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PETASE/MHETASE BIOCATALYSIS FOR PLASTIC DEGRADATION IN MUNICIPAL WASTEWATER SYSTEMS: OPPORTUNITIES, CHALLENGES, AND FUTURE PROSPECTS

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Abstract

Polyethylene terephthalate (PET) plastic pollution has emerged as a significant environmental problem because it is not biodegradable and because of its harmful tendencies of accumulation in water bodies. Municipal waste water systems are also seen to be critical pathways in the distribution of microplastics, which demonstrates the necessity of novel remediation methods. The critically analyzed information presented in this review indicates that PETase and MHETase enzymatic biocatalysis have potential to be used as a sustainable method of PET degradation in wastewater infrastructure. The synergistic interaction between MHETase to convert intermediates to assailable monomers and PETase to depolymerize plastics is addressed as one of the biochemical control over recycling of plastics. Existing wastewater treatment regimens such as the preliminary, primary and secondary treatment are appraised to find constraints in microplastic removal. The review also examines bioaugmentation techniques, enzyme inhibition aspects and operational issues like hydraulic retention time and matrix related inhibitors that determine catalytic ability. Economic evaluations suggest that enzymatic crushing or recycling of PET can save on energy needs, production of greenhouse gases, and the expenses of producing petroleum-based products than the traditional PET manufacturing. Also, recent in situ remedies like engineered microbial biofilms, dual-enzyme cascade systems, and thermostable hydrolases are discussed as having the potential to enhance the stability of enzymes and catalytic efficiency in dynamic wastewater environments. Although enzyme engineering and bioprocess design have made great progress, large scale application is hampered by environmental variability and system integration issues. The present review highlights the fact that the optimization of enzyme systems, strong microbial consortia, and enhanced reactor designs are required to process laboratory-scale PET biodegradation into the applications of sustainable wastewater treatment.

INTRODUCTION

Water is crucial for life, an essential component of production and a vital resource that encourages the prolonged growth of society and economy (Bai et al., 2025). But the global water security is presently facing significant threats from anthropogenic water pollutants. As the rate of urbanization increases, municipal waste water treatment plants (WWTPs) are now the major source of complex chemical contaminants, particularly microplastics (Shahid & Ali, 2023). For remediating wastewater of a variety of pollutants the

physical, chemical and biological treatment strategies are available. For the increasing issue of microplastic expansion, the enzymatic biodegradation has been recommended as a remediation technique which is ecofriendly, effective and sustainable (Çaloğlu et al., 2023).

An enzyme, PETase that decomposes polyester and has promising capability for use in wastewater treatment (Zurier & Goddard, 2023). The overall world production of polyethylene terephthalate (PET) is predicted to touch 1,800 million tons by 2050, further aggravating the environmental crisis that is estimated to result in over 12,000 million metric tons of plastic waste in the environment Polyethylene terephthalate (PET) is considered to be one of the most common types of polyester plastic used across the world (Shahid & Ali, 2023). This plastic is a synthetic material that is produced on a massive scale to be used as a raw material for various plastic products such as plastic bottles, carpeting, clothing and plastic beverage containers (Taniguchi et al., 2019). Plastic materials that are manufactured in massive quantities include polyethylene terephthalate, accounting for 70% of the total plastic manufactured. Fibers made from PET plastic materials are known to be extremely durable, strong, elasticity and resistant to chemicals. However, the properties of this material make it hard to degrade, thus accumulating in the environment (Shahid & Ali, 2023).

The *Ideonella sakaiensis*, microorganism uses two vital enzymes, PETase and MHETase. These enzymes are required to breakdown PET in two steps through MHET, producing components needed for another round of PET production, the TPA and ethylene glycol (Palm et al., 2019). Polyethylene terephthalate (PET), a non-degradable pollutant, is accumulated in environment, has become a global concern. The plastic products constitute PET have already accumulated in considerable amounts in the environment, causing challenges towards various endangered species ultimately threatening the biodiversity and ecosystem (Maity et al., 2021). This paper critically analyzes the limitations of current wastewater treatment strategies and analyzes PETase/MHETase biodegradation as a putative remedy and to increase treatment productivity.

