens*, share structural similarities with PETase, possessing an α/β-hydrolase fold and a catalytic triad. However, they generally feature a more open active site, allowing for the accommodation of bulkier substrates such as PET oligomers and low-crystallinity PET films (Shirke *et al.,* 2018). Some engineered cutinases, such as Leaf-Branch Compost Cutinase (LCC) and Thermobifida fusca cutinase, have demonstrated enhanced thermal stability and catalytic performance, making them viable candidates for industrial-scale PET recycling applications (Tournier *et al.,* 2020).

Lipases and esterases also contribute to PET biodegradation, albeit with more limited efficacy compared to PETase or cutinases. Lipases, typically known for catalyzing the hydrolysis of long-chain triglycerides, have shown some activity on PET under specific conditions, particularly when the polymer is pretreated or present in low-crystallinity form (Barth *et al.,* 2015). Esterases, by contrast, exhibit a broader substrate range and act on short-chain ester bonds, aiding in the degradation of PET oligomers and facilitating the breakdown of residual by-products in the PET degradation pathway.

Collectively, these supporting enzymes expand the enzymatic toolbox for PET biodegradation and underscore the potential of synergistic enzyme cocktails for efficient plastic waste treatment. Ongoing efforts in protein engineering, directed evolution, and metagenomic screening continue to uncover and optimize novel variants of cutinases, lipases, and esterases with improved PET-degrading capabilities (Singh *et al.,* 2021). Their integration into multi-enzyme systems holds promise for enhancing the speed and completeness of PET hydrolysis, ultimately contributing to a more sustainable bioremediation strategy.

Biotechnological Approaches for Enhancing Enzymatic PET Degradation

Advances in structural and computational studies have provided critical insights into the mechanisms underlying PET biodegradation. X-ray crystallography and cryo-electron microscopy have been instrumental in elucidating the high-resolution structures of PET-hydrolyzing enzymes such as PETase and MHETase. These structural data reveal the enzyme's active site architecture, substrate-binding pockets, and key catalytic residues, which are essential for understanding how the enzymes interact with PET substrates (Chen *et al.,* 2018). Notably, PETase from *Ideonella sakaiensis* has been extensively studied, and its crystal structure has revealed a unique α/β-hydrolase fold that accommodates the terephthalate ring of PET, positioning it for efficient hydrolysis (Yoshida *et al.,* 2016). Understanding the spatial configuration of the active site allows for the identification of potential targets for enzyme engineering, such as residues that contribute to substrate specificity, binding affinity, and catalytic efficiency.

In addition to experimental structural analyses, computational approaches like molecular dynamics simulations and quantum mechanical calculations have significantly advanced our understanding of the enzymatic mechanism. Molecular dynamics (MD) simulations allow for the exploration of enzyme-substrate interactions at atomic resolution, helping to predict how conformational changes in the enzyme influence substrate binding and catalysis (Joo *et al.,* 2018). For example, MD simulations have been used to study the dynamic motions of PETase during the hydrolysis of PET, revealing the enzyme's flexible active site that adapts to the substrate's geometry during catalysis (Feng *et al.,* 2021). These studies also provide insights into the role of water molecules in the reaction, highlighting their involvement in the formation of the tetrahedral intermediate, a crucial step in the hydrolysis of PET ester bonds.

Computational tools such as molecular docking and virtual screening are increasingly being used to identify potential inhibitors of PET-hydrolyzing enzymes. These tools facilitate the screening of large compound libraries for molecules that can modulate enzyme activity, either by inhibiting or enhancing their performance (Wei *et al.,* 2022). By simulating the binding affinity of potential ligands to the enzyme's active site, researchers can prioritize compounds for further testing and optimize conditions for enzymatic PET degradation. Additionally, computational methods have enabled the design of engineered variants with improved thermostability, substrate specificity, and resistance to product inhibition. These

design strategies are particularly useful for improving the efficiency of PETase and other PET-degrading enzymes in industrial applications.

Computational predictions and structural studies also support the development of hybrid or multi-enzyme systems for PET biodegradation. By combining enzymes such as PETase and MHETase with cutinases or lipases, researchers can engineer more robust and efficient systems for breaking down PET at scale. These hybrid systems can synergistically degrade the polymer into smaller, more manageable intermediates, which can be further processed into monomers or other valuable compounds (Tournier *et al.,* 2020). The ability to predict enzyme behavior and interaction through computational models is therefore invaluable for advancing the field of PET biodegradation and optimizing enzymatic recycling technologies.

