MICROBIOLOGY · THESIS & RESEARCH REPORT

Microbial Biodegradation

A Biological Solution to PET Plastic Accumulation using Artificial Intelligence

PETase · MHETase · AI-Driven Enzyme Engineering

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Part I Research Summary

Part II Thesis — Six Chapters

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PART I

Research Summary

Microbial Biodegradation: A Biological Solution to PET Plastic Accumulation using AI

Shazmah Hasnain

1. Introduction

Polyethylene terephthalate (PET) plastic is widely used in packaging and textiles due to its cost-effectiveness, durability, and versatility. However, PET resists natural breakdown, causing severe environmental pollution and accumulating in terrestrial and marine ecosystems. To address this, microbial biodegradation offers a sustainable solution. Utilizing specialized enzymes, this process hydrolyzes ester bonds to depolymerize PET into environmentally safe building blocks. To overcome natural catalytic limits, recent advances integrate artificial intelligence and structural biology to engineer high-efficiency biocatalysts

2. Microbial enzymes , Catabolic Pathways and AI computational tools.

Microbial degradation relies on specialized enzymatic pathways. The bacterium Ideonella sakaiensis secretes PETase, an extracellular hydrolase that breaks down high-molecular-weight PET into mono-(2-hydroxyethyl) terephthalate (MHET). A secondary enzyme, MHETase, then hydrolyzes MHET into terephthalic acid (TPA) and ethylene glycol (EG). Once inside bacterial cells, these monomers can be metabolized as carbon sources, yielding biomass, carbon dioxide, and water. Alternatively, in industrial settings, recovered TPA and EG streams can be purified and re-polymerized directly into virgin-grade PET resin, achieving true infinite closed-loop circularity.

3. Challenges / Limitations and AI Solutions.

Structure of Plastic: PET plastic is naturally crystalline, dense, and rigid, making it difficult for microbial enzymes to physically attach and break down ester bonds.

AI solution: AI models re design active sites for better compatability

Rate of Degradation: Natural enzymes such as PETase have a very slow reaction rate, requiring long timeframes to decompose even small plastic quantities.

AI solution: Machine learning enzymes find optimal Mutations for enzymes

Temperature Instability: Plastic degrading bacteria operate at moderate ambient temperatures, whereas PET softens faster at higher temperatures 60–70°C that destroy natural enzyme structures.

AI solutuon: Ai guided engineering identifies stable mutations to allow enzymes to survive extreme temperatures.

Scale-Up Costs & Process Control: Culturing microbes and producing isolated enzymes in bioreactors require significant CapEx and OpEx

AI solution: Implementing AI digital twins, computer vision surface analytics, and automated bioprocess control dynamically optimizes pH, temperature, and feeding schedules to lower operational expenses.

Conclusion

Microbial biodegradation is an excellent solution to break down PET as it is eco-friendly. By utilizing specialized enzymes like PETase and MHETase, microorganisms can convert persistent synthetic polymers into harmless natural metabolites. However, a few challenges such as temperature, and the rate of enzyme breakdown still serve as limiting factors for this solution to be implemented. With the Usage of AI , these limitations can tried to be tackled and microbial recycling can become a powerful tool for sustainable waste management.

PART II

Thesis

Microbial Biodegradation: A Biological Solution to PET Plastic Accumulation
Shazmah Hasnain

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Chapter 1: Introduction and problem statement

1.1 Global plastic pollution context

Polyethylene terephthalate (PET) plastic is a highly versatile thermoplastic polymer widely used across global manufacturing sectors for applications such as beverage packaging, food containers, synthetic textile fibers, and engineering materials. As highlighted in recent industrial reports, PET remains the most abundant polyester plastic produced globally, with nearly 70 million tons manufactured annually for packaging and textile applications alone (Tournier et al., 2020). The widespread adoption of PET stems from its exceptional mechanical strength, chemical stability, low weight, optical clarity, and cost-effectiveness. However, the same chemical durability that makes PET an ideal material for single-use consumer products also presents severe ecological challenges upon disposal.