Synergy between PETase and MHETase enzymatic machinery

PET can be used as carbon source by a bacterium *Ideonella sakaiense* which previously known as PET hydrolase (PETase), a type of enzyme that needs to biodegrade polyethylene terephthalate (PET). PET hydrolase (PETase) catalyzed the initial degradation of polyethylene terephthalate (PET) and soluble intermediates, Monoterephthalate (MHET) and trace levels of bisterephthalate (BHET) are released on the action of PETase in the polymer matrix disintegrates the ester bonds (Burgin et al., 2024). The action of the enzyme MHETase hydrolyze MHET into ethylene glycol and terephthalate Releases MHETase totally to assailable monomers. Structural analysis reveals that this enzyme (MHETase) possesses a hydrolase fold and a lid area, which ensures high selectivity of MHET towards this enzyme (Palm et al., 2019). Breakdown intermediates require MHETase that is elevated during the process of PET metabolism (Tanaka et al., 2024). PETase and MHETase are to co-operate to be effective in PET biodegradation. PETase leads to soluble intermediates and MHETase to the conversion of MHET to ethylene glycol (EG) and terephthalic acid (TPA), which enter microbial metabolism upon the simultaneous presence of PETase and MHETase (Yoshida et al., 2021).

Even though these two enzymes are required to activate efficiently in the assimilation of PET, experimental gene disruption proves that PETase is required to be active in PET hydrolysis (Yoshida et al., 2021). MHET accumulation is capable of inhibiting PETase activity indicating that MHETase is required to continue with depolymerization and prevent feedback inhibition. The enzymatic breakdown of PETs has protein engineering methods used to develop variants of PEPTase that have a higher depolymerization rate, better binding toward substrates, and enhanced thermostability (Yin et al., 2024). Temperature, surface area, product inhibition, and polymer crystallinity influence the efficacy of enzymatic PET breakdown. On the one hand, polymer

chains can be more mobile and enzyme activity can increase with the rise of temperatures, but on the other hand, highly crystallized PET does not suffer as much under enzyme attack (Wei et al., 2024).

Critical insight

The synergistic effect of PETase and MHETase denotes significance of the enzymatic synergy in the biodegradation of PET. Although PETase is a catalyst that triggers the depolymerization of polymer, the presence of the intermediate product of polymer depolymerization, which is MHET, can hinder the catalyst efficiency by taking the form of a form of inhibition that is feedback. MHETase thus is a highly important regulatory mechanism that quick changes MHET to ethylene glycol and terephthalic acid that maintains a consistent PETase activity instead of accumulating intermediates. Although there is promising development of enzyme engineering, which increase the stability of enzymes and their catalytic abilities, there is still difficulty in degrading crystalline PET and in its use at a large scale. To achieve a better recycling of plastics, future studies must be aimed at optimization of enzyme formulations, increasing thermostability, and developing coupled biocatalytic systems that effectively use the PETase and MHETase to recycle plastics.

