Scientific Research Publishing

Open Journal of Applied Sciences, 2026, 16(5), 1916-1939
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Enzymatic Biorecycling of Polyethylene Terephthalate (PET): Mechanisms, Microbial Resources, and Protein Engineering Strategies

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How to cite this paper: Meza-Puebla, R.N., Mulia-Rodríguez, J., Velázquez-Rodríguez, A. and Osorio-González, D. (2026) Enzymatic Biorecycling of Polyethylene Terephthalate (PET): Mechanisms, Microbial Resources, and Protein Engineering Strategies. *Open Journal of Applied Sciences*, **16**, 1916-1939.
https://doi.org/10.4236/ojapps.2026.165106

Received: April 17, 2026
Accepted: May 24, 2026
Published: May 27, 2026

Abstract

Polyethylene terephthalate (PET) is one of the most extensively used synthetic polymers worldwide due to its favorable mechanical strength, transparency, chemical resistance, and low manufacturing cost. However, its persistence in natural environments and the continuous increase in global plastic production have intensified the need for sustainable recycling technologies. Conventional PET recycling routes, including mechanical and chemical processing, remain constrained by polymer quality loss, high energy demand, and significant environmental burdens. In this context, enzymatic biorecycling has emerged as a promising alternative based on selective depolymerization under mild operating conditions. PET-hydrolyzing enzymes such as PETases, cutinases, lipases, and related polyester hydrolases catalyze the cleavage of ester bonds, releasing terephthalic acid (TPA), ethylene glycol (EG), bis (2-hydroxyethyl) terephthalate (BHET), and mono (2-hydroxyethyl) terephthalate (MHET), which can be recovered and reintroduced into manufacturing value chains. Recent studies have demonstrated substantial progress in the discovery of microbial degraders, metagenomic screening, structural biology, and computational protein engineering. Machine learning, directed evolution, rational design, and semi-rational mutagenesis have significantly improved enzyme thermostability, catalytic turnover, and tolerance to industrially relevant conditions. Nevertheless, several challenges remain, including PET crystallinity, substrate heterogeneity, additive interference, product inhibition, and scale-up limitations. This review summarizes the physicochemical basis of PET recalcitrance, the diversity of PET-degrading microorganisms, catalytic mechanisms of enzymatic depolymerization, and state-of-the-art enzyme engineering approaches driving next-generation PET circularity technologies.

Keywords

Polyethylene Terephthalate (PET), Enzymatic Depolymerization, Biorecycling, Protein Engineering

1. Introduction

Plastic materials have become indispensable in modern society because of their low cost, versatility, durability, and scalability. However, the same physicochemical properties that make synthetic polymers commercially valuable also make them highly persistent environmental contaminants. Unlike naturally occurring polymers, most petroleum-derived plastics are poorly integrated into biogeochemical cycles, resulting in long residence times in terrestrial and aquatic ecosystems and increasing risks for human and environmental health [1] [2].

Among commercial plastics, polyethylene terephthalate (PET) is particularly relevant because of its widespread use in beverage bottles, food packaging, textiles, medical containers, and engineering materials. PET combines high tensile strength, transparency, dimensional stability, and chemical resistance, which explains its dominant market position. Nevertheless, these same characteristics also hinder natural degradation and complicate end-of-life management [3] [4].

Global plastic waste generation has reached hundreds of millions of metric tons annually and is projected to continue increasing under current production and consumption trends. International policy scenarios indicate that unmanaged plastic dispersal may intensify significantly by 2060 unless systemic interventions are implemented [5]. Consequently, governments and industries are increasingly adopting recycled-content targets and circular economy strategies, especially for PET-based packaging streams.

Current PET recycling technologies are mainly divided into mechanical and chemical routes. Mechanical recycling preserves the polymer backbone but often reduces material quality due to thermal history, chain scission, discoloration, and crystallinity changes after repeated processing cycles. Chemical recycling can recover monomers with high purity through glycolysis, methanolysis, hydrolysis, or related treatments, but these processes usually require high temperatures, aggressive reagents, and substantial energy inputs [6] [7].

Biotechnological approaches have therefore gained strong attention as lower-impact alternatives. Enzymatic depolymerization uses highly selective biocatalysts to hydrolyze PET ester bonds under comparatively mild conditions, enabling recovery of terephthalic acid (TPA) and ethylene glycol (EG), the original monomeric building blocks of the polymer. This strategy offers compatibility with circular manufacturing models while potentially reducing greenhouse gas emissions and process severity [8] [9].

A major milestone in the field was the discovery of *Ideonella sakaiensis*, a bac-

terium capable of using PET as a carbon source through the coordinated action of PETase and MHETase enzymes. Since then, a broad diversity of PET-active enzymes has been identified from bacteria, fungi, compost microbiomes, marine systems, and metagenomic datasets. These include cutinases from *Thermobifida*, thermostable polyesterases, fungal lipases, and engineered variants with substantially enhanced performance [10]-[12].

Catalytic performance is strongly influenced by substrate morphology. Amorphous PET is generally more accessible to enzymes than highly crystalline PET, while additives, dyes, multilayer packaging, and mixed waste streams further reduce process efficiency. Product inhibition by intermediates such as MHET can also limit depolymerization rates. These factors explain why enzyme discovery alone is insufficient for industrial deployment [11] [13].