Environmental Applications of Enzymatic PET Bioremediation

The enzymatic degradation of PET holds great promise for revolutionizing PET waste management, offering a sustainable alternative to traditional recycling methods. PET is one of the most widely used plastics in the world, particularly in the form of beverage bottles and packaging materials, and its accumulation in the environment poses significant ecological challenges. Conventional recycling methods, such as mechanical recycling and incineration, often suffer from limitations related to energy consumption, contamination, and a lack of efficiency in breaking down the polymer completely. In contrast, enzymatic biodegradation offers a more environmentally friendly and potentially more efficient way to break down PET into its constituent monomers, which can then be repurposed into new PET products (Wei *et al.,* 2022).

One of the most promising applications of PET-hydrolyzing enzymes is in the development of biotechnological recycling processes. These processes involve the use of enzymes like PETase and MHETase to degrade PET waste at a molecular level. When PET waste is exposed to these enzymes, it is broken down into its monomers—terephthalic acid (TA) and ethylene glycol (EG)—which can be purified and re-polymerized to produce high-quality PET, thereby closing the recycling loop. This enzymatic recycling process has the potential to reduce the environmental impact of PET by enabling closed-loop recycling, where PET is continuously reused without degradation of quality (Yoshida *et al.,* 2016). Additionally, enzymatic recycling could help alleviate the

pressure on landfills and reduce the environmental footprint of traditional recycling methods.

In addition to closed-loop recycling, the use of PETase and related enzymes in industrial-scale PET biodegradation offers promising avenues for bioremediation of PET waste in landfills and other polluted environments. Enzyme-based bioremediation could be deployed in the treatment of PET-contaminated soil or marine environments, where plastic waste is often found. Enzymes can break down large quantities of PET over time, converting it into smaller, more biodegradable components that can be further degraded by other microorganisms in the ecosystem. This approach could help mitigate the long-term environmental impacts of plastic pollution and accelerate the natural degradation of PET in ecosystems where it is typically resistant to microbial breakdown (Tournier *et al.,* 2020).

To scale enzymatic PET recycling and bioremediation to industrial levels, several challenges remain to be addressed. These include improving the stability, activity, and cost-effectiveness of PET-degrading enzymes under industrial conditions. For instance, the thermal stability of PETase needs to be enhanced to allow for effective degradation of PET at higher temperatures, which are typically used in industrial processes (Joo *et al.,* 2018). Furthermore, the costs associated with enzyme production and enzyme recovery after the degradation process must be reduced to make enzymatic recycling economically competitive with conventional recycling methods. Advances in protein engineering, such as directed evolution and rational design, offer promising solutions to these challenges by optimizing enzyme properties for large-scale applications (Wei *et al.,* 2022).

Another significant application of PETase and related enzymes lies in the synthesis of valuable products from PET waste. Beyond recycling the monomers into new PET, enzymatic processes can also be used to produce chemicals, biofuels, and other bio-based materials from PET waste. For example, the hydrolysis of PET into its monomers can be combined with fermentation processes to generate bio-based chemicals like bioethanol or bioplastics, offering an integrated approach to waste-to-resource conversion (Shrimpton-Phoenix *et al.,* 2022). This approach not only addresses the plastic waste problem but also contributes to the development of sustainable bioeconomies.

Environmental and Economic Implications

Despite the promising potential of enzymatic PET degradation, several significant challenges must be overcome before these biotechnological approaches can be widely implemented at an industrial scale. One of the primary challenges is the efficiency of the enzymes involved in PET degradation. Enzymes like PETase and MHETase are effective at breaking down PET, but their activity is often limited by several factors, including the complex and crystalline nature of PET, which can hinder enzyme access to the polymer chains (Joo *et al.,* 2018). This limitation means that even the most advanced enzymes require optimization to enhance their catalytic efficiency, especially for degrading PET in real-world conditions such as those found in landfills or marine environments.

Additionally, environmental conditions, such as pH and temperature, can significantly affect the efficiency of enzymatic degradation. While some enzymes, such as PETase, show promise in a variety of pH and temperature ranges, many still operate best under specific conditions that are difficult to replicate on a large scale (Yoshida *et al.,* 2016). For example, PETase works effectively at moderate temperatures, but it may not retain its activity in the high temperatures typically used in industrial settings. Efforts to engineer more heat-stable enzymes through directed evolution or rational design have shown success but remain a critical area of research (Wei *et al.,* 2022). Achieving a balance between enzyme stability, activity, and cost-efficiency will be crucial for the widespread use of these bioremediation techniques.

Another challenge lies in the cost of enzyme production. Current methods for producing enzymes at scale, such as recombinant protein expression systems, can be expensive and resource-intensive. While research has made significant strides in improving enzyme yield and reducing production costs, the economic feasibility of large-scale enzymatic recycling or bioremediation remains a critical barrier to widespread adoption (Tournier *et al.,* 2020). To make enzymatic PET degradation commercially viable, new strategies for cost-effective enzyme production, such as using microbial cell factories or optimizing fermentation processes, must be explored.