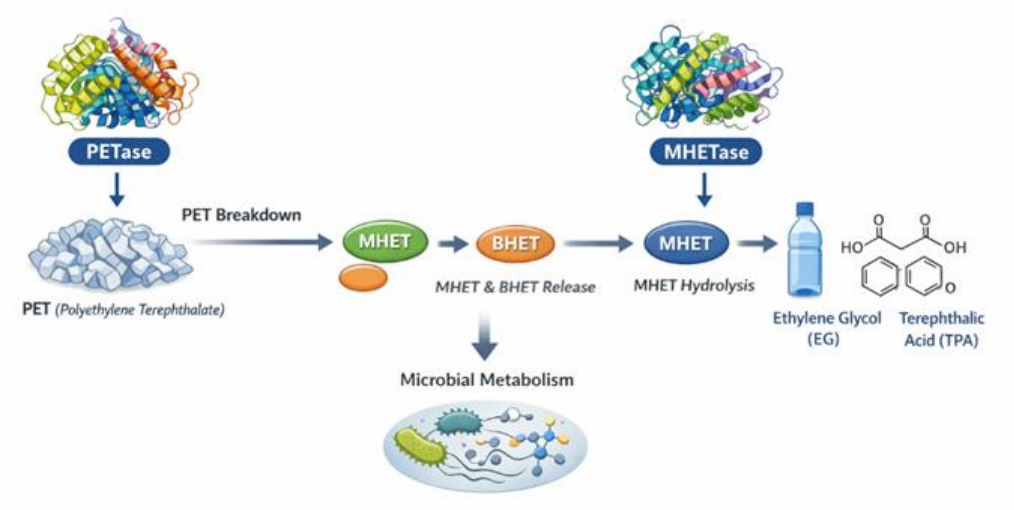

Fig. 01. Synergy between PETase and MHETase in PET degradation

Systematic treatment

The first step to remove large and heavy waste in wastewater treatment is preliminary treatment. This treatment takes place in two steps, first is screening in which large floating substances like rags (~60%), plastic (~5%) and paper (25%) are removed from debris by using screens. Second step is grit removal which mainly removes inorganic particles such as sand, gravel and other heavy particulate matters (bone parts, coffee and corn) through grit channels (Templeton & Butler., 2011) Next step is primary treatment in which suspended solids are separated by sedimentation process. For several hours wastewater effluent is allowed to stay in tanks for the removal of smut from top and sludge from bottom. Around 40% of Biological Oxygen Demand (BOD) and 80-90% of suspended solids and 55% of fecal coliforms is removed by primary treatment. (Kalfa et al., 2020).

In secondary treatment, the primary treated water which contains activated sludge is processed through oxidation ditches, bio filters and moving biological contactors. Municipal wastewater having large amount of organic materials is treated through combination of methods which are discussed above .With the help of large number of thriving microorganisms, most important includes bacteria and protozoa organic contaminants can be removed. Microorganism perform this task through their natural metabolic activity (Verlicchi et al., 2012).

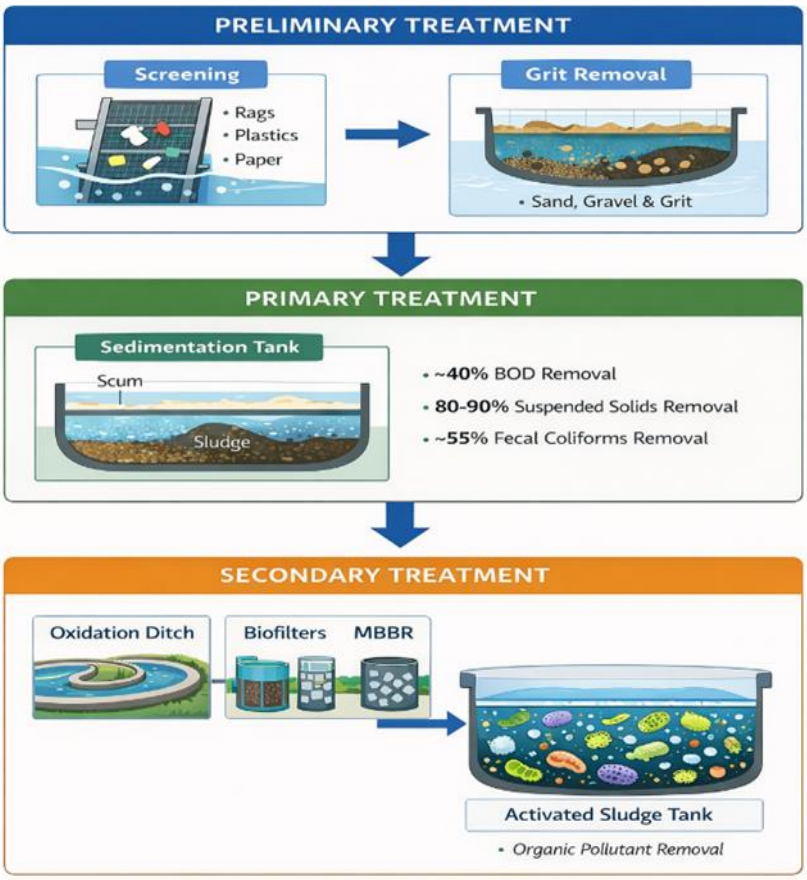

Fig. 02. Treatment methods

Bioaugmentation

This is in situ biological process to remove contamination in polluted environment by using important microbial diversity which has capacity to increase the degradation process. To intensify the pollutant degradation conventional bio augmentation strategies are used. In this strategy free floating microbial cell either as natural strains, bioengineered microbes or vector with particular biodegradation genes are introduced in polluted systems. However, natural strain is mostly used. Mixed consortia is preferred because of exceptional metabolic diversity, stability and plasticity under changing environmental conditions. Thus it increases the validity of bio augmentation strategies. Overall, bio augmentation has become vital method to scale up the biological treatment process. In PET degradation, cutinases, lipases and carboxylases are the vital hydrolytic enzymes. Ester bonds are broken by esterases and target PET surface is modified. Cutinases perform hydrolysis of ester bond in PET and released intermediate product metabolized and degrade the pollution causing agents. (Wahalathanthrige et al., 2026)

