

## Review On Microbial Biodegradation Of Microplastic

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### Abstract :

Plastic wastes are gradually increasing in our environment due to heavy use plastic product , industrial waste. Mechanical abrasion, UV radiation, weathering, and animal grazing etc. all contribute to the breakdown of plastic waste into microplastics. Now-a-days microplastic pollution is a growing worldwide environmental concern which is now threats to ecosystem and human health. This review aims to find Microbial biodegradation of microplastic to reduce it's toxicity in our environment to make sustainable and ecofriendly and find an alternate of recycling of plastic which is costly process and provide an overview of importance of fungi and bacteria. Fungi and bacteria have the ability to enzymatically breakdown various types of microplastic. *Candida rugosa* can degrade Polybutylene Succinate (PBS), marine fungi *Zalerionmaritimum* can degrade polyethylene (PE). *Aspergillus fumigatus* can degrade polyethylene succinate (PES), marine Bacteria like *Alcanivoraxxenomutans* and *Halomonastitanicae*, degrade polystyrene (PS) and generate microplastic or nano plastic. Polystyrene as sole energy source when associated with bacteria and fungus during growth. During this process, plastic weight loss, the creation of carboxylic and carboxyl acid groups, a reduction in heat resistance, and the production of PS metabolic intermediates in cultures were all noted. During the experiments there are some challenges like- microorganisms often forms biofilms on the surface of microplastic, variations in experimental conditions such as temperature, pH, nutrient levels, and microbial inoculum can be challenging achieving consistent and reproducible results across different studies. In conclusion by offering a thorough overview of the state of the art regarding the microbial biodegradation of microplastics and emphasizing the potential of bacteria and fungi as efficient agents in the remediation of microplastic pollution. The information shared aids in the creation of environmentally responsible and long-lasting methods for controlling microplastic pollution in a variety of environments. The main purpose is to understanding of microbial interaction with microplastic.

**Keywords** –Enzymatic degradation , Microplastic , Toxicity ,Degradation mechanism.

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### I. Introduction-

In this modern era plastics have become the part of human life. Day to day life we use different plastic products in many ways. As per recent data the production of plastic is 400 metric ton per year worldwide in 2021, and waste generated 353 metric ton, only 15% plastic wastes are recycled (Bruna Alves, 2024). Plastics are synthetic polymer consists of long chain of carbon with hydrogen , nitrogen , oxygen, Sulphur (Shah et al., 2008). The production of plastic first began in the year of 1950, since then the uses of this polymer has increased vigorously. Plastics which are less than 5mm in size are called Microplastics. Mechanical abrasion, UV radiation, weathering, and animal grazing etc. all contribute to the breakdown of plastic waste into microplastics. Microplastics found in air, terrestrial environment as well as marine. Microplastic pollution directly impacts on human health as well as marine life. Microplastic pollution effects fish, which also impacts on human food chain (Galloway et al., 2017).

Microplastics are insoluble in water and are synthetic solid particles of polymeric matrix, generally 1µm to 5mm in size with regular or irregular shape (Frias & Nash, 2019). And plastic particles 1 to 1000nm in size are called nanoplastics which can behave like colloidal (Gigault et al., 2018). In our environment various type of microplastics have been identified. The wide range of plastic goods used in daily life is reflected in the variety of shapes and compositions of microplastics. Microbeads found in personal hygiene products, broken plastic bottles, fibers from synthetic textiles, and degraded particles from bigger plastic objects like packaging materials are typical instances of microplastics. The most common polymers in microplastics are polystyrene (PS), polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Polybutylene succinate (PBS), polylactic acid (PLA). These polymers are incredibly durable, which not only extends the lifespan of plastic items but also makes them impervious to natural deterioration processes. (Kaushal et al., 2021a).