In addition to enzyme optimization and cost reduction, the effective integration of enzymatic processes into existing recycling infrastructures poses another hurdle. Traditional mechanical recycling methods are well-established and involve large-scale processes that are optimized for materials like PET. Integrating enzymatic steps into these existing systems

requires careful consideration of how enzymes will be incorporated into the overall recycling workflow without disrupting efficiency. Developing modular enzyme-based systems that can be added to current recycling lines, or creating entirely new systems specifically for enzymatic recycling, will be necessary for achieving large-scale implementation (Shrimpton-Phoenix *et al.,* 2022).

Despite these challenges, the future of PET biodegradation through enzymatic processes is bright. Researchers are exploring a variety of strategies to address these limitations, including the use of multi-enzyme systems, the discovery of novel enzymes with better stability and activity, and the application of synthetic biology techniques to engineer highly efficient microbial consortia for PET degradation. Furthermore, the growing interest in circular economy models, where products are continuously recycled into new forms, is driving innovation in sustainable plastic waste management. As these research areas evolve, the potential for enzyme-based PET recycling and bioremediation could revolutionize the way we manage plastic waste and help mitigate the environmental impacts of plastic pollution.

In the long term, enzymatic PET biodegradation could be integrated with other waste-to-resource technologies, such as those based on microbial fuel cells or biorefineries, to create a holistic and sustainable approach to managing plastic waste. By unlocking the full potential of PET-degrading enzymes, researchers and industries can contribute to a more sustainable future, where plastic pollution is reduced, and valuable resources are reclaimed from waste.

Current Challenges and Knowledge Gaps

While enzymatic degradation of polyethylene terephthalate (PET) offers a promising approach to addressing plastic pollution, several challenges must be overcome before it can be fully optimized and widely adopted. These challenges range from technical difficulties related to enzyme performance to environmental and regulatory concerns. Identifying and addressing these challenges will be key to advancing this bioremediation strategy (Kawai, *et al.,* 2025).

Enzyme Stability in Harsh Environments

One of the primary challenges in enzymatic PET biodegradation is ensuring enzyme stability and activity in real-world, harsh environmental conditions. PET degradation often requires enzymes to function under conditions that differ significantly from the laboratory settings in which they are typically optimized. Enzymes such as PETase must maintain stability at high temperatures, varying pH levels, and the presence of organic solvents and detergents commonly found in plastic waste. Developing more robust enzymes capable of withstanding such conditions is crucial for large-scale industrial applications. Techniques such as enzyme immobilization, protein engineering, and the use of stabilizers are being explored to improve enzyme stability under these challenging conditions (Kawai, *et al.,* 2025).

Efficiency and Product Recovery

The efficiency of PET degradation is another key challenge that impacts the scalability of enzymatic recycling processes. While PETase and related enzymes have shown promising results in laboratory experiments, the actual degradation rate in real-world conditions often falls short of the required standards for industrial applications. Additionally, recovering the products of enzymatic PET breakdown, such as monomers like terephthalic acid (TPA) and ethylene glycol (EG), poses another challenge. These monomers are valuable for the chemical industry, but separating them from the reaction mixture efficiently without contaminating them with by-products or residual plastic is complex and costly. Advances in reactor design, process optimization, and product recovery technologies are necessary to improve overall efficiency and economic viability (Ghosal, and Nayak, 2022).

Standardization and Regulatory Hurdles

As with any emerging technology, enzymatic PET degradation faces regulatory and standardization challenges. The lack of internationally recognized standards for evaluating enzyme performance, safety, and environmental impact is a significant barrier to large-scale implementation. Regulatory bodies must establish clear guidelines for the use of enzymes in biodegradation processes, including protocols for testing enzyme efficacy, ensuring product safety, and minimizing environmental risks. Standardization efforts, combined with the

development of harmonized regulatory frameworks, are essential to facilitate the widespread commercialization of enzymatic PET recycling technologies (European Food Safety Authority, 2021).

Future Perspectives and Research Directions

The enzymatic degradation of polyethylene terephthalate (PET) represents an innovative and promising solution to the growing plastic pollution crisis. However, several areas remain to be explored and developed to enhance the efficiency and scalability of this bioremediation strategy. Future research directions are crucial to addressing existing challenges and accelerating the application of PET biodegradation on a global scale. The following sections explore key advancements and emerging trends in the field (Ghosh, and Patel, 2023).