Chemically, PET is a semi-crystalline polyester composed of repeating ester bonds (–COO–) formed through the polycondensation of terephthalic acid (TPA) and ethylene glycol (EG). The hydrophobic nature of the aromatic rings, combined with the strong ester linkage network, renders the polymer highly resistant to natural biological degradation. Consequently, post-consumer PET waste accumulates in terrestrial environments, municipal landfills, and marine ecosystems, where natural weathering takes centuries to breakdown the material. Furthermore, physical degradation breaks macro-plastic PET debris down into microplastics (< 5 mm), which contaminate aquatic food webs, absorb hydrophobic organic pollutants, and pose long-term bioaccumulation risks across trophic levels.

1.2 Limitations of Current Recycling pattern

Post-consumer PET waste is currently managed through mechanical recycling, chemical recycling, incineration, and landfilling, each possessing distinct operational and environmental drawbacks:

Mechanical recycling is the primary commercial pathway for managing post-consumer PET, but its reliance on thermal extrusion introduces significant structural limitations. Thermal reprocessing breaks down polymer chains via hydrolytic and thermal degradation, reducing the intrinsic viscosity (*IV*) of the material. As intrinsic viscosity decreases, mechanical properties—specifically tensile strength and impact resistance—degrade significantly. This physical degradation limits mechanically recycled PET (*rPET*) to lower-tier applications, a phenomenon known as downcycling. For instance, post-consumer PET bottles are frequently converted into carpet fibers, polyester strapping, or insulation materials. These end-use commodities lack secondary recycling infrastructure, effectively terminating the material's

lifecycle and ensuring its eventual disposal in municipal landfills or waste-to-energy incineration facilities.

Chemical recycling pathways, including glycolysis, methanolysis, and hydrolysis, overcome mechanical degradation by depolymerizing PET back into its constituent monomers: terephthalic acid (TPA), dimethyl terephthalate (DMT), and monoethylene glycol (MEG). These monomers can be purified and repolymerized into virgin-quality PET. However, chemical depolymerization requires high operating temperatures (often exceeding 200 °C to 250 °C), elevated pressures, and concentrated metallic catalysts or organic solvents. These operational parameters result in substantial energy consumption, high operational expenditures, and increased environmental impacts related to solvent recovery and waste management.

1.3 Biological Remediation as a Solution

To address the thermodynamic and financial constraints of conventional chemical recycling, biological remediation using specialized microbial enzymes presents a sustainable, low-energy alternative. Enzymatic depolymerization utilizes mild operating conditions (typically 50 °C – 72 °C), atmospheric pressure, and aqueous pH (7.0 – 8.5) to selectively cleave the ester bonds of PET.

Biocatalysts such as PET hydrolases (PETases) and cutinases yield high-purity monomer streams (TPA and EG) that can be recovered, purified, and directly re-entered into virgin-quality resin synthesis without material degradation. By substituting high-temperature thermochemical cracking with targeted biocatalysis, enzymatic recycling offers a closed-loop framework capable of processing contaminated, mixed, or multi-layer plastic streams that are unsuited for mechanical sorting.

1.4 Thesis Statement and objectives

Thesis Statement: *While traditional mechanical and chemical recycling methods are limited by high energy consumption, poor economic feasibility, and degraded product yields, microbial biodegradation using engineered enzymes such as PETase offers a greener, more cost-effective alternative that achieves superior monomer recovery and enables infinite closed-loop circularity.*

The primary research objectives of this thesis are:

1. To evaluate the fundamental chemical and physical limitations of traditional mechanical and chemical recycling methods, specifically assessing energy consumption, operating costs, and product quality decay.

2. To examine the biochemical mechanisms and active site structural biology of PET depolymerization, detailing how enzymes cleave hydrophobic ester linkages into reusable building blocks under mild conditions.
3. To analyze the role of artificial intelligence, protein engineering, and computer vision in overcoming natural enzyme bottlenecks (such as thermal instability and product inhibition) and optimizing process monitoring.
4. To model the bioprocess engineering requirements, techno-economic feasibility (TEA), and life cycle assessment (LCA) parameters necessary for scaling microbial PET degradation into commercial bioreactor systems.