Microplastics will be exposed to humans through a variety of channels after being released into the environment, potentially posing health hazards. Furthermore, microplastics may be introduced to species in soil

and aquatic settings, and their direct toxicity has been thoroughly studied. It should be mentioned that some inorganic and organic substances are being released into the environment, such as tributyltin, Pb, Zn, and bisphenol A (BPA). Coexisting pollutants, such as PCBs and PAHs, have the ability to adsorb on microplastics, which may result in synergistic toxicity. Therefore, it was also impossible to ignore the indirect harm that microplastics cause (Wang et al., 2021). Microorganisms, such as fungus and bacteria, are fundamental to ecosystems because they take part in vital biogeochemical processes. On the other hand, microbial populations and their functional functions may be disturbed by the introduction of microplastics into their environments. According to studies, microplastics may influence microbial growth, diversity, and metabolic processes by acting as physical and chemical stressors. Changes in microbial communities can have an impact on soil health, ecosystem resilience, and nutrient cycling in addition to individual microorganisms. (Zettler et al., 2013). Invertebrates play a crucial role in the food chain of aquatic habitats by acting as prey for a variety of species and maintaining the stability of the ecosystem. These species are seriously threatened by microplastics because they can consume polluted food sources and get directly or indirectly consumed. Physical injury, decreased eating efficiency, and interference with reproductive processes are among the effects on invertebrates. The risk is further increased by the possibility of biomagnification, as microplastics consumed by their prey may be accumulated by predators at higher trophic levels. (Wright et al., 2013). The consumption of microplastics poses significant dangers to both marine and terrestrial species. Concerns regarding physical injury, digestive system obstruction, and the possible leaching of harmful compounds are raised by the ubiquitous presence of microplastics in the digestive tracts of different animals. The existence of plastic fibers and debris in seafood sold for human consumption was brought to light by research conducted by Rochman et al. (2015), who also emphasized the possible health risks associated with consuming polluted marine species. (Rochman et al., 2015). Microplastics' possible effects on human health are a developing field of study that needs close attention. Humans can come into touch with microplastics through their diet, breathing, or skin. Numerous food products, drinking water sources, and even the air have been found to contain microplastics. Generally human exposure to microplastics by consuming contaminated food and beverages. A study suggests that the microplastics particles can pass through the gastrointestinal tract and perhaps build up within the human body. Human health are affected because of presence of microplastics in seafood, salt honey and other consumables so it needs to explore the health effect of dietary microplastic exposure (Schwabl et al., 2019).

### **What is Microplastics?**

Plastics are synthetic polymer consists of long chain of carbon with hydrogen, nitrogen, oxygen, Sulphur. The production of plastic first began in the year of 1950, since then the uses of this polymer has increased vigorously. From day to day life to industry plastics are used in different forms. Plastics which are less than 5mm in size are called Microplastics. Plastic wastes are gradually increasing in our environment due to heavy use of plastic product, industrial waste. Mechanical abrasion, UV radiation, weathering, and animal grazing etc. all contribute to the breakdown of plastic waste into microplastics.

Generally microplastics are categorized in two types – Primary microplastic and secondary microplastic.

Primary microplastics-1) Nurdles or pellets- small preproduction plastic pellets which are used in manufacturing of plastic products. 2) Microbeads – Tiny spherical plastic particles generally used in personal care products, such as toothpaste, 3) Microfibers – small fibers which are released from textile while washing mainly synthetic fabrics like polyester washing.

Secondary microplastics – Microfragments – small pieces of plastics breakdown from larger plastic items like bottles, bag due to environmental factors like sunlight, waves.

Materials – Microplastics are composed of various types of plastic.

Polyethylene (PE)- PE is a polymer of ethylene monomer. This is the most common type of polymer widely used, every year million tons of PE is produced for using in different ways like packaging material, bottle, carry bag etc.

Polypropylene (PP)- PP is the most commonly used polymer consists of propylene monomer. It is a thermo plastic so it can be reheated without deforming. So it is used in large scale in packaging industry, textile industry, automobile industry and many other ways.

Polyvinyl chloride (PVC) – PVC is the third most used polymer after PE and PP. It is widely used in production of pipes, cables, construction material etc.

Polystyrene (PS) – PS is a polymer composed of repeating units of styrene. It is found in disposable foam products and packaging. PS is a non toxic polymer so it is widely used in food packaging.

Polyethylene Terephthalate (PET) – PET is a very tough polymer made by polymerisation of ethylene and terephthalic acid through the formation of ester linkages. It is generally used in beverage bottles and food containers.