Advances in Enzyme Discovery and Design

The discovery of novel enzymes capable of efficiently breaking down PET is a focal point for future research. Although PETase and MHETase have garnered significant attention, researchers are investigating other microbial sources and enzyme classes with similar or superior plastic-degrading capabilities. Advances in metagenomic approaches will likely uncover additional enzymes, expanding the toolbox available for PET degradation. Furthermore, the engineering of enzymes through directed evolution, mutagenesis, and rational design is expected to improve their stability, catalytic efficiency, and range of action. These advancements will be vital for scaling up PET biodegradation processes and making them more cost-effective (Chow *et al.,* 2023).

Integration with Circular Bioeconomy

One of the most exciting future perspectives for PET biodegradation is its integration into the circular bioeconomy. As industries move towards more sustainable practices, enzyme-based PET recycling can play a crucial role in creating closed-loop systems where plastic waste is continuously recycled and reused. Enzymatic PET recycling could reduce the need for new petrochemical production, helping to decrease carbon footprints and reliance on fossil fuels. Developing technologies that integrate enzyme-based degradation with other recycling processes, such as mechanical and chemical recycling, will be essential for maximizing the efficiency and sustainability of plastic waste management (Salvador, *et al.,* 2019).

Potential of AI and Machine Learning in Enzyme Engineering

Artificial intelligence (AI) and machine learning (ML) are poised to revolutionize the field of enzyme engineering. By analyzing vast datasets from enzyme structure-function relationships and predicting enzyme performance, AI and ML can significantly accelerate the identification of potential candidates for PET biodegradation. These technologies can also help optimize enzyme design and predict the impact of specific mutations, enabling faster development of more efficient enzymes for industrial applications. Additionally, AI can play a key role in modeling biodegradation processes, providing insights into reaction mechanisms and guiding experimental designs for better performance under various environmental conditions (Ali, and Zhang, 2024)..

Global Collaborations and Policies for Bioremediation

The scale of the global plastic pollution issue requires international collaboration and concerted efforts from multiple stakeholders, including governments, industries, and research institutions. Collaborative research efforts that bring together experts in biotechnology, environmental science, and industrial engineering will be key to developing scalable bioremediation technologies. Global policies and regulations that promote the use of sustainable waste management practices and encourage the development of green technologies will provide the necessary support for large-scale enzyme-based PET recycling initiatives. Governments can also play a crucial role in funding research, creating incentives for industries to adopt environmentally friendly technologies, and setting up frameworks for collaboration across borders (Agnelli, and Tortora, 2022).

Conclusion

The enzymatic biodegradation of polyethylene terephthalate (PET) represents a promising and innovative solution to the growing global plastic pollution crisis. The discovery and characterization of enzymes such as PETase and MHETase, along with supporting enzymes like cutinases and lipases, have provided critical insights into the mechanisms through which plastics can be broken down biologically. These enzymes are capable of efficiently degrading PET, offering an environmentally friendly alternative to traditional methods such as incineration and landfilling, which often exacerbate environmental harm. The stepwise breakdown of PET, facilitated by these enzymes, not only presents a

potential route for plastic waste management but also opens the door to more sustainable recycling processes.

However, despite significant advancements in enzyme research, several challenges remain. These include improving the efficiency, stability, and scalability of PET-degrading enzymes to make them commercially viable. The ability to produce enzymes in sufficient quantities, under cost-effective conditions, and at temperatures suitable for large-scale industrial processes, are critical factors that need further optimization. Moreover, integrating enzymatic degradation systems into existing recycling infrastructures remains a key hurdle that requires thoughtful consideration of both technical and economic factors.

As research continues to advance, the potential for enzyme-based PET recycling systems to revolutionize the management of plastic wastes grows. The future of this field lies in overcoming these challenges through innovative solutions, such as enzyme engineering, multi-enzyme systems, and cost-effective enzyme production methods. Furthermore, aligning enzymatic recycling with the principles of a circular economy could lead to a future where PET waste is no longer a pollutant but a valuable resource. By advancing the science of PET biodegradation, we are not only moving closer to addressing the environmental impacts of plastic wastes but also contributing to the development of more sustainable and efficient recycling technologies.

In other word, while the enzymatic degradation of PET shows immense promise, it requires continued innovation and collaboration between researchers, industries, and policymakers to realize its full potential. As we move towards a more sustainable future, the integration of enzymatic bioremediation techniques into global plastic waste management systems could play a pivotal role in reducing plastic pollution and fostering a more circular and sustainable plastic economy.

Recommendations

Avoid reusing single-use PET bottles, especially for hot liquids. Store PET containers away from heat and direct sunlight. Opt for alternatives like glass or stainless steel, especially for food and beverages and support and push for biodegradable or refillable packaging systems.

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