To overcome these barriers, protein engineering has become a central driver of innovation. Directed evolution, rational mutagenesis, semi-rational redesign, molecular dynamics, and machine learning have generated next-generation enzymes such as HotPETase, Fast-PETase, DuraPETase, TurboPETase, and optimized LCC variants capable of near-complete PET depolymerization under industrially relevant conditions [14]-[18].

This review discusses the physicochemical properties that determine PET recalcitrance, the diversity of PET-degrading organisms, catalytic mechanisms of enzymatic hydrolysis, current enzyme engineering strategies, and the remaining technical bottlenecks for large-scale implementation of enzymatic PET biorecycling.

2. Physicochemical Analysis of the PET Molecule

Polyethylene terephthalate (PET) is an organic molecule produced by the esterification of terephthalic acid and ethylene glycol, which forms the monomers and dimers of bis (2-hydroxyethyl) terephthalate (BHET). Subsequently, through a polycondensation reaction, BHET is polymerized into long PET chains. This material belongs to the polyester group because its monomers contain an ester functional group. It also possesses physical and chemical properties that make it a material of high technological value, particularly because its glassy form, which has less organized and more flexible fibers than its crystalline counterpart, is highly valuable for the food industry [19].

The material itself shows chemical resistance to substances that would damage the composition of other materials, such as acids, bases, and solvents, making it an ideal candidate for packaging, pharmaceutical and food containers, and for the manufacture of devices that require above-average durability and resistance at a low production cost. Physically, PET can be used as thermoformed sheets to construct containers or extruded to form fibers, which are then used to make fabric commonly referred to as polyester. This makes PET an extremely versatile material. In addition, it has considerable temperature resistance, being able to withstand up to 70˚C in its crystalline form and 50˚C in its glassy form without losing

its mechanical properties [3].

These same physicochemical properties that confer resistance, flexibility, and durability at low cost also make PET a persistent emerging contaminant in ecosystems due to its widespread use and negligent handling. It has become the second most discarded plastic material, and efforts to reincorporate it through recycling have proven insufficient [4].

PET as a material is composed of units of the polymer it composed them, but the arrangement of this chains determine physical and chemical properties of interest in in the process of recycling, on general the proportion of crystalline and amorphous parts are vital to determine the quality of the material for a determinate purpose, the amorphous part being a mobile fraction giving the material transparency and flexibility, in the other hand crystallinity is the fraction of the material aligned coherently in a way they interfere with light and disperse it, giving the material an opaque coloration and in comparation with the amorphous part is less flexible and more fragile [20].

This proportion has an importance when the glass transition temperature (Tg) of PET enters in the process being approximately 70˚C, at this temperature polymers changes from a brittle an ordered alignment to a rubber and viscous state the latter being vital for the PET industrial application because this is the way in how industry create bottles and other recipients; therefore increasing the temperature above that increase the proportion of crystallized Pet in the mixture each time making this process unsuitable for continuous recycling. In the other hand enzymatic recycling have an increase opportunity of catalysing reaction exactly around and above the Tg of PET, this happens because the enzymes utilized to degrade PET can be designed to operate at that temperature where the chain of amorphous pet unfolds and became more accessible for catalytic reactions [21] [22].

3. PET Recycling

At present, PET recycling follows two main routes. In the first, clean and separated material is treated with heat to return it to a semi-liquid state and reshape it, a process commonly referred to as mechanical recycling. This route is highly effective, but it is limited by the process itself, since repeated thermal treatment increases the proportion of crystalline PET in the recycled product, resulting in inferior mechanical and optical properties compared with the original material. A second recycling route involves chemical treatments using acids, strong bases, high temperature, and pressure to depolymerize the plastic into its constituent components, allowing it to be reintroduced into production as new material, although at a high ecological cost [6].

Current technological advances aimed at mitigating the damage caused by PET also include its conversion into diesel-like fuels that may be used directly in industry. However, this solution is not optimal because of the associated carbon

emissions and the loss of PET as a valuable material. Other alternatives include the complete degradation of PET into compounds that can be naturally assimilated, which addresses the ecological perspective but also implies the loss of the material itself [23].

It should be noted that although the methodologies currently used for PET recycling are diverse and many involve considerable environmental risk, they are not the only processes through which PET may be returned to circulation. Trophic chains themselves may also play an important role in their transformation. Although this material was once considered essentially non-recyclable, with estimated reincorporation times of decades to centuries, that view is only partially correct because it does not take into account the ability of living organisms to convert this synthetic material into a source of sustenance. Over the last two decades, this capacity has been reported in different research groups around the world in taxonomic groups such as algae, bacteria, and fungi [3].

4. PET-Biodegrading Organisms

The capacity of living organisms to transform PET has significantly changed the perception of synthetic plastics as completely inert materials. Several biological systems are capable of colonizing PET surfaces, secreting extracellular enzymes, and converting the polymer into smaller molecules that may subsequently enter into their metabolic pathways. The main groups reported to participate in this process are bacteria, fungi, and algae.

4.1. Bacteria

The bacterial ability to depolymerize polymers is well known in more labile materials such as cellulose and cutin, which are structural and protective components of plants. These substrates were once considered highly resistant from the evolutive point of view, much as synthetic plastics were initially thought to be but now are degraded by these microorganisms.

Bacterial has one of the most solid pieces of evidence of degradation of polymers including PET in which is possible define two approaches of this mechanism happening, only after the colonization of the surface of the material and the other directly affecting the material.