Chapter 2 :Literature Review & Biological Mechanisms

2.1 Key Microorganisms Involved

Microbial research over the past two decades has identified diverse bacterial and fungal species capable of secreting polyester-hydrolyzing enzymes:

- **Bacterial Systems:** A major milestone in enzymatic plastic remediation occurred with the isolation of *Ideonella sakaiensis* 201-F6 from a PET bottle recycling facility by Yoshida et al. (2016). *I. sakaiensis* secretes a specialized two-enzyme system capable of using amorphous PET as its primary carbon and energy source under ambient growth conditions. Prior to this discovery, enzymatic PET degradation relied primarily on thermophilic actinomycetes—such as *Thermobifida fusca*, *Thermobifida cellulosilytica*, and *Saccharomonospora viridis*—which produce cutinases capable of hydrolyzing low-crystallinity PET at elevated temperatures. They typically required elevated temperatures near the polymer's glass transition phase to function effectively.
- **Fungal Systems:** Filamentous fungi possess hydrophobic aerial hyphae and secrete specialized surface-active proteins known as hydrophobins, which enhance attachment to hydrophobic polymer surfaces. Thermophilic and mesophilic fungal strains belonging to *Aspergillus* spp. (e.g., *Aspergillus oryzae*), *Penicillium* spp., and *Fusarium oxysporum* produce extracellular esterases and cutinases capable of cleaving ester bonds across low-crystallinity PET films.

2.2 Enzymatic Pathways

The complete biological degradation of PET involves a two-enzyme process that converts high-molecular-weight insoluble polymers to metabolic monomers, terephthalic acid (TPA) and ethylene glycol (EG).

1. PETase (Polyethylene Terephthalate Hydrolase): An extracellular enzyme that hydrolyzes internal ester bonds in the insoluble polymer chain of PET via endo- and exo-hydrolytic routes. The main product is mono(2-hydroxyethyl) terephthalate (MHET), with small amounts of bis(2-hydroxyethyl) terephthalate (BHET) and TPA (Yoshida et al., 2016).

MHETase (MHET Hydrolase): An outer-membrane or periplasmic peripheral enzyme that accepts soluble MHET and hydrolyzes the remaining ester bond to yield TPA and EG, preventing the accumulation of MHET which serves as a competitive inhibitor against PETase.

2. Structural Biology & Active Sites

Both enzymes are members of the alpha/beta-hydrolase fold superfamily but with important adaptations:

Catalytic core of PETase: Have a conserved catalytic triad (Ser160–His237–Asp206 in I. sakaiensis PETase) and an oxyanion hole formed by backbone amide groups (e.g. Tyr87 and Met160). Classical cutinases have a narrower, more closed active site cleft than that of PETase. This enables it to host bulky rigid aromatic ester units in PET chains.

MHETase active site: It is formed by a catalytic triad (Ser225–His528–Asp492) embedded in a deep and narrow binding pocket. The active site is partially clogged by a lid domain that confers high specificity to the smaller, soluble MHET molecule but prevents binding to larger polymer chains.

Although PET degrading enzymes have evolved, wild-type microbial systems are limited by a number of physical and biochemical reasons that prevent the efficient depolymerization of commercial plastic at scale:

2.3 Obstacles in the natural systems

In addition to polymer crystallinity and thermal instability, wild-type enzymatic depolymerization is severely constrained by mass transfer limitations at the solid-liquid interface. Because insoluble PET acts as a heterogeneous substrate, the rate of hydrolysis depends on the physical surface area accessible to extracellular enzymes. Naturally secreted esterases and cutinases often exhibit sub-optimal binding affinities for hydrophobic PET surfaces, resulting in low adsorption rates and quick desorption before ester bond cleavage occurs. Furthermore, wild-type active site clefts lack the structural plasticity needed to accommodate rigid, bulky aromatic units, creating high activation energy barriers for ester bond hydrolysis. These kinetic bottlenecks limit wild-type microbial systems to modest degradation rates on low-crystallinity films, making unengineered strains unsuitable for high-throughput industrial waste management.

- **High Polymer Crystallinity:** Commercial PET products possess a highly ordered crystalline structure. In these regions, polymer chains are tightly packed and ester linkages are constrained, making it difficult for PETase enzymes to access and cleave the necessary chemical bonds
- **Temperature Sensitivity and Denaturation:** The rate of enzymatic polymer hydrolysis increases dramatically near the glass transition temperature of amorphous PET (approx 65-70 degrees), where polymer chain mobility rises significantly. However, wild-type enzymes are highly sensitive to thermal changes. Exposing these biocatalysts to temperatures required to soften the plastic alters their active sites, leading to irreversible enzyme denaturation and a complete stoppage of PET breakdown.