Polylactic acid (PLA) - PLA is a biodegradable and bioactive thermoplastic which is made from renewable resources, usually derived from plant-based sources like corn starch or sugarcane. It is a naturally biodegradable bioplastic, so it is considered more environmentally friendly than the other traditional plastic.

Polybutylene succinate (PBS) – PBS is a biodegradable bio based thermoplastic which is used in the production of biodegradable plastics and packaging materials.

#### Chemical nature of different Microplastics-

Based on chemical composition plastics are grouped in three categories, polymer with carbon-carbon backbone like PE, PP, PS, PVC, polymer with heteroatoms in main chain that means polymer in which the backbone contains element such as oxygen, nitrogen, sulfur, or silicon in addition to carbon, like PET, PU, and polymer with ester-bond backbone like PHA, PLA.

Polyethylene(PE)- PE  $-(C_2H_4)_n$ -is made up of a linear carbon chain joined by hydrogen bonds. PE typically has a semi-crystalline structure and a very high biodegradation resistance. PE polymers can have a range of densities and physical and three-dimensional structures depending on the manufacturing procedures used and the resulting linear chain configurations. Due to these there are different types of PE, like low molecular weight polyethylene(LMWPE), low density polyethylene(LDPE), high density polyethylene(HDPE), linear low-density polyethylene(LLDPE).Commercially PE are used in many ways due to their different density and molecular weight, generally used in garbage bag, grocery bag, insulation of wires, bottle, toy etc. It is also used for food packaging.(Mohan et al., 2020).

Polypropylene(PP) – Among synthetic polymers PP  $(C_nH_{2n})$ is the most commonly utilized linear hydrocarbon polymer.Three stereoisomeric forms - atactic, isotactic, and syndiotactic are present in PP due to the methyl group that replaces one of the hydrogens found in PE on every other carbon Using propylene as the monomer, J. Paul Hogan and Robert L. Banks created this polymer for the first time in 1951 (Stinson, 1987). For it's synthesis, Metallocene catalysts has been used. Its characteristics are comparable to those of polyethylene, but it is stronger, more heat resistant, and more chemical resistant. After polyethylene, polypropylene is the commodity plastic that is produced on a larger scale and is frequently utilized in labeling and packaging.Numerous industries use these polymers, such as food packaging, textiles, laboratory apparatus, and vehicle parts. Together with PE, PP are classified as polyolefins and are inert materials that are resistant to microbes due to their hydrophobic backbones made of lengthy carbon chains, high molecular weights (between 10,000 and 40,000 g/mol), and the addition of antioxidants and stabilizers during the manufacturing process, which keeps polyolefins from oxidizing in the atmosphere.(Mohan et al., 2020).

Polystyrene(PS)- PS  $(C_8H_8)_n$  is a homopolymer composed of od repeating units of styrene. Depending on its kind, it can be either a thermoplastic or a thermosetting plastic.The monomer styrene, also known as ethenylbenzene, consists of a vinyl group attached to a benzene ring.Polymerization is the process by which styrene molecules join together to create long chains, giving polystyrene its distinctive linear and unbranched structure with a high molecular weight(Kaushal et al., 2021a). PS polymers have remarkable stability due to their large molecular weight and strong hydrophobic nature, rendering them highly impervious to biodegradation. By using the oxidation-reduction process, PS's carbon-carbon backbone is extremely resistant to enzymatic cleavage.Based on its many uses, PS has been divided into four product categories -General purpose polystyrene (GPPS)/oriented polystyrene (OPS), PS foam, expanded polystyrene (EPS) foam, and high impact polystyrene (HIPS). PS is used in many ways , it is used in production of disposable cutlery, hot beverage cups as it is thermostable, insulation materials for electric components, packaging materials etc.(Mohan et al., 2020).

Polyvinylchloride(PVC) – PVC $(C_2H_3Cl)$  is a thermoplastic polymer consisting of repeating vinyl chloride monomer units.Vinyl chloride, the monomer, is made up of a chlorine atom and a vinyl group  $(CH_2=CH-)$ . Its chemical formula is  $(CH_2=CHCl)$ . These monomer units are joined together to create a polymeric chain during the polymerization of vinyl chloride.The vinyl chloride monomer is repeated throughout the polymer chain of PVC, as indicated by the chemical structural representation  $(CH_2=CHCl)_n$ . Usually, free radical polymerization is used to accomplish the polymerization process, which results in the formation of a linear and comparatively rigid polymer structure. Worldwide PVC is the 3<sup>rd</sup> most used polymer. The rigid and along with plasticizer like phthalates, the flexible both form of PVC are used commercially to produce pipes, bottles, cables etc.(Kaushal et al., 2021b).