The degradation process begins with colonization, which occurs when bacteria adhere to the surface of the material and secrete enzymes that facilitate its breakdown. This process can be understood in two stages. First, the affinity of the substrate surface for water is increased through the action of enzymes such as carboxylases, lipases, and cutinases acting on the PET surface this increase hydrophobicity and allow the continuous degradation of the material from the ends to the center of the molecule until complete the depolymerization. This preliminary weakening prior to hydrolysis has been reported in *Moesziomyces antarcticus*, whose lipase can convert BHET into MHET and subsequently into TPA and EG. A similar effect has also been described for *Thermomyces lanuginosus*, whose li-

pase, when used in consortium with *Thermobifida fusca* and *Fusarium solani*, enabled the degradation of PET fibers and films [24] [25].

Other processes do not involve a preliminary increase in PET surface hydrophilicity and instead directly attack the ester bonds of the polymer. This is the case for cutinases, which directly hydrolyze PET into water-soluble fragments such as BHET and MHET. Examples include cutinases from *T. fusca* and *F. solani*, as well as leaf-branch compost cutinase (LCC), which is capable of degrading PET near the polymer transition temperature with efficiencies close to 90% [26]. Likewise, the PETase and MHETase system from Ideonella sakaiensis can hydrolyze amorphous PET at room temperature, although with lower efficiency than counterparts better adapted to elevated temperatures [13] [14].

4.2. Fungi

Among fungi, *Aspergillus oryzae* has been reported to possess a lipase capable of weakening the plastic surface in polyester fabric considering a step similar of the found-on bacteria. When combined with ethylene glycol, this enzyme increases PET affinity for water and facilitates degradation by reducing the contact angle with water facilitating the PET degradation reported [27]. Other examples such as *Thermomyces insolens* also show behaviors similar to those described in bacteria when interacting with the material, either by changing its hydrophobicity.

On the other hand, fungi of the family Fusarium show capaibilities of degradate directly the PET by directly attacking ester bonds when their enzymes are modified. A notable difference is that fungal enzyme variants may have greater resilience to adverse conditions in comparison with other organisms [28].

4.3. Algae and Photosynthetic Systems

More recently, the use of chlorophytic algae for PET treatment has shown promising progress. Organisms capable of attacking this plastic have been reported when the material is previously treated with ultraviolet radiation, extreme pH conditions, or heat. However, although these approaches can demonstrate material deterioration, they have not yet provided definitive evidence of complete chemical degradation [29].

4.4. Environmental Consortia

Mixed microbial communities often outperform isolated strains because they combine complementary metabolic and catalytic functions. Compost, marine, and wastewater consortia have shown synergistic PET depolymerization and assimilation of PET-derived intermediates in which have been found capable of complete depolymerization who can be implemented on bioreactors or their enzymes integrated on other biological systems.

Representative PET-degrading biological systems and their associated enzyme classes are summarized, see **Table 1**.

Table 1. Reported microorganisms with potential PET-degrading activity, including bacterial, fungal, algal, and microbial consortium systems. Listed enzymes correspond to polyester hydrolases, cutinases, esterases, lipases, PETases, MHETases, or related catalysts described as participating in PET hydrolysis, surface modification, or depolymerization.

Microorganism	Enzyme	Degradation process	Ref.
Aspergillus oryzae	—	CD/SM	[27]
Streptomyces sp.	SM14est	CD/SM	[30]
Thermomyces insolens	HiC; PmC; FsC	CD/SM	[24]
Fusarium oxysporum	LCH;1	CD	[31]
Fusarium solani	FsC	CD	[24] [32]
Penicillium citrinum	—	CD/SM	[33]
Ideonella sakaiensis	IsMHETase; IsPETase	CD/HE	[13] [14] [34]
Alteromonas macleodii	—	NCD/HE	[35]
Thermobifida fusca	Thf42_Cut1; TfH; BTA2; Tfu_0882; TfCut1; TfCut2	CD/HE	[36]-[39]
Thermobifida halotolerans	Thh_Est	CD/SM	[34] [40]
Celeribacter neptunius	—	CD	[37]
Oleispira antarctica	PET5	CD/HE	[23] [37]
Pseudoalteromonas citrea	—	CD/HE	[23]
Pseudomonas sp.	jmPE13; jmPE14	CD/HE	[41]
Pseudomonas stutzeri	PsM1	NCD/HE/PT	[42]
Candida antarctica	CALB	CD/HE	[43]
Bacillus subtilis	BsEstB	CD	[44]
Saccharomonospora viridis	Cut190	CD/HE	[45]
Thermobifida cellulosilytica	Thc_Cut1; Thc_Cut2	CD/HE	[34]-[36]
Thermobifida alba	Tha_Cut1	CD/HE	[34]
Acidovorax delafieldii	AdCut	CD	[46]
Moraxella sp.	MoPE	CD	[47]
Deinococcus maricopensis	DmPETase	CD	[48]
Moesziomyces antarcticus	CALB	CD/HE	[25]
Thermomonospora curvata	Tcur0390; Tcur1278	CD	[49]
Vibrio gazogenes	PET6	CD	[23]
Polyangium brachysporum	PET12	CD	[23]
Chlamydomonas reinhardtii	—	NCD/HE/PT	[50] [51]
Leaf-branch compost	LCC	CD/SM/HE	[52]
Marine microbial consortium	Ple629	CD/HE	[12] [53]

CD: Confirmed degradation, NCD: Not experimental confirmed degradation, SM: PET Surface modification, HE: enzyme used in heterologous expression, PT: Used as pretreatment for degradation.