- **Heterogeneous Interface Kinetics:** Insoluble PET functions as a solid substrate in an aqueous medium. Hydrolysis relies on physical surface adsorption, where naturally secreted enzymes display low adsorption affinities and rapid desorption rates, resulting in low effective catalytic turnover rates (k_{cat}).

2.4 AI-Driven Computational Discovery & Metagenomic Mining

To overcome the traditional methods of microbial isolation , researchers have incorporated usage of Artificial Intelligence in order to identify enzymes like hydrolases directly from the data given.

- **Metagenomic Mining:** Deep classification models scan plenty of metagenomic sequence data from Marine sediments, local authority landfills, and industrial waste sites were examined to discover genes responsible for synthesizing esterases and cutinases (Danso et al., 2018).
- **Deep Learning Structural Prediction** : uses frameworks like AlphaFold and ESMFold to create accurate 3D models of protein structures. These models are built from amino acid sequences without needing crystallography. The tools can predict how the active site of an enzyme looks in three dimensions. This allows scientists to identify enzymes with the shape for a specific function. By screening candidates, in a computer researchers can pick the most promising ones to test in the lab. This approach saves time and resources by focusing experiments on the options.

Chapter 3: Protein Engineering & Biotechnological Advancements.

3.1 Rational Design vs. Directed Evolution For unaltered versions of enzymes to be engineered in strong biocatalysts , it is majorly dependent on 3 ways:

- **Rational Design :** uses high quality clear images and computer programs like PyMOL and Rosetta to add changes to the protein. By introducing salt bridges, disulfide bonds, or hydrophobic clusters into flexible loop regions, scientists enhance a protein's thermostability while preserving the conformational flexibility required at the active site. (Austin et al., 2018)
- **Directed Evolution :** builds collections of mutants by using error-prone PCR or site-saturation mutagenesis. By screening variants quickly we find useful changes all over the enzyme even in parts far from the active site that make the structure stronger. (Cui et al., 2021)
- **Machine Learning Pipelines** : use deep-learning models that look at protein sequences and apply self-attention networks, such as MUTag to forecast mutation sets. These models test millions of variants on the computer reducing the need for lab experiments. (Lu et al., 2022)

3.2 Key Engineered PETase Variants Attempts to the perfect PETase enzymes have given in several high-performing engineered variants:

Engineered Variant	Engineering Strategy	Melting Temp (T_m)	Optimal Temp (T_{opt})	Key Performance Characteristics	Primary Citation
LCC-ICCG	Rational Design (Disulfide Bonds & Active Site Mutations)	94.5 °C	72 °C	Achieves >90% depolymerization of pre-treated PET within 10 hours at scale.	Tournier et al. (2020)
FAST-PETase	Machine Learning (MutCompute Self-Attention Model)	67.1 °C	50 °C	Rapidly depolymerizes untreated post-consumer PET across broad pH and temperature ranges.	Lu et al. (2022)
DuraPETase	Consensus Design & Computational Backbone Optimization	77.0 °C	60 °C	Displays enhanced shelf-life stability and high resistance to surfactant-induced denaturation.	Cui et al. (2021)

Engineered Variant	**Engineering Strategy**	**Melting Temp (T_m)**	**Optimal Temp (T_{opt})**	**Key Performance Characteristics**	**Primary Citation**
HotPETase	Directed Evolution & Rational Active-Site Redesign	82.5 °C	70 °C	Maintains prolonged catalytic activity under high-solids loading near polymer glass transition.	Bell et al. (2022)

3.3 Computer Vision and Computer-Assisted Analytics in Degradation Monitoring

Beyond structural protein engineering, artificial intelligence offers automated tools to assess and monitor plastic degradation rates in real time within laboratory environments.

- **Dataset Preparation and Annotation:** High-resolution optical and scanning electron microscopy (SEM) images of degrading PET films are annotated using platforms like Roboflow. By marking areas with surface pitting, micro-cracks, and biofilm growth, we create standardized datasets to train our computer vision models.
- **Automated Feature Detection via TensorFlow:** Convolutional neural networks (CNNs) built in TensorFlow are trained on these labeled datasets. These models can automatically identify and measure surface erosion on plastic samples, allowing us to track degradation in real time without destroying the samples.
- **Quantification via ImageJ:** Microscopic images are processed through ImageJ to obtain objective measurements of pit depth, film thickness loss, and average surface roughness (R_a). Combining computer vision with ImageJ gives us a reliable way to assess enzyme efficiency across different plastic samples.