Hurdles in treatment

Hydraulic retention time affects the nutrient extraction efficiency, biomass yield and reactivity balance. Shorter hydraulic retention time has negative effect on above mentioned factors due to volumetric loading rates, duration of contact and length of physiochemical cycling during day and night. It also favors the nutrient input and biomass yield; on the other hand, it reduces the wastewater quality. In short hydraulic retention time causes poor waste water treatment and nutrient extraction as compared to longer HRTs. But controversial findings were showed by some other studies. (Thiruchchelvam et al., 2026).

Inhibitory matrix effect is shown by some organic solvents and organic acids (ethanol, methanol acetone, formic acid, acetic acid and oxalic acid). This affect the micropollutant

treatment which is aided by enzymes. These compounds perform competitive inhibition for active site of enzyme. Rearrangement of amino acid structure and lowering enzyme activity is done by fluctuation in PH of these compounds. Another factor is surface active agents. These agents increase the solubilization of compounds. There are two categories of surfactants, non-ionic surfactant and ionic surfactants. Non-ionic surfactants include Triton X-100 and these do not have good effect on structure of enzyme, just hydrophobic interaction occurred. Ionic surfactants like sodium dodecyle sulphate, causes enzyme distortion. Modification in enzyme structure cause less indole removal from waste water when Triton X-100 is present. (Alshabib & Onaizi, 2020).

Critical insight:

The use of hydraulic retention time (HRT) and matrix-associated inhibitors are important factors that determine the efficiency of the biological wastewater treatment systems. Longer HRT will tend to enhance nutrient removal, and biomass productivity by maximum microbial contact and metabolic activity, but the findings in the different studies have not been consistent, indicating that the performance of the system is also dependent on working conditions including the type of the microbial community, and the composition of the influent. Equally, inhibitory effects are introduced by the presence of organic solvents, acids and surfactants such that they may change the enzyme structure and catalytic activity. The ability to bind competitively to enzyme active sites and disrupt the structure as a result of pH changes or ionic surfactants can decrease the efficiency of pollutant degradation. Thus, reduction of wastewater treatment processes involves multidimensional control over the work parameters and structure of the matrix to reduce the level of enzymatic inhibition and preserve the activity of biocatalysts in a stable state.

Economics of PET Recycling

Enzymatic polyethylene terephthalate (PET) depolarization for commercial possibility largely depends on efficiency of enzyme production, pretreatment intensity and requirements of downstream purification. Evaluation based on Techno-economic shows that at industrial scale when the processes are optimized, recycling of Enzymatic PET has the ability to compete economically with virgin PET manufacturing, because it makes purification stages easy and basic by reducing dependency on feedstock derived from fossils (Singh et al., 2021).U.S production cost of virgin PET is approximately $1.87 per kilogram and Industrial-scale modeling suggests that PET produced from recycling of monomers by enzyme action could cost around $1.51 per kilogram and its lower than the U.S production cost. It shows that after the process integration challenges are meet recycling by enzyme action can become financially suitable. (Singh et al., 2021). For assessing the environmental feasibility of pathways like bio catalytic recycling there is a critical parameter which is energy demand. By studying the life cycles of different methods like normal fossil derived TPA production routes and enzymatic PET recycling it is revealed that enzymatic PET recycling can lower the total supply chain energy consumption by approximately 69-83% relative to usual fossil derived TPA production routes. (Singh et al., 2021). Life cycle modeling shows that in comparison with TPA, enzymatic PET recycling can reduce emissions of greenhouse gas by approximately 17% to 43% per kg of the greenhouse gas produced by TPA, but the results are influenced by source of electricity and process configuration (Singh et al., 2021). The catalytic efficiency and thermal stability can be improved greatly by enzyme engineering which directly affect the economic sustainability. Engineered PET hydrolases which have better and improved thermostability shows increased rate of degradation near the glass transition temperature of PET, it decreases time of reaction and requirements for reactor size (Kawai et al., 2024). The recovery efficiency of monomer improved by dual enzyme cascade systems which combines both PETase and MHETase (Lu et al. (2024).