5. Bioremediation and Biorecycling of PET

Biodegradation is a biological process in which living organisms or their enzymes are used to reduce, eliminate, or transform contaminants present in the environment to reincorporate them into the biogeochemical cycles Bioremediation consists of the utilization of microorganisms, plants, animals, and enzymes to neutralize or eliminate contaminants, transforming them into innocuous o less toxic substances. Biorecycling takes the approximation of bioremediation of using living organisms or enzymes but with the difference of the goal to recuperate substances of interest from the process and use it to create a new product of value [2].

In the specific case of polyethylene terephthalate (PET), this process represents a promising strategy, because it allows the treatment of this highly persistent synthetic polymer through biological systems who capable of attacking their ester bonds and generating simpler compounds that may later be assimilated and incorporated to the environment with less impact (Biodegradation) or recovered for further use and the creation of products based on the building block recovered from this substance (Biorecycling).

Likewise, biorecycling extends this concept beyond contaminant removal. Instead of considering PET waste only as an environmental burden, this approach recognizes it as a source of reusable carbon and chemical building blocks. Through microbial metabolism or enzymatic depolymerization, PET-derived compounds may be reincorporated into productive chains, reducing dependence on virgin petrochemical resources and supporting circular economy models (see **Table 2**).

Different strategies have been proposed for PET bioremediation and biorecycling, including the use of purified enzymes, whole-cell biocatalysts, engineered microorganisms, microbial consortia, and hybrid systems combined with physicochemical pretreatments to improve substrate accessibility and catalytic efficiency.

Among the microorganisms capable of degrading PET, the enzymes identified to date share a common feature: their ability to degrade the cuticle of plant cells. This cuticle is a polymeric structure composed of fatty acids linked by ester bonds, which confer water impermeability to plants and function as a physical barrier against microbial attack. In response, many microorganisms have evolved enzymes specifically adapted to cleave the ester bonds that form the cuticular polymer, with varying degrees of efficiency. This same catalytic principle explains why cutinases and their evolutionary relatives are also able to degrade PET, since the bonds that hold this polymer together are likewise ester bonds, making them suitable targets for depolymerization and subsequent biological assimilation as an energy source. From a biotechnological perspective, this property enables PET degradation at a substantially lower chemical and energetic cost than more aggressive methods such as pyrolysis or alkaline hydrolysis [9].

Despite their great potential, industrial implementation still faces important challenges related to catalytic efficiency, operating costs, process scale-up, and the heterogeneity of plastic waste streams [54].

Table 2. Table type styles (Table caption is indispensable). Representative enzymes involved in the biodegradation of synthetic and biobased plastics. The table summarizes major catalytic classes and their proposed mechanisms of action for the cleavage, oxidation, or depolymerization of polymeric substrates such as PHA, PHB, PE, PET, and PCL.

Plastic	Enzyme type	Mechanism of action	Ref.
PHA	Hydrolase	Through the action of extracellular hydrolases, polyhydroxyalkanoate can be converted into its monomers. These products may subsequently be assimilated by the cell through β-oxidation pathways.	[55]
PHB	Hydrolase	Similar to PHA hydrolases are able to depolymerize polyhydroxybutyrate into hydroxybutyric acid, which can enter the Krebs cycle for energy generation.	[55]
PE	Amylase	Polyethylene is not readily degradable under normal conditions; however, when starch is incorporated during production, microorganisms may degrade the material through amylase activity. Starch hydrolysis leaves shorter polyethylene chains that become more susceptible to further degradation.	[55]
PE	Laccase (Phenol Oxidase)	This enzyme promotes oxidation-reduction cycles on UV-pretreated polyethylene, enabling depolymerization into lower molecular weight fragments that can then undergo further oxidation into metabolizable products.	[56]
PE	Alkane Hydrolase	The enzyme acts by cleaving terminal bonds in the ethylene chain through hydration and hydroxyl group insertion. Subsequent reactions convert the products into organic acids that may be utilized through β-oxidation.	[57]
PE	Monooxygenase	Monooxygenases introduce hydroxyl groups into internal regions of the ethylene chain, followed by oxidation steps that convert terminal groups into aldehydes and later into carboxylic acids. Final products can be metabolized through β-oxidation.	[58]
PET	Esterase	Enzymatic hydrolysis of PET ester bonds generates a mixture of terephthalic acid (TPA) and ethylene glycol (EG), together with smaller amounts of incomplete hydrolysis products such as BHET and MHET.	[15]
PET	Aromatic Polyesterase	Enzymatic hydrolysis acts on ester bonds associated with the aromatic rings of PET, producing BHET, MHET, and terephthalic acid.	[34]
PCL	Lipase	Lipase acts on the ester bond linking each monomer when the substrate is present at an interface between hydrophilic and hydrophobic phases, releasing 6-hydroxycaproic acid, which may be converted into acetyl-CoA and used for energy generation.	[59]

6. Reaction Mechanism

To implement the modifications required to make enzymatic PET degradation a practical reality and to overcome the intrinsic limitations of current enzymes, it is first necessary to understand the catalytic mechanism by which this process occurs.

PET-active cutinases perform their function by binding the polymer chain in such a way that the substrate aligns with the serine residue located in the active site, while the hydrophobic region of the catalytic pocket interacts with the aromatic ring of the PET chain. A nucleophilic attack then takes place in which the serine residue becomes activated through the histidine of the catalytic triad (Ser-His-Asp/Glu). This attack occurs on the carbonyl carbon of the ester group within the polymer chain. As a consequence, generating an acyl-enzyme intermediate. Subsequently, histidine activates a water molecule that attacks the acylated intermediate, leading to cleavage of the acyl-enzyme bond, release of terephthalic acid, and regeneration of the enzyme so that the catalytic cycle can begin again (see

Figure 1).