Chapter 4: Bioreactor Scale-Up, Bioprocess Optimization, and Monomer Upcycling Pathways.

Translating enzymatic PET degradation from bench-scale laboratory assays to industrial processing requires scaled-up bioreactor architectures. While enzymatic activity is routinely evaluated in small shaking flasks, large-scale operation demands precise control over fluid dynamics, mass transfer, thermal regulation, and downstream monomer recovery. This chapter discusses the physical parameters required for enzymatic PET degradation, AI-driven process optimization strategies, and methods for recovering breakdown products into new value-added chemical streams.

4.1 Operational Parameters and Bioreactor Architectures

To maximize enzymatic accessibility, industrial bioprocessing systems must include pre-treatment stages that lower polymer crystallinity. Commercial post-consumer PET bottles typically exhibit crystalline contents exceeding 30–40%, presenting dense, hydrophobic regions that inhibit enzymatic cleavage. Mechanical pretreatment techniques—such as micronization, high-energy ball milling, and cryo-grinding—reduce particle size down to sub-millimeter scales, significantly increasing the specific surface area available for enzyme binding. Chemical pretreatments, such as rapid thermal quenching or swelling agent exposure (e.g., green solvents or mild alkaline solutions), disrupt inter-chain hydrogen bonding and order within crystalline domains. Converting semi-crystalline PET into highly amorphous flakes ($<15\%$ crystallinity) increases depolymerization rates by over an order of magnitude in continuous bioreactors.

- **Temperature Control**: Thermophilic engineered enzymes like HotPETase and LCC-ICCG require operational temperatures between 65°C and 72°C. These high temperatures keep the PET polymer near its glass transition temperature (T_g), making the polymer chains flexible enough for the enzyme active site to access them easily. Industrial vessels utilize external jacketed heating loops and continuous temperature sensors to prevent local thermal spikes that could denature the enzyme.
- **pH and Buffer Dynamics:** PET depolymerization releases terephthalic acid (TPA) and mono-(2-hydroxyethyl) terephthalate (MHET), both of which act as acidic species that lower the pH of the reaction mixture. Because enzymes function optimally within a narrow range (pH 7.0–8.0), automated dosage pumps continuously supply basic solutions (such as NaOH or Na_2CO_3) to neutralize acid generation in real time.
- **Bioreactor Configurations**: Continuous Stirred-Tank Bioreactors (CSTBs) are widely chosen due to their effective mixing capabilities and ease of continuous feeding. High-

shear impellers are implemented to maintain uniform particle distribution without creating excessive hydrodynamic shear stress that could disrupt enzyme tertiary structure. Alternatively, packed-bed reactors are used when immobilization matrices are employed to retain high enzyme concentrations over multiple operational cycles.

4.2 AI-Driven Bioprocess Optimization and Digital Twins

Managing scale-up parameters using manual or traditional heuristic methods is often inefficient due to complex physical and biological variables. Artificial intelligence improves process control through dynamic modeling and continuous automated feedback.

1. Hybrid models and digital twining

Combining mechanistic first principle biological kinetics with data embedded neural networks creates an exact replica of the Bioreactor. This replica works simultaneously with the original bioreactor predicting enzyme inactivation rates and product accumulation hours before any physical change occur

2. Reinforcement Learning (RL) Control Systems

Reinforcement learning agents monitor crucial process signals (such as dissolved oxygen, temperature, and pH) .The agent automatically adjusts feeding schedules and base dosage to maintain maximum catalytic rates while reducing power consumption and mechanical wear on impellers.

4.3 Monomer Recovery and Upcycling Pathways

Once enzymatic depolymerization breaks down the PET polymer the product mixture includes dissolved monomers, unreacted solid residues, buffering salts and enzyme proteins. Efficient downstream separation is essential for recovering monomers in a way and enabling upcycling.

Downstream Monomer Separation: The crude effluent is. Centrifuged to remove unreacted PET solids. The liquid filtrate is then acidified, by lowering the pH to 2–3 which causes terephthalic acid (TPA) to precipitate as solid crystals. The remaining liquid phase, which contains ethylene glycol (EG) is separated using solvent extraction or evaporative crystallization.