Polyethylene Terephthalate (PET) – PET  $(C_{10}H_8O_4)_n$  is a thermoplastic polymer that is a member of the polyester family. Repeating units produced from terephthalic acid and ethylene glycol characterize its chemical structure. Terephthalic acid, ethylene glycol, and monomers combine to generate ester bonds, which produce a linear, highly crystalline polymer chain. It's polyester structure gives it advantageous qualities including chemical resistance, mechanical strength, and transparency.Glass transition temperatures (Tg) of aromatic polyesters in PET are high, ranging from around 75-80°C in air. In contrast, Tg falls to 60–65°C in an aqueous solution. The amorphous PET sections become more pliable and susceptible to enzymatic and/or microbial

assault at temperatures higher than Tg. Because shorter chains have higher motility than longer chains, a drop in average chain length with polymer breakdown was found to result in a decrease in Tg. PET generally used to manufacture bottles, containers, textile fibers, films etc. (Mohan et al., 2020).

**Polyurethane (PU)** - The chemical structure of polyurethane (PU), a multipurpose polymer, is produced when isocyanates and polyols combine. Urethane linkages are created when isocyanates, such as diisocyanates, combine with polyols, which can be polyester or polyether polyols. The general chemical structure is  $(C_{27}H_{36}N_2O_{10})_n$ . Carbamate linkages are used to bind organic molecules together to create polyurethane. They might be an ether or an ester. They don't dissolve in water. It is among the most versatile plastic types with a wide range of uses in adhesives, insulation, coats, tires, sponges, paints, and fibers etc. (Kaushal et al., 2021b).

**Polyhydroxyalkanoates (PHA)** - PHA are biodegradable polymers which are produced by microorganism under limited nutrient condition. PHA composed of hydroxyalkanoate monomer units. Typically, these monomers consist of an alkyl chain linked to hydroxyl (-OH) and carboxyl (-COOH) groups. The following represents the overall structure:  $\{(R)-O-CO-CH_2-CH(R')-CO-\}$ . Here R and R' in this case stand for different alkyl groups. The variation in PHA characteristics is partly due to the variety of alkyl side chains. PHA are either homo or copolymers depending on the structural differences in the monomers that make up the polymer. The characteristics of PHA are, it is insoluble in water, resistant to UV rays, and hydrolytic attack, which help anaerobic biodegradation in sediments. PHA are widely used in many different sectors, including the biomedical one. Examples of these industries include tissue engineering, bio-implant patches, drug delivery, surgery and wound dressing. (Raza et al., 2018).

**Poly(lactic acid) (PLA)** - PLA is a linear aliphatic thermoplastic polyester that is made from renewable resources like sugarcane or maize starch and is both biodegradable and biobased. PLA is a member of the polyester family that is created by polymerizing lactic acid monomers. The chemical structure of PLA is linked with ester, and the formula is  $(C_3H_4O_2)_n$ . PLA may be found in two stereoisomeric forms: poly(L-lactic acid) and poly(D-lactic acid). The monomer unit is made up of a chiral carbon. Furthermore, poly(DL-lactic acid), a racemic combination, can also be generated. It generally takes six weeks to twenty four weeks to degrade, and also cost effective to produce so it is a very good alternative of PP, PS, PE. Worldwide PLA are used in biomedical, packaging and agriculture fields. (Carrasco et al., 2010).