This process creates intermediates generated for the brake of the unions in each monomer generating TPA, BHET and MHET being the latest the principal intermediate generated when the reaction occurs. BHET generates when the acylation process attack the ester bond on the PET polymer released on the cut generated by this process and leaving the rest of the polymer into an intermediate state where the rest of the PET chain (MHET part of the molecule) still bonded to enzyme by the serine on the catalytic triad, then the deacylation reaction occur and liberates the MHET (mayor proportion) or the TPA (minor proportion) from the bonding with the Ser on the triad [60].

On the second part of the degradation MHETase transform the MHET into ethylene glycol an terephthalic acid using a similar pathway of acylation and deacylation, during the acylation reaction the a catalitic triad conformed by Ser-His-Asp generate an attack on the carbonyl C of the MHET the alcohol portion of the bond (ethylene glycol) is released and generate an acylated intermediate with the TPA bonded to the Ser, after the process the histidine restore the Ser transferring a proton from a water molecule and releasing TPA. This process is similar to the other cutinase who degrade PET but specific for the MHET molecule [61].

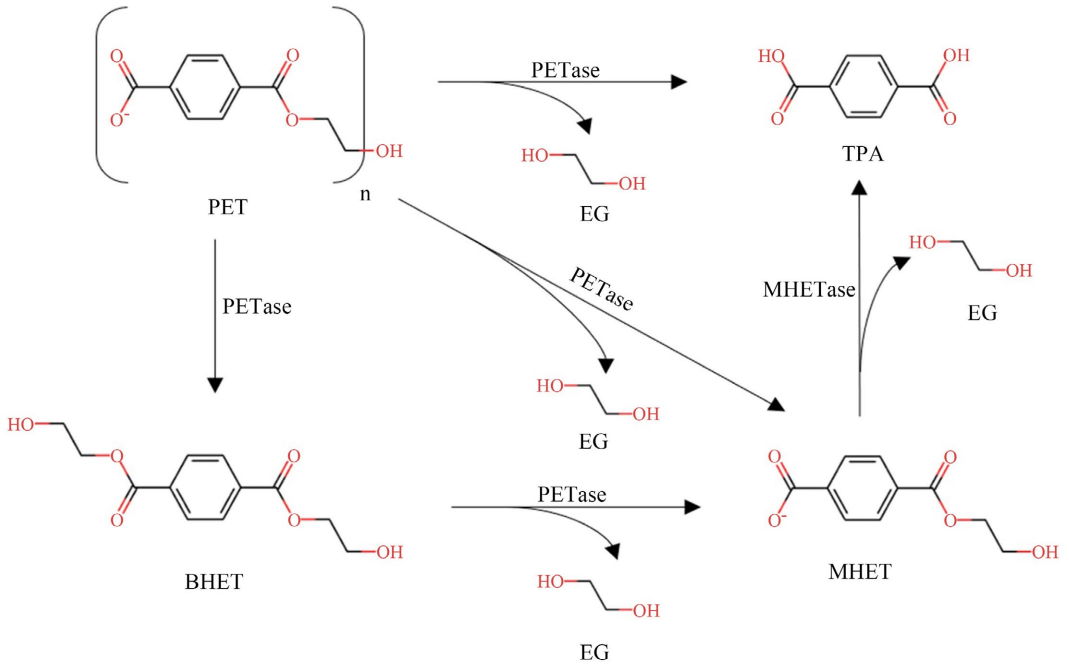

Figure 1. Reaction scheme of PET depolymerization carried out by Ideonella sakaiensis through the coordinated action of PETase and MHETase. PETase hydrolyzes polyethylene ter-ephthalate (PET) into the intermediate products bis (2-hydroxyethyl) terephthalate (BHET) and mono (2-hydroxyethyl) terephthalate (MHET), while MHETase further converts MHET into terephthalic acid (TPA) and ethylene glycol (EG), completing the biological breakdown of the polymer.

During PET degradation into its constitutive components, bis (2-hydroxyethyl) terephthalate (BHET) and mono (2-hydroxyethyl) terephthalate (MHET) are the

principal intermediate products. The former may be directly reused for the synthesis of new virgin PET or may continue along the same enzymatic pathway until complete conversion into terephthalic acid and ethylene glycol. However, MHET represents a major bottleneck because it can slow down or even inhibit enzymatic depolymerization by acting as a competitive intermediate in the reaction system. For this reason, efficient conversion of MHET is essential for achieving long-term viable biorecycling processes, although many of the proposed solutions substantially increase operational costs [62].

Under natural conditions, PET degradation by Ideonella sakaiensis occurs through an extracellular process in which PETase and MHETase act on the polymer surface, generating ethylene glycol, terephthalic acid, and MHET. The first two products are subsequently assimilated by the bacterium: ethylene glycol is converted into glycerate, whereas terephthalic acid is transformed into β-ketoadipate. Both intermediates enter central metabolic pathways and are ultimately funneled into the Krebs cycle as cellular energy sources. At this stage, however, the industrial value of the degradation products is significantly reduced, because once they enter bacterial metabolism, they are further mineralized into CO_2 and water. Consequently, although this pathway is less attractive for raw-material recovery, it still retains substantial potential for environmental bioremediation [63].