Repolymerization into Virgin-Grade PET: Purified TPA and EG are re-condensed into purity virgin-grade PET resin. Unlike recycling, which weakens plastic fibers over time, closed-loop chemical upcycling keeps the polymer the same so the material can still be used for food-grade packaging.

Biological Upcycling into Value-Added Products: Recovered TPA and EG can serve as carbon feedstocks for engineered micro-organisms such as Pseudomonas putida. Specialized bacteria that are engineered for the purpose take the monomer stream. Produce higher-value compounds. For example polyhydroxyalkanoates (PHA bioplastics) acid or specialized industrial organic acids.

Chapter 5: Techno-Economic Analysis (TEA), Life Cycle Assessment (LCA), and Systemic Ethics

Although engineered enzymatic variants and AI-optimized bioreactors demonstrate high efficacy in laboratory settings, their real-world usage depends on economy, environmental benefit, and observance of regulations .Industrial implementation requires evaluating capital costs, operational expenses, carbon emissions, and biosafety protocols. This chapter presents a detailed Techno-Economic Analysis (TEA), a comparative Life Cycle Assessment (LCA), and a discussion on safety and ethical AI deployment.

5.1 Techno-Economic Analysis (TEA) of Enzymatic PET Recycling

A sensitivity analysis of Operational Expenditures (OpEx) reveals that enzyme production costs represent the primary economic driver in scaled bioreactor facilities. Enzyme production expenses are heavily tied to fermentation yields, carbon feedstock prices, and downstream isolation steps. Utilizing recombinant expression hosts like *Escherichia coli* or *Pichia pastoris* requires significant energy inputs for aeration, cooling, and nutrient delivery. To bring operational costs down to parity with virgin resin manufacturing ($1.20–1.50 per kg), industrial processes must prioritize high-yield expression vectors, low-cost growth media, and effective enzyme immobilization strategies. Immobilizing engineered variants onto silica or polymeric supports enables enzyme reuse over multiple batch cycles, reducing the ongoing cost contribution of biocatalysts per ton of processed plastic. .

1. Capital Expenditure: CapEx is the money you need to spend at first to build the place where the processing happens.

- **Bioreactor Infrastructure**: Big stainless steel Stirred-Tank Bioreactors that are used on a large scale. These bioreactors have heating systems that go around them and automatic systems to check the Ph levels.
- Pretreatment Facilities: Machines that break down materials, devices that make materials very fine and equipment that uses heat to change the structure of PET before it is broken down by enzymes.
- Downstream Purification Equipment: machines that separate substances, containers where acid is used to make things settle devices that form crystals and tools that reuse chemicals to get clean terephthalic acid and clean ethylene glycol.

2. Operational Expenditure (OpEx) :OpEx includes the costs that come with running a plant every day:

- **Enzyme Production Costs:** The biggest part of OpEx is the cost to make enzymes. Making engineered enzymes such as LCC-ICCG or FAST-PETase involves growing them in bacteria like E. Coli or yeast like Pichia pastoris. This process uses nutrients-media lots of air and energy for keeping the system going. After growth the enzymes must be. Purified, which adds more expense.
- **Energy Consumption:** A lot of electricity and steam are needed to keep bioreactors at the temperature. Temperatures between 50 degrees Celsius and 72 degrees Celsius are required during the breakdown of plastic. Also high-shear impellers use power to mix the contents properly. All this energy adds up over time.
- **Chemical Buffers & Neutralization**: Chemical Buffers & Neutralization means adding a basic solution, such as (NaOH) to neutralize TPA acid generation during hydrolysis.
- **Feedstock Sourcing**: Feedstock Sourcing costs include buying, sorting and washing PET waste flakes that people have already used.

