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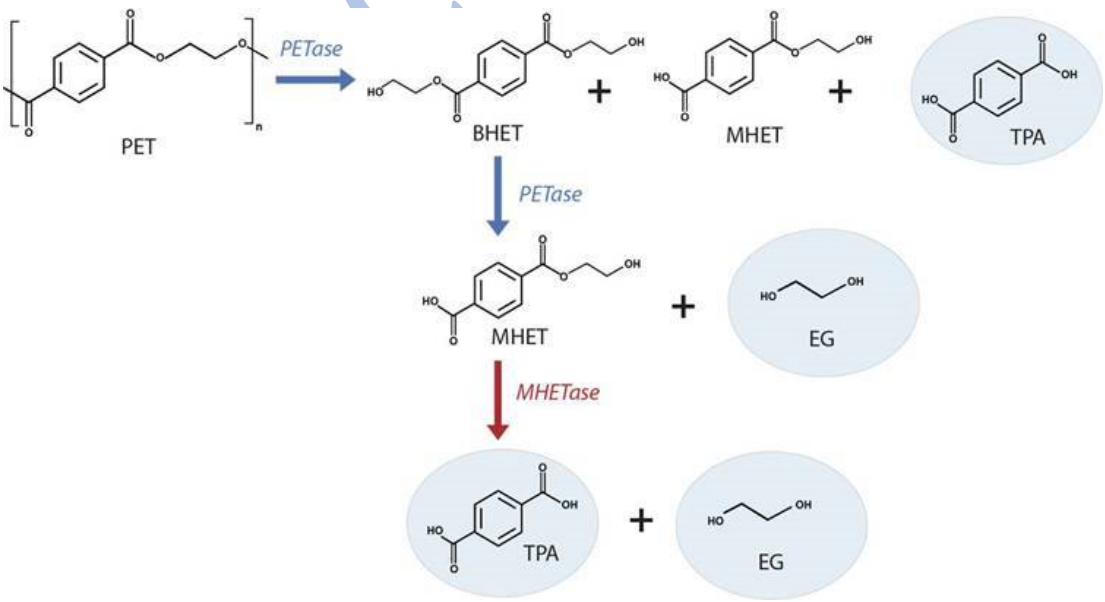
Enzymatic degradation of PET polymer is the most studied and tried out process worldwide. The generic microbial enzymes are well established and hence I suggest we go ahead first with the trials involving PET film and bottle flakes. PET hydrlolase enzyme (a cutinase-like serine hydrolase that attacks the PET polymer) is the foremost in this field. The second enzyme MHET hydrolase works with the former enzyme in conjunction to breakdown PET polymer. PETase and MHETase are able to breakdown PET polymer within a few days incubation period into TPA & EG. These are generic enzymes that may be available commercially from select suppliers and not liable for patents. Genetically modified microbial enzymes based on the above have been patented by some companies, notably Carbios.

In 2016 the discovery and characterization of the soil bacterium strain, *Ideonella sakaiensis* 201-F6 was reported, found growing in PET-contaminated sediment near a plastic recycling facility in Japan. This gram-negative, aerobic, rod-shaped bacterium has the remarkable ability to use PET as its major carbon and energy source for growth. *I.sakaiensis* employs a two-enzyme system to depolymerize PET to its building blocks, TPA and EG, which are further catabolized to a carbon and energy source. One of the two enzymes, ISF6_4831 protein, hydrolyzes and breaks ester linkages. With a preference for aromatic rather than aliphatic esters, and a specific inclination towards PET, it is designated as a **PET hydrolase (PETase)**. The PETase enzyme in *I. sakaiensis* is a cutinase-like serine hydrolase that attacks the PET polymer, releasing **bis(2-hydroxyethyl) terephthalate (BHET)**, **mono(2-hydroxyethyl) terephthalate (MHET)** and TPA. PETase further cleaves BHET to MHET and EG. The second enzyme, ISF6_0224 protein, MHET hydrolase (MHETase), further hydrolyzes the soluble MHET to produce TPA and EG (Fig. 2). Both enzymes can be used synergistically, to enzymatically convert PET into its two environmentally benign monomers, TPA and EG4, making it possible to fully recycle PET.