7. Enzyme Design as a Proposed Solution

The use of enzymes to carry out degradation processes or to produce valuable substances from specific substrates is a well-established biotechnological strategy that takes advantage of proteins obtained from diverse organisms. However, because these enzymes emerged through natural evolution rather than industrial selection, they are not inherently optimized for manufacturing environments. As a result, substantial efforts in design and optimization are required. Historically, most approaches have focused more on adjusting the industrial process conditions surrounding the enzyme than on redesigning the proteins themselves to better adapt them to production environments [8].

With the increasing characterization of enzymes in databases worldwide, the logical next step has been to use current biochemical, microbiological, and computational tools to adapt these biological resources toward specific objectives. The combination of bioinformatics and molecular biotechnology has therefore enabled the development of numerous mutant proteins capable of degrading PET at higher rates and under more demanding conditions of temperature, pressure, pH, and salinity than their native counterparts. Several engineered enzymes specifically developed for PET degradation have already demonstrated promising laboratory performance.

Bioinformatics plays a central role in this adaptation process. Through molecular simulation, structural prediction, docking analysis, and rational redesign, it is possible to engineer cutinases, hydrolases, and related enzymes with improved catalytic performance under industrially relevant conditions. These strategies also

reduce research costs by decreasing uncertainty during experimental screening and limiting the need for extensive reagent-intensive trial-and-error procedures. Such redesign approaches have generated results that would not be achievable with native enzymes alone, thereby expanding the feasibility of PET biorecycling within practical timeframes [18] [64].

Computationally guided enzyme engineering has already been successfully applied to PET-active proteins and other polymer-degrading systems, creating a growing knowledge base upon which future developments can build. These methods not only reduce costs, but also democratize access to advanced enzyme biotechnology by lowering operational barriers while increasing the probability of obtaining functionally useful variants [65].

The use of mutant proteins derived from known PET-degrading enzymes has also demonstrated that these methodologies can solve substrate-specific challenges. PET exists in multiple structural forms, and an enzyme that performs efficiently on amorphous PET may not function equally well on highly crystalline PET. Temperature is another critical factor, since optimal degradation is frequently sought near the polymer transition region. Likewise, substrate accessibility can be hindered by fiber packing, surface morphology, and polymer entanglement. For these reasons, additives and pretreatment strategies are often combined with enzyme engineering to enhance catalytic efficiency [66].

Current structural studies indicate that many PET-degrading enzymes belong to the α/β-hydrolase superfamily. Their catalytic activity is generally more effective on amorphous PET than on crystalline structures, although the precise mechanism by which surface erosion and polymer accessibility are controlled remains under active investigation [67].

Protein modifications may target multiple objectives either individually or in combination: increasing substrate-binding affinity, improving physicochemical tolerance, enhancing thermostability, introducing accessory domains to optimize substrate interactions, altering hydrophobicity, accelerating turnover, reducing intermediate accumulation, or constructing multifunctional fusion proteins and chimeric systems. Such approaches greatly expand the design space available for next-generation PET biocatalysts [68].

7.1. Major Strategies for Improving PET-Degrading Enzymes

Directed Evolution

When the complete structure-function relationship of an enzyme is unknown, directed evolution provides a powerful alternative. Random mutagenesis, error-prone PCR, or degenerate codon libraries are used to generate large mutant populations. These variants are then subjected to selective pressure, and improved enzymes are repeatedly enriched over multiple cycles, mimicking artificial selection until the desired trait is achieved [69].

Rational and Semi-Rational Protein Engineering

This strategy relies on molecular knowledge of the enzyme structure and cata-

lytic mechanism to introduce targeted modifications at active sites, flexible loops, or substrate-binding regions. Successful examples include IsoPETase variants redesigned using thermostability determinants identified in TfCut2, as well as mutants capable of degrading more crystalline PET, pigmented PET, and PET microparticles. Semi-rational approaches expand this concept by introducing controlled diversity into selected regions followed by screening for superior variants [70].

Machine Learning-Assisted Engineering

When sufficiently large datasets are available, machine learning can identify beneficial mutations that might be overlooked through conventional human analysis. Combined with molecular docking or directed evolution, these methods can generate highly efficient variants from modest starting templates. One notable example is the GRAPE framework, which prioritizes stabilizing mutations while filtering those predicted to be structurally harmful. Experimental validation remains essential, but tools such as AlphaFold2 and RoseTTAFold have greatly expanded the potential of AI-assisted enzyme design for PET degradation [71].

Those techniques have been used to overcome the different bottlenecks who has limited the implementation of PET biorecycling from which thermostability, crystallinity tolerance, enzyme loading, and MHET inhibition are the most prominent.

The thermostability of the enzymes at disposal who limit the operational temperature of the enzymes can be responded with rational engineering and directed evolution introduction mutation who increase the rigidity of the structure allowing to maintain structure on temperature close to Tg (65˚C - 70˚C), the introduction disulfide bonds or replacing heat-sensitive regions with heat resistant version of themselves without compromise the catalytic function is a viable approach with this techniques.

The optimization of the preprocessing of the material also has an important part of the process debilitating the structure at macroscopic level and establishing an ideal point of temperature, concentrations for the enzymes or the addition of metallic ions to increase efficiency and recovery between reactions.

The problem with access to the substrate and crystallinity can be challenged with improved hydrophobic regions on the binding site who can increase the capacity of the enzyme to interact with the substrate and loading with an increased efficiency and is possible redesign the biding site to accept the crystalline conformation part of PET.