#### Enzymes involved in degrading of Microplastics –

Name of Enzyme	Name of MP degraded by the Enzyme	Microorganism	Mechanism of Degradation	Structural characteristics of degradation	References
PETase	PET	<i>Ideonella sakaiensis</i>	Hydrolysis of ester linkages, leading to monomeric breakdown	Ester bonds in PET chains	(Temporiti et al., 2022a)
Cutinase	PET	<i>Humicola insolens</i>	Hydrolysis of Ester bond	Amorphous region of the polymer	(Sui et al., 2023)
Esterase	PE	<i>Pseudozyma antarctica</i> <i>Bacillus subtilis</i>	Hydrolysis of carbon chain ester linkage	By attacking amorphous region- weaker point of polymer	(Mohan et al., 2020)
Laccases	PE	<i>Trametes versicolor</i> <i>Rhodococcus ruber</i>	Oxidation introducing functional groups	By attacking amorphous region- weaker point of polymer	(Fujisawa et al., n.d.)
Cutinase Alkane hydroxylases, Cytochrome P450	PP	<i>Thermobida sp.</i>	Hydrolysis of ester bond	Amorphous region of the polymer	(Temporiti et al., 2022a)
Cytochromes P450	PS	<i>Bacillus subtilis</i>	Oxidation of carbon backbone	$\beta$ carbon of polymer	(Hou & Majumder, 2021a)
Monoxygenase	PS	<i>Pusillimonas sp.</i>	Oxidation of carbon backbone	$\beta$ carbon of polymer	(Hou & Majumder, 2021a)
Alkane hydroxylase	PS	<i>Pseudomonas putida</i>	Oxidation of carbon backbone	U ring carbon of polymer	(Hou & Majumder, 2021a)
Cutinase	PLA	<i>Cryptococcus sp.</i>	Hydrolysis of	Amorphous	(Mohan et al., 2020)

		Strain S-2	Ester linkage	region of the polymer	
Lignin peroxidase	PVC	<i>Phanerocheate chrysosporium</i>	Oxidation of carbon backbone	Attack on C-C backbone	(Temporiti et al., 2022a)
Lipases	PU	<i>Candida rugosa</i>	Hydrolysis of Ester linkage	Nucleophilic attack on Ester bond	(Temporiti et al., 2022b)
Esterase	PU	<i>Chaetomium globosum</i> <i>Aspergillus terreus</i>	Hydrolysis of Ester bond	Hydrolysis on polymers containing ester and urethane link	(Temporiti et al., 2022b)

### Mechanism of Enzymatic Degradation of Microplastics-

**Mechanism of PET degradation by IsPETase** – IsPETase has been shown to facilitate the breakdown of PET into its individual monomers, such as bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), and terephthalate (TPA). Moreover, IsPETase can also degrade BHET, a compound resembling PET. The enzyme's interaction with BHET leads to the formation of MHET without further decomposition of the substance. Within IsPETase's active site lies a catalytic triad comprising three residues (Ser160, His237, and Asp206), with Ser160 believed to function as a covalent nucleophile targeting the carbonyl carbon in the ester bond, a process observed in other carboxylesterases. Examination of the ester bond of 2-HE(MHET)<sub>4</sub> reveals that the substrate-binding site in IsPETase can be divided into two distinct subsites - subsite I and subsite II, accommodating one and three MHET units, respectively.

Based on the structural and biochemical analyses, PET degradation mechanism. Initially, PETase from the bacterium *Ideonella sakaiensis* attaches to the PET surface utilizing its flat hydrophobic surface with a substrate binding cleft. The degradation process of PET can be categorized into two distinct stages: nick generation and terminal digestion. In the nick generation phase, four MHET moieties are linked to each subsite, with the ester bond positioned between subsite I and II near the catalytic Ser160 residue. Subsequently, cleaving one ester bond results in the creation of a nick in PET, yielding two PET chains with different terminals. The terminal digestion stage involves the breakdown of the two PET chains with Hydroxy Ethyl(HE) and TPA termini into MHET monomers in slightly varying methods. For HE-terminal PET digestion, the terminal MHET and the subsequent three MHET moieties bind to subsite I and subsite II, respectively, resulting in the production of one MHET monomer and <sup>HE</sup>PET<sub>n-1</sub>. The digestion of TPA-terminal PET follows a similar pattern, producing one TPA molecule and <sup>HE</sup>PET<sub>n-1</sub>. Alternatively, PET degradation can also occur by binding PET polymer chains and the enzyme in the reverse direction, although this method may be less efficient. This process can lead to the formation of various PET monomers and dimers, ultimately resulting in the production of MHET, TPA, and Ethylene glycol(EG). Continuous digestions of <sup>HE</sup>PET and <sup>TPA</sup>PET progress in a combinational manner, leading to the accumulation of MHET, TPA, BHET, and EG. BHET can further degrade into MHET and EG, ultimately accumulating MHET, TPA, and EG due to PET degradation. It is important to note that the degradation of PET film by IsPETase generates a significant amount of TPA, primarily through the terminal digestion step of TPAPET.(Joo et al., 2018).