PET depolymerization scheme: PETase catalyzes the depolymerization of PET to bis(2-hydroxyethyl)-TPA (BHET), mono(2-hydroxyethyl) terephthalate (MHET), and terephthalic acid (TPA). MHETase converts MHET to monomers TPA and ethylene glycol (EG).

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When MHETase enzyme is added to the reaction, the enzyme mixture breaks down PET twice as fast as PETase on its own. The degradation trend observed within the tested enzyme loadings range shows increasing levels of constituent monomers as the concentration of both enzymes increases. This indicates that the reactions are enzyme, rather than substrate, limited. The synergy analysis also indicates that degradation rates increase with PETase loading and that the presence of MHETase, even at low concentrations relative to PETase, improves total degradation. The current experiments do not indicate an optimal ratio of PETase to MHETase.

Another promising enzyme is TfCut2 – a thermostable cutinase from Thermobifda fusca microbe. The enzyme has provided the fastest degradation rate for a 200 micron low crystallinity PET film along with application of a cationic surfactant. This enzyme is not available on a commercial scale. However, we will try to procure this enzyme from commercial sources or from biotechnology department of an institution.

Microbial & Enzymatic biodegradation of polyolefins – brief background

Polymers with hydrolysable ester bonds in their backbones (PET, PUR) are more susceptible to biodegradation than polymers with carbon chain backbones like PE, PS, PP and PVC. Enzymes that degrade the high-molecular weight polymers of PET and ester-based PU are known and details of their activities have been characterized. However, there is a growing body of research on microbial (and enzymatic) biodegradation of polymers with carbon chain backbones (PE, PP, PS etc.)

Most of the microbial species known to degrade PE are capable of hydrolyzing and metabolize linear n-alkanes, like paraffin molecules (e.g. C44H90, Mw 618). **Alkane hydroxylases** (AHs) are the key enzymes involved in aerobic degradation of alkanes by bacteria. The first step involves hydroxylation of C-C bonds to release primary or secondary alcohols, which are oxidized to ketones or aldehydes, and subsequently to hydrophilic carboxylic acids. Microbial oxidation reduces the number of carbonyl-groups due to the formation of carboxylic acids. Carboxylated n-alkanes are analogous to fatty acids, which are catabolized by bacteria via the β-oxidation system pathway. The oxidation products released by the action of enzymes in this process are absorbed by microbial cells where they are catabolized.[1,2,3,4]

The most important enzymes of interest in the alkane hydroxylase system are the **monoxygenases**. The number and types of AHs vary greatly in different bacteria, which itself differs in the amount of carbon in the alkane chains. The **Rhodococcus sp**. TMP2 genome encodes 5 AHs (alkB1, alkB2, alkB3, alkB4, and alkB5) while the **P. aeruginosa** genome encodes two AHs: alkB1 and alkB2. The Alkane hydroxylase system has also been well studied in **P. putida** GPo1, where the enzyme is involved in the hydroxylation of the terminal carbon-first step of the n-alkane oxidation pathway. Studies have shown that AlkB enzyme of **P. aeruginosa** strain E7 played a central role in the mineralization and thus, biodegradation

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of LMWPE into CO_2. The alkB gene was cloned in **Pseudomonas sp.** E4, and the AlkB enzyme expressed from the recombinant strain participated in the early stage of LMWPE biodegradation, in the absence of the other specific enzymes like rubredoxin and rubredoxin reductase. A class of multi- copper enzymes, known as **Laccase enzymes** (phenol oxidases), expressed by **Rodococcus rubber**, have also been shown to play an important role in PE biodegradation.[1,2,3,4]

1. “Biodegradation of polyethylene: a brief review”, Ghatge et al. - Applied Biological Chemistry (2020) 63:27 (The Korean Society for Applied Biological Chemistry)
2. “Biodegradation of plastics – a brief review”, Muthukumar et al. - International Journal of Pharmaceutical Sciences Review and Research (2015) 31:2
3. “Recent insight into enzymatic degradation of plastics prevalent in the environment: A review”, Kaushal et al. – Cleaner Engineering & Technology (2021) 2
4. “Review on the current status of polymer degradation: a microbial approach”, Pathak et al. - Bioresources & Bioprocessing (2017) 4:15

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