Finally, inhibition by MHET can be addressed through protein engineering, by designing enzymes with lower affinity for this substance at its binding site compared to PET, or by creating bifunctional chimeras capable of degrading MHET as soon as it forms.

All bottlenecks have solutions for each case but the implementation of each one for separate with leave the others unsolved or not optimally solved, there is no unique "magic bullet" engineered enzyme who can solve every problem presented, from a methodological perspective, no single engineering strategy has proven uni-

versally superior for complete PET depolymerization. However, available evidence strongly suggests that combining multiple complementary approaches often yields the best overall results.

7.2. PETase as the Main Innovation Model

Metagenomic analyses and laboratory studies have shown that PETase can degrade PET efficiently when enzyme loading is increased to approximately 0.6 mg of enzyme per gram of PET, reaching degradation efficiencies close to 90% after 16 h. The MHET released during the reaction may also be further transformed, generating terephthalic acid and ethylene glycol. These results indicate that PETase-based systems may become viable technologies for PET depolymerization and recycling [72].

One of the most effective solutions proposed for the accumulation of MHET has been the use of a second specialized enzyme: MHETase, first identified in Ideonella sakaiensis. This enzyme hydrolyzes MHET into ethylene glycol and terephthalic acid, thereby completing PET conversion into its fundamental monomers. However, simultaneous industrial use of PETase and MHETase remains challenging because PETase is considerably more temperature-sensitive than MHETase. This limitation has driven the development of fast PETases, thermostable PETases, and engineered MHETases able to operate under shared process conditions, although current combined systems still underperform compared with the most efficient stand-alone PETases [60].

More recently, multifunctional protein systems have been proposed in which several catalytic activities are fused into a single enzyme. These chimeric proteins combine structural elements from different biological sources while maintaining compatibility between domains. Such systems may offer new opportunities to improve catalytic efficiency, increase tolerance to harsh conditions, and fine-tune substrate specificity for future PET biorecycling applications (see **Table 3**).

Table 3. Table type styles (Table caption is indispensable). Engineered enzymes developed for PET depolymerization and biorecycling. Reported variants include catalysts obtained through directed evolution, rational design, semi-rational engineering, machine learning-guided redesign, or process optimization. Maximum operating temperature and degradation efficiency values correspond to the conditions described in the cited studies.

Enzyme	Source organism	Technique	Max. Temperature	Efficiency (%/h)	Ref.
DuraPETase	Ideonella sakaiensis	Machine learning/Directed evolution	37˚C	15%/240 h	[71]
Turbo-PETase	Ideonella sakaiensis	Machine learning	65˚C	99%/10 h	[73]
HotPETase	Ideonella sakaiensis	Directed evolution	82.5˚C	31%/5 h	[16]
Fast-PETase	Ideonella sakaiensis	Mutagenesis	67.4˚C	100%/168 h	[17]
IsoPETase	Ideonella sakaiensis	Protein engineering	40˚C	26%/48 h	[64]
DepoPETase	Ideonella sakaiensis	Directed evolution	50˚C	100%/96 h	[18]
Delta-LCC	Clostridium thermocellum	Semi-rational protein engineering	55˚C	97%/90 h	[74]
LCC (ICCG)	Clostridium thermocellum	Rational protein engineering	72˚C	90%/9.3 h	[26]

Continued

LCC (ICCG_RIP)	Clostridium thermocellum	Rational protein engineering	74˚C	100%/24 h	[75]
LCC (ICCG_16M)	Clostridium thermocellum	Machine learning/Rational protein engineering	72˚C	90%/9.3 h	[76]
HiC	Thermomyces insolens	Process-condition optimization	62.6˚C	22.3%/336 h	[77]
TfCut1/TfCut2	Thermobifida fusca	Directed evolution/Rational protein engineering	65˚C	100%/36 h	[64]
ThcCut1	Thermobifida cellulosilytica	Mutagenesis	70˚C	99%/70 h	[78]
FoCut5a	Fusarium oxysporum	Process-condition optimization	40˚C	6%/18 h	[79]
Mors1	Moraxella sp.	Rational protein engineering	25˚C	60%/24 h	[80]
Cut190	Saccharomonospora viridis	Rational protein engineering	65˚C	95%/96 h	[45]

8. Limitations

Over the last two decades, scientific efforts aimed at discovering, characterizing, and applying biotechnological tools for the development of bioreactors have yielded promising results at the laboratory scale, including the identification of organisms capable of metabolizing plastics to sustain their biological functions. However, these advances remain insufficient for implementation beyond controlled experimental conditions. If meaningful progress is to be achieved, it is not realistic to wait for natural evolution alone to generate enzymes capable of solving the PET problem within an undefined timeframe. Instead, accelerated discovery and engineering strategies are required to obtain solutions within timelines compatible with the urgency of the current environmental challenge.

One of the major limitations of PET degradation is the ability of enzymes to remain active at the temperatures required to approach the transition region of crystalline PET, typically between 60˚C and 75˚C. At this stage, PET loses its amorphous arrangement and reorganizes into a crystalline structure which, although mechanically stronger, becomes less flexible and more opaque, thereby reducing its commercial value compared with the amorphous form. This creates a major bottleneck because a significant proportion of recycled PET ultimately reaches this state, and there are still insufficient strategies for recovering its raw materials without subjecting it to aggressive chemical treatments involving pressure, temperature, and extreme pH. Enzymatic biodegradation is also constrained because substrate affinity is significantly lower when PET is in a crystalline conformation than when it is amorphous. Conversely, enzymes that perform well on crystalline PET often show limited activity on the amorphous counterpart [10].