**Mechanism of PET degradation by Cutinase** - The single- enzyme cutinase derived from the fungus *Humicola insolens* (HIC) exhibit significant hydrolytic activity and stability at 70°C. The cutinase from HIC can efficiently breakdown both the amorphous and crystalline regions of PET without requiring prior treatment, when it is applied in reactions involving moist-solid mixtures instead of dilute aqueous solution. Once bound to PET cutinase undergo structural modification to enhance the accessibility of the active site for hydrolysis of ester bond. Serine, histidine, glutamate, the essential catalytic residue, constitute the catalytic triad for the cleavage of ester bonds. A temporary covalent intermediate is formed between the enzyme and the substrate when catalytic serine residue attacks ester bond at active site of PET. The catalytic triad aids in the nucleophilic attack on the ester bond, leading to the degradation of PET into smaller components like MHET, TPA.(Sui et al., 2023).

**Mechanism of PE degradation by Laccases** - Most microbial enzymes are capable of degrading lignin polymer, which contain oxidizable C-C bonds, that plays a pivotal role in the biodegradation of polyethylene(PE). Laccase from *Trametes versicolor* can oxidize of functional groups in PE. A system known as the laccase mediator system (LMS) combined with mediator 1- hydroxybenzotriazol (HBT) can effectively degrade high molecular - weight polyethylene (Fujisawa et al., n.d.). Laccase generally attacks on amorphous region (weaker region) of carbon bond in PE. Laccase cleaved at amorphous region found in the PE film, which leads to formation of easily accessible carbonyl groups. That causes significant reduction in the weight of the PE film. It was observed that there was a noticeable decrease in the molecular weight of a PE membrane after treating with laccase. The oxidative activity of laccase caused PE breakdown, it may catalyzed oxidation of PE, leading cleavage of polymer chains, resulting smaller fragments. Wang 2021.

**Mechanism of PE degradation by Esterase-** Esterase from *Bacillus subtilis* cause the polymer to break into smaller pieces by attacking the ester bonds found in polyethylene chains. In order to develop effective enzymatic degradation processes, it is essential to understand the substrate specificity and ideal conditions for these enzymes (Temporiti et al., 2022c). The initial attachment of the esterase occurs to the surface of PE by a series of hydrophobic interaction and /or electrostatic forces. After attachment the enzymes modifies the property of surface of PE, ultimately enhancing it's susceptibility to enzymatic activity. Ester bonds of PE are hydrolyzed by the catalytic action of esterase, results the breakdown of the polymer into smaller molecules such as oligomers and monomers. As the hydrolysis reaction progresses, the enzyme may eventually detach from the polymer surface, potentially enabling it to move to other areas of the material and continue its degradation process. This detachment and release mechanism allows for the enzyme to exert its catalytic effect on adjacent regions of the polymer, contributing to the overall degradation of the PE material.(Zhang et al., 2023).

**Mechanism of PP degradation by Cutinase,Alkane hydroxylases, Cytochrome P450** -The degradation mechanism of PP is almost similar to PE degradation. To degrade, firstly polymer converted into oligomers, due to an increase in oxygenated functional groups, chain scission, and the formation of methyl ketones as byproducts.(Lv et al., 2024). The degradation of PP exhibits an exceptionally slow rate when exposed to the natural environment, a phenomenon primarily attributed to the robust structural stability and the hydrophobic characteristics inherent in these polymers. These attributes stem from the backbone chains of PP, which consist solely of C-C and C-H bonds, further enhancing their resistance to degradation processes. The challenging nature of PP degradation has impeded a comprehensive understanding of the various routes, mechanisms, and enzymes essential for this process, with current knowledge remaining at a nascent stage. The degradation of PP is conceptualized to take place in three distinct stages: Firstly, the initial stage involves the creation of hydrolyzable functional groups within the C-C backbones of PP. Enzymes such as Cutinase, Alkane hydroxylases, and cytochrome P450 are identified as potential catalysts capable of hydroxylating PP to kickstart the biodegradation process. Secondly, the subsequent stage encompasses the hydrolysis and fragmentation of the carbon chain, with enzymes like cutinases, lipases, and esterases being identified as potential candidates for breaking down hydroxylated PP into shorter-chain alkanolic acids and alkanols. These processes in the first and second stages are reiterated until the chain length of the PP degradation products reaches a level suitable for microbial utilization. Finally, the third stage involves the assimilation of degradation products to support microbial biomass formation and other metabolic pathways.(Lee et al., n.d.)