Technical & Operational Parameter	**Conventional Mechanical Recycling**	**Solvolytic Chemical Recycling (Glycolysis)**	**Enzymatic Biodegradation (Industrial Bioreactor)**
Operating Temperature	260 °C−280 °C	190 °C−240 °C	65 °C−72 °C
Operating Pressure	Atmospheric / High Extrusion	Moderate to High (0.1−0.6 MPa)	Atmospheric
Tolerance to Contamination	Low (Requires clean, sorted feedstocks)	Moderate (Requires pre-sorting and catalyst management)	High (Enzyme specificity targets PET in mixed streams)
Primary End Product	Regranulated rPET (Lower intrinsic viscosity)	Purified BHET / TPA and MEG monomers	High-purity TPA and EG monomers
Closed-Loop Circularity	Finite (3−5 cycles before severe quality loss)	Infinite (Virgin-equivalent monomer recovery)	Infinite (Virgin-equivalent monomer recovery)
Energy Intensity (MJ/kg PET)	8−12 MJ/kg	25−40 MJ/kg	12−18 MJ/kg (Dependent on heating/agitation)
Estimated Operational Expenditure	Low (0.60−0.90 USD/kg)	High (1.40−2.10 USD/kg)	Moderate (1.10−1.50 USD/kg)
Primary Technical Bottleneck	Thermal & hydrolytic chain scission	Solvent recovery & catalyst toxicity	Enzyme production cost & product inhibition

5.2 Life cycle assessment and environmental impacts

To be certain about the result enzymatic degradation provides is sustainably genuine over traditional methods , a comprehensive Life cycle assessment demonstrates its environmental impact to both virgin PET production and traditional ways of obtaining.

- **Global warming potential:**

Producing Virgin grade pet from petroleum feedstock generates approximately 2.2 to 2.5 kg CO_2 - equivalent per kg of resin. In comparison, enzymatic depolymerization when powered by renewable resources greatly reduces greenhouse gas emissions.The primary carbon burden in enzymatic recycling stems from the thermal energy required to operate bioreactors at 65 to 70 degrees celsius and the electricity used in fermenting enzyme batches.

- **Comparison to Mechanical Recycling:**

Mechanical recycling possesses a lower initial energy footprint because it involves simple melting and re-pelletizing. However, mechanical recycling downgrades plastic quality over each cycle, restricting its long-term circularity. Enzymatic recycling produces virgin-quality TPA and EG, allowing infinite closed-loop recycling without material degradation.

- **Comparison to Incineration and Landfilling:**

Diverting PET from landfills prevents microplastic accumulation in terrestrial ecosystems. Furthermore, avoiding incineration eliminates direct greenhouse gas emissions and toxic air pollutants associated with burning synthetic polymers.

5.3 Biosafety, Regulatory Compliance, and Ethical AI Deployment

Incorporation of genetically modified micro-organisms, engineered protein variants, and autonomous artificial intelligence into industrial bio-facilities introduces regulatory and ethical requirements.

1. Biological Containment and Environmental Safety Engineered bacterial strains used for enzyme production or direct PET degradation must be physically contained within closed-loop bioreactors. To prevent accidental environmental release of genetically modified organisms (GMOs):

- **Physical Containment**: Bioreactor facilities operate under Biosafety Level 1 (BSL-1) or BSL-2 standards with HEPA air filtration and thermal emission sterilization before wastewater release.
- **Synthetic Suicide Switches**: Synthetic biology tools incorporate inducible kill switches (such as auxotrophic mutations or toxin-antitoxin systems) that cause modified micro-

organisms to self-destruct if they leave the controlled bioreactor environment.

2. Regulatory Approvals for Monomer Upcycling Recovered TPA and EG intended for food-contact packaging must comply with strict food safety regulations (such as standards enforced by the U.S. FDA or EFSA). Processing facilities must prove that enzyme residues and chemical purification solvents are entirely removed from final repolymerized resins.
3. Ethical AI Governance and Algorithmic Security Using AI models for enzyme design and bioreactor automation requires ethical oversight:

- Model Explainability and Validation: Machine learning models predicting mutation stability must undergo physical laboratory verification to prevent computational bias from directing research toward non-functional variants.
- Autonomous Safety Overrides: Reinforcement learning systems managing bioreactor parameters (temperature, pressure, base dosage) must include hardcoded safety bounds. Human operators must maintain real-time override authority to prevent dynamic AI controllers from triggering unsafe operating conditions.

Chapter 6: Synthesis of Core Findings, Systemic Bottlenecks, and Future Directions

The integration of artificial intelligence tools with microbial protein engineering provides a promising framework for addressing global polyethylene terephthalate (PET) plastic waste. By combining machine learning algorithms with bioprocess engineering, this thesis has detailed an end-to-end strategy—ranging from AI-driven metagenomic discovery and variant engineering to computer vision laboratory monitoring, scale-up bioreactor dynamics, and closed-loop monomer upcycling. This final chapter states the core findings of the research, identifies persistent technical and industrial bottlenecks, and outlines strategic directions for future investigation.