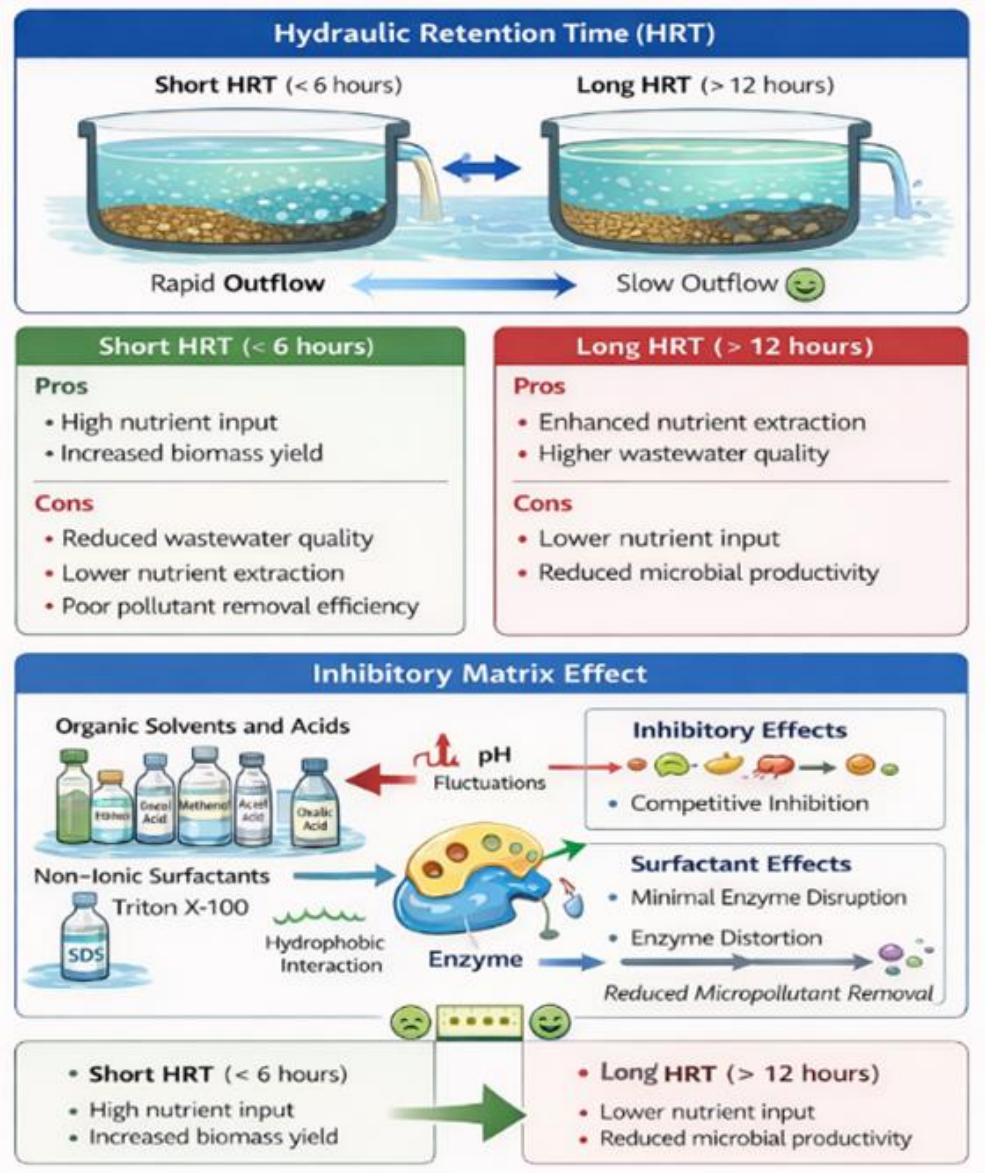

Fig. 03. Factors affecting wastewater treatment efficiency

Table 01: Economic comparison between virgin PET and Enzymatically Recycled PET

Parameter	Virgin PET Production	Enzymatic PET Recycling	Source
Production cost (USD/kg)	~$1.87	~$1.51	Singh et al., 2021
Energy consumption	High (fossil-based)	69–83% lower	Singh et al., 2021
GHG emissions	High	17–43% lower	Singh et al., 2021
Feedstock source	Fossil fuel	Plastic waste	Singh et al., 2021

Critical insight

According to this table enzymatic PET recycling is less costly, consumes less energy and less costly to manufacture greenhouse gas emissions than virgin PET manufacture. Effect on cost and environmental performances in municipal wastewater infrastructure are caused by low plastic concentration, diverse operating conditions, electricity source and stability of enzyme. Hence practical use hinges on the increased stability of enzymes and efficiency of integration of systems.