However, there are very few enzymes are obtained and identified, which can degrade PP. So PP degradation mechanism is not properly identified. Exploring the microbial communities capable of degrading PP and identifying the enzymes involved represent intriguing and valuable avenues for further research in this field.(Temporiti et al., 2022b).

**Mechanism of PS degradation by Cytochromes P450, MonooxygenaseAlkane hydroxylase-** Alkane hydroxylase, monooxygenase, and cytochrome P450 are enzymes that primarily target the  $\beta$ carbon of the carbon chain and play a crucial role in cleaving the main chain. They may also play a significant role in an unidentified degradation pathway that further breaks down the PS polymer. Cytochromes P450, an enzyme superfamily with heme cofactors, are found widely across different life kingdoms. The main oxidative reaction facilitated by cytochrome P450 is the monooxygenation of a substrate. Cytochrome P450 CPY152A1 (from *Bacillus subtilis*) can catalyze the hydroxylation of ethylbenzene and the epoxidation of styrene in the presence of hydrogen peroxide. This initial oxidation process may be controlled by alkane-degrading cytochromes P450 to convert alkane into a primary alcohol, which is further oxidized to an aldehyde and ultimately transformed into a fatty acid(Rojo, 2009). Subterminal oxidation may also take place in certain microorganisms where the resulting secondary alcohols are oxidized to ketones. A Baeyer–Villiger monooxygenase can then convert these ketones to esters, which are subsequently broken down to form a primary alcohol and a common fatty acid. Consequently, cytochromes P450 have the ability not only to break C-C bonds, leading to the production of single-ring aromatic compounds, but also to oxidize these compounds.(Hou & Majumder, 2021b).

Monooxygenases from *Pseudomonas* sp. that introduce a single oxygen atom into the substrate may also play a role in the degradation of PS. These particular enzymes, which can degrade alkanes, have the potential to participate in breaking down the main chain of PS, which is an alkane with substitutions. Additionally, monooxygenases like 4-hydroxybenzoate 3-monooxygenase and 3-hydroxybenzoate 6-monooxygenase might aid in the oxidation of cyclic aromatic compounds (e.g., 4-hydroxybenzoate and 3-hydroxybenzoate) formed during the breakdown of the PS main chain(Hou & Majumder, 2021b).

Alkane hydroxylase from *Pseudomonas putida*, by performing dioxygenation with the incorporation of two atoms of dioxygen into the aromatic ring, facilitates dihydroxylation. These enzymes are responsible for introducing a hydroxyl group into alkanes. Activation: The alkane hydroxylase activates the normally unreactive carbon-hydrogen bonds of PS through hydroxylation. Degradation: The hydroxylated PS then

undergoes subsequent enzymatic processes, resulting in its breakdown into smaller compounds (Hou & Majumder, 2021b).

**Mechanism of PLA degradation by Cutinase-** PLA is commonly known biodegradable plastic. Various enzymes involved in the degradation of PLA have been extensively studied. The breakdown of PLA occurs through enzymatic hydrolysis of its ester bond at amorphous region, followed by the absorption and processing of lactic acid oligomers, dimers, and monomers. The primary facilitator of PLA biodegradation is an enzyme such as cutinase derived from *Cryptococcus sp.* Strain S-2. (Lee et al., n.d.). Essentially, the degradation of PLA is driven by a series of processes. Firstly, Cutinase enzyme binds to the surface of PLA at amorphous region, aligning its active site with polymer chains. Secondly the enzyme catalyzes the hydrolytic cleavage of ester bonds in PLA, leading to the breakdown of the polymer into lactic acid monomers. Finally, the resulting monomers are released from the enzyme's active site, enabling further degradation or integration into natural metabolic pathways. (Sourkouni et al., 2023).