PET used in industrial applications exists in two principal forms whose proportions vary according to the intended use: opaque crystalline PET and transparent vitreous or amorphous PET. For efficient recycling, the proportion of crystalline PET should ideally be reduced because this form is substantially more resistant to degradation, to the extent that PETase and other polyester hydrolases may become ineffective against it. Additional variables such as reaction temperature, water absorption, molecular orientation, material topology, and especially crystallinity

must be carefully considered during PET recycling. Among these factors, temperature is both one of the most decisive and one of the most problematic variables, since the optimal operating window is generally above the glass transition temperature but below the temperature at which PET begins to recrystallize (approximately 70˚C) [11].

Another major limitation is that PET waste is commonly mixed with additives that modify its physicochemical and organoleptic properties, making it more flexible, altering its color, or increasing resistance to photodegradation. These additives complicate recycling processes by making the substrate highly heterogeneous for chemical, physical, and biotechnological treatments alike. Consequently, proper separation, cleaning, and pretreatment become just as important as the recycling process itself. This challenge has slowed the development of new technologies because each industrial sector that uses PET introduces additional complexity to an already difficult recycling problem [7].

Nevertheless, the integration of more methodologies in the same process can substantially increase the effectiveness at industrial level and overcome the limitations of each technique can't overcome it is implemented alone. When the industrial process takes into consideration the limitations can generate workflow to compensate handicaps on their technologies and leverage available technology strengths for example: The intake of the PET waste can be classified by their characteristics before be treated, in this way problems related with the mixture of polymers and the additives on the mixture can be avoided preparing specific treatments for each; a pretreatment who uses physical and mild chemical attack to the PET can reduce significantly the crystalline fraction on the material and neutralize the additives when are identified allowing the engineered enzymes act over their best performance substrate either be the amorphous of crystalline fractions to archive higher initial reactions; the design of custom made specialized enzymes who can perform in the controlled mixtures implemented has the potential of degrade PEY efficiently if are made knowing the environmental characteristics they have to tolerate, the same can be used to design enzymes who target crystalline portions in other mixtures or the degradation of intermediates who can halter the degradation process as the MHET, increasing the amount of desired product obtained in each catalysis process; finally the recovery of the monomers can be archived using membrane filtration from the bioreactor collecting the TPA, EG and residual MHET or BHET allowing enzyme recycling, while specialized process can recover the TPA and EG with activated carbon with high purity (>95%) to recycling and up-cycling porpoises, as mentioned there is no magic bullet who can resolve everything.

9. Conclusions

Polyethylene terephthalate (PET) remains one of the most strategically important synthetic polymers because of its favorable mechanical performance, chemical resistance, transparency, and broad industrial applicability. At the same time, these

same properties are directly responsible for their environmental persistence and for the increasing complexity of their end-of-life management. As a result, PET can no longer be addressed exclusively through conventional recycling paradigms; rather, it must be considered within an integrated technological framework that combines materials science, biotechnology, process engineering, and circular economy principles.

Current evidence indicates that enzymatic PET depolymerization is no longer merely a conceptual alternative, but a technically credible route for selective polymer breakdown and monomer recovery. The identification of PET-active microorganisms, the structural characterization of PETases, cutinases, lipases, and related hydrolases, and the elucidation of their catalytic mechanisms have collectively established a robust foundation for the development of biologically driven PET recycling platforms. In particular, the ability of these enzymes to operate under comparatively mild reaction conditions offers a significant advantage over aggressive thermochemical routes, especially when process selectivity and product quality are key considerations.

Nevertheless, the transition from laboratory-scale proof of concept to industrial deployment remains constrained by several critical factors. PET crystallinity, substrate heterogeneity, additive interference, limited enzyme thermostability, mass-transfer restrictions, and inhibition by hydrolysis intermediates continue to impose significant barriers to process efficiency. These limitations underscore that successful PET biorecycling will depend not only on enzyme discovery, but also on the rational integration of pretreatment methods, reactor design, substrate conditioning, and downstream product recovery.

In this context, protein engineering has emerged as the central enabling technology for next-generation PET biocatalysis. Directed evolution, rational and semi-rational design, structural bioinformatics, and machine learning-assisted optimization have already produced enzymes with markedly improved thermal tolerance, catalytic turnover, substrate accessibility, and operational stability. These advances demonstrate that the performance gap between native biological catalysts and industrial process requirements can be narrowed through systematic molecular redesign. Importantly, this engineering paradigm also allows catalytic properties to be tuned according to substrate class, reaction environment, and target application, thereby expanding the practical scope of enzymatic PET conversion.

From a broader technological perspective, the future of PET treatment will likely rely on hybrid processing models in which biological, chemical, and physical strategies are combined rather than treated as mutually exclusive alternatives. Within such systems, enzymatic depolymerization may serve not only as a monomer-recovery tool, but also as a platform for waste valorization, carbon recirculation, and low-impact materials management. Thus, PET biorecycling should be understood not simply as a waste treatment approach, but as a key component of emerging circular manufacturing systems.

Ultimately, the significance of enzymatic PET recycling lies in its capacity to transform a persistent environmental liability into a recoverable resource stream. Continued progress will depend on the coordinated development of enzyme engineering, computational design, techno-economic optimization, and industrial implementation pathways. The evidence reviewed here supports the conclusion that PET biorecycling has moved beyond theoretical feasibility and is now positioned as a realistic and strategically relevant route for sustainable plastic management.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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