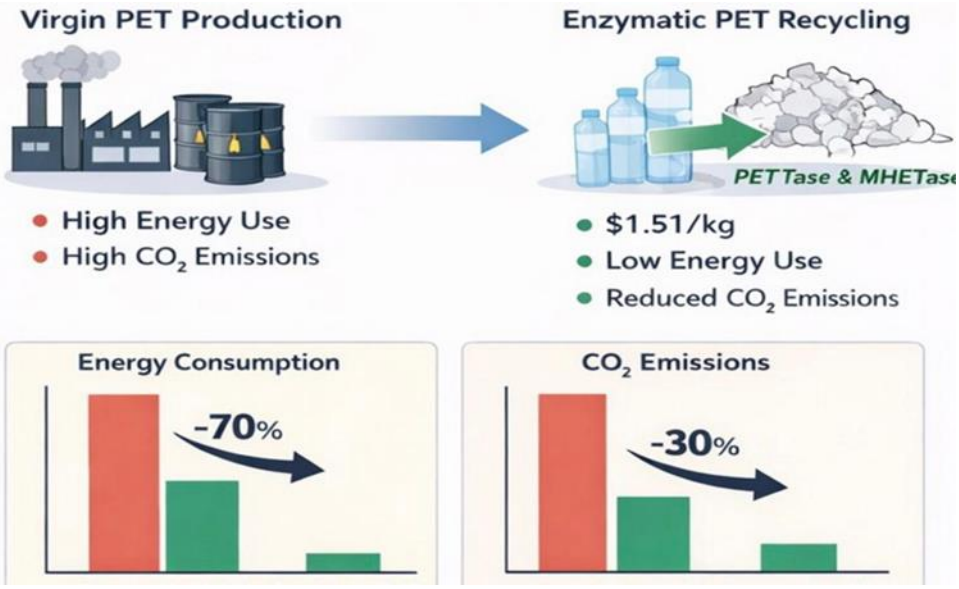

Fig. 04. Enzymatic PET production and recycling

In Situ solutions

In municipal wastewater systems implementing PETase/MHETase bio catalysis directly need strategies that under variable environmental conditions maintains activity of enzyme. For the implementation of plastic depolymerization systems in situ for wastewater infrastructure and contaminated environment engineered microbial biofilms are promising strategy. Enzyme retention, substrate contact, and tolerance to environmental stresses are all improved by biofilms, which offer a structurally stable microbial consortia embedded in extracellular polymeric materials (Flemming et al., 2016). These systems can be made to express PETase/MHETase to express at same time which allows depolymerization in sequence in wastewater channels. Natural microbial consortia which shows greater degrading efficiency as compared to monocultures are modeled by this spatial arrangements of enzyme (Danso et al., 2019). Under different temperature and load of contaminants biofilms provide protection of structure and enhanced activity of catalysis (Flemming et al., 2016). Substrate interactions have been further defined by structure-based insights into MHETase, which allows for logical enzyme optimization (Palm et al., 2019). Continuous degradation performance is improved by combining PETase/MHETase systems which minimizes gathering of inhibitory intermediates (Lu et al., 2024). The use of engineered hydrolases with increased thermostability and operational robustness is of particular promise to be added to sludge digestion units or terrestrial treatment units. (Kawai et al., 2024).

Critical insight

Engineered biofilms and dual-enzyme systems of PETase / MHETase are a promising solution to in-situ depolymerization of plastics in wastewater conditions, but there are a number of convenient drawbacks that can be identified. Environmental factors like temperature, contamination load, and hydraulic dynamic changes can have a robust effect on the functionality of these systems by impacting the expression of enzymes and stability over an extended duration. Also, it is imperative to ensure controlled expression of the two enzymes in microbial consortia to avoid a build-up of the inhibitory chains that might slow down the catalytic performance. The generation of controlled microbial consortia and improved reactor designs will consequently be essential in terms of transferring any laboratory-based biocatalytic systems and other applications into trustworthy use in the environment.