The degradation of a polymer through hydrolysis typically results from a combination of chemical hydrolysis and the diffusion of water and oligomers. In the field of organic chemistry, this process can be defined as a bimolecular nucleophilic substitution, which can be catalyzed by either acids or bases. It is important to mention that the pH of the surrounding environment affects the mechanism and rate of hydrolytic degradation of PLA. Specifically, under neutral or basic conditions, hydrolytic degradation predominantly occurs through backbiting reactions, although there is a minor contribution from random scission hydrolysis. Conversely, in basic environments, the hydrolytic degradation of D, L-lactic acid oligomers primarily involves random ester cleavage, which is influenced by factors such as the rate constant, water absorption, diffusion coefficient of chain fragments, and solubility of degradation byproducts like lactic acid and lactide. (Teixeira et al., 2021).

**Mechanism of PVC degradation by Lignin peroxidase** -The process of fungal PVC biodegradation is challenging due to the material's hydrophobic nature, resistance to wear, and structural durability. Only a limited number of studies have reported on the alteration of PVC molecular weight by microorganisms. Studies have shown that the degradation of PVC is linked to the breakdown of lignin, as evidenced by the modification of PVC structure by fungal lignin peroxidase from *Phanerochaete chrysosporium*. This enzyme, with a molecular weight of 46 kDa, peaks in production after four weeks at 25°C and pH 5, resulting in a 31% reduction in PVC film weight and the observation of alkenyl C-H stretching in the PVC structure through FTIR analysis (peak at 2943 cm<sup>-1</sup>). (Temporiti et al., 2022b)

The degradation process of polyvinyl chloride (PVC) by lignin peroxidase from *Phanerochaete chrysosporium* consists of several key steps: 1) Oxidative Cleavage: Lignin peroxidase initiates the oxidative decomposition of PVC by breaking chemical bonds, with a specific focus on carbon-carbon (C-C) and carbon-chlorine (C-Cl) bonds. This process is enabled by the enzyme's heme group and necessitates the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as an oxidizing agent. 2) Formation of Radicals: The enzyme produces radical species capable of attacking the PVC polymer, causing its structural disintegration. 3) Degradation of Products: The reaction leads to the creation of smaller molecules derived from the PVC polymer, which may consist of chlorinated and non-chlorinated hydrocarbons. 4) Enzyme Regeneration: Following the reaction, the enzyme goes through a series of reduction and oxidation steps to restore its active form, enabling it to catalyze subsequent reactions. (Lee et al., n.d.).

**Mechanism of PU/PUR degradation by Lipase and Esterase** - Lipases responsible for the degradation of polyester polyurethane primarily come from yeasts within the *Candida* genus. Gautam et al. first proposed kinetics and mathematical models for the degradation of polyurethane by *Candida rugosa* (Gautam et al., 2007). The enzymes from *Candida rugosa* catalyze the hydrolysis or synthesis of triglycerides, depending on the specific reaction conditions. These enzymes possess an  $\alpha/\beta$ -hydrolase fold structure that includes a hydrophobic cavity covered by a movable lid, allowing for conformational changes during activity. This lid can shift from a closed to an open position to accommodate the substrate. The degradation process involves the lipase binding to the substrate interface between an insoluble substrate phase and an aqueous phase. Then the enzymes facilitate the hydrolysis of ester linkage in long-chain triacylglycerols, leading to the release of free fatty acids and glycerol. Generally this enzymatic process is applied in various biotechnological fields, for breakdown of PU materials (Álvarez-Barragán et al., 2016).

There are many mechanisms of enzymatic hydrolysis play a role in degradation of PU. Most of the research papers discuss the breakdown of ester bonds found in poly(ester-urethane), resulting in the liberation of compounds containing hydroxyl and carboxylic acid functional groups (Lee et al., n.d.). Initially, esterases sourced from *Chaetomium globosum* and *Aspergillus terreus* identify PU as a substrate and subsequently adhere to the PU polymer's