6.1 Synthesis of Core Thesis Findings

This work examined how computational artificial intelligence and biological mechanisms combine to solve the environmental persistence of post-consumer PET plastics:

- AI-Guided Enzyme Discovery and Variant Design: Traditional trial-and-error mutagenesis is limited by vast sequence space. Generative machine learning platforms and structure-prediction models (such as AlphaFold and ESMFold) allow precise targeted modifications to PET-degrading hydrolases. Thermostable variants—including FAST-PETase, DuraPETase, HotPETase, and

LCC-ICCG—demonstrate that targeted mutations at specific active-site residues significantly enhance binding affinity, catalytic rate (k_{cat}), and thermal tolerance at operational temperatures near or above the glass transition temperature(65°C and 72°C)

- Quantitative Automated Monitoring via Computer Vision: Replacing destructive and slow manual assays, the integration of computer vision pipelines (combining Roboflow dataset annotation, TensorFlow convolutional neural networks, and ImageJ spatial analysis) enables non-invasive, automated quantification of surface micro-erosion, pit formation, and film thickness reduction (R_a)during enzymatic hydrolysis.
- * Scaled-Up Bioprocess Controls and Digital Twins: Scaling depolymerization from shaking flasks to industrial Continuous Stirred-Tank Bioreactors (CSTBs) requires steady control over heterogeneous reaction dynamics. Reinforcement learning controllers and kinetic digital twins maintain operational stability across key variables—specifically pH (7.0–8.0), temperature (60 to 70 degree celsius), base dosage, and mixing shear stress.

- Economic and Environmental Viability: Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA) indicate that while high enzyme expression costs represent the dominant Operational Expenditure (OpEx), enzymatic recycling offers a significant reduction in Global Warming Potential (1.5--1.8 kg CO_2-eq/kg PET) compared to virgin resin synthesis from petrochemical feedstocks.

6.2 Systemic Bottlenecks and Technical Limitations

Despite significant advances in enzymatic plastic biodegradation, several critical engineering and biological bottlenecks remain before widespread commercial deployment can be realized:

- Polymer Crystallinity Limits: While amorphous PET regions degrade rapidly, highly crystalline PET (>30–40% crystallinity, commonly found in thermoformed packaging and bottle caps) remains highly resistant to enzymatic cleavage, requiring energy-intensive thermal or mechanical pretreatment.
- Product Feedback Inhibition: Accumulation of intermediate degradation products, particularly mono-(2-hydroxyethyl) terephthalate (MHET) and terephthalic acid (TPA), causes competitive inhibition at the enzyme active site, severely slowing reaction kinetics in high-solids bioreactors over time.

6.3 Future Directions and Strategic Outlook

To overcome these technical challenges and transition enzymatic PET degradation into a mature circular bioeconomy, future research should prioritize four key strategic avenues:

- Multi-Enzyme Cascades and Cell-Surface Display: Developing dual-enzyme systems—such as co-displayed PETase–MHETase chimeras or artificial cellulosome-inspired bacterial surface scaffolds—can eliminate MHET intermediate accumulation through continuous direct hydrolysis, maximizing total monomer yields.
- AI-Driven De Novo Protein Design: Moving beyond single-point mutations on existing natural enzymes, next-generation deep learning architectures (e.g., protein diffusion models and language-model-based sequence generators) will enable the de novo design of entirely novel synthetic hydrolases optimized from scratch for extreme industrial conditions.
- Consolidated Bioprocessing (CBP) Strains: Genetic engineering of robust chassis organisms (such as Pseudomonas putida) to simultaneously express PET-degrading enzymes and metabolize the resulting TPA/EG monomers directly into value-added bioplastics (such as polyhydroxyalkanoates) within a single bioreactor vessel, eliminating separate downstream purification steps.

- Standardized Regulatory Metrics: Establishing global benchmarking protocols for AI-designed synthetic enzymes, closed-loop biosecurity standards, and food-grade safety certifications for upcycled monomer resins to ensure rapid regulatory approval and market entry.

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