Table 2: Engineering Strategies for Municipal Application of PETase/MHETase Systems

Strategy	Functional Benefit	Limitation in Wastewater Context	Source
Engineered Biofilms	Improved enzyme retention and structural protection under flow conditions	Risk of excessive biofilm accumulation and mass transfer limitations	Flemming et al., 2016
PETase/MHETase Co-expression (Dual-Enzyme Cascade)	Sequential depolymerization; prevents MHET accumulation	Requires genetic stability and balanced expression levels	Lu et al., 2024
Thermostable Engineered Hydrolases	Higher degradation rates near PET glass transition temperature; reduced reaction time	Energy input constraints in low-temperature municipal systems	Kawai et al., 2024
Structure-Based MHETase Optimization	Enhanced substrate specificity and catalytic precision	May require continuous adaptation for mixed plastic streams	Palm et al., 2019
Microbial Consortia Engineering	Synergistic degradation and improved system resilience	Community dynamics may shift under environmental stress	Danso et al., 2019

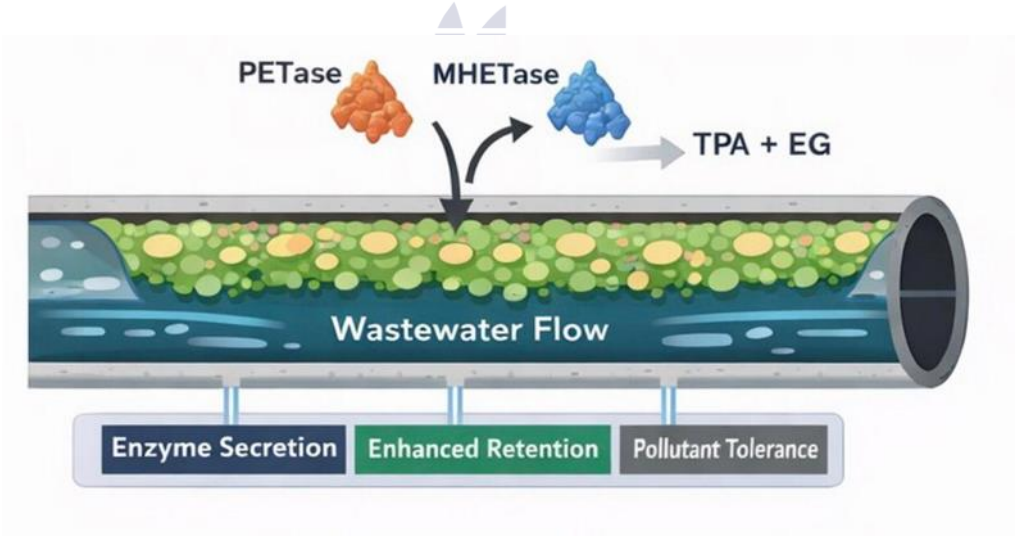

Fig. 05. PETase, MHETase and Wastewater flow chart.

Conclusion

The increasing deposition of microplastics derived in the form of PETs in the waters and the oceans requires the use of new and sustainable approaches to treatment in addition to the traditional ways of wastewater treatments. PETase and MHETase is a prospective enzymatic system that will allow converting persistent PET polymers to environmentally assailable monomers in serial catalytic reactions. The power of multi-enzymes demonstrates the possibility of biological depolymerization as an alternative to the mechanical or chemical traditional recycling approaches. Nevertheless, the effective incorporation of PET-degrading biocatalysts on a municipal-scale wastewater system is not an approach that is simple because of operation constraints like enzyme instability, the presence of inhibitory wastewater matrices, hydraulic retention, and environmental variations. Enzyme engineering, bioaugmentation and

microbial biofilm technology have been developed and have greatly enhanced catalytic efficiency and stability which implies that someday engineered biocatalytic systems can be used to treat large amounts of wastewater. The economic and life-cycle analysis further indicates that enzymatic PET recycling may provide significant savings of energy and greenhouse gas emissions relative to using the conventional method of producing PET. However, there is the need to translate the laboratory breakthrough to credible field

operations through some interdisciplinary work based on translating laboratories into wastewater biochemical applications, the perspective of environmental microbiology, and wastewater process optimization. Further studies in this area need to aim at increasing the thermostability of the enzymes, increasing the accessibility of the substrates to crystalline PET, and establishing controlled microbial consortia that can be used in complex wastewater environment. Lenient bioreactors based on the concept of adding PETase/MHETase systems to existing systems may offer an ultimate drive in scalable plastic biodegradation. Finally, enzymatic innovation in the company of better approaches of managing wastewater will be necessary to tackle the world challenge of plastic pollution in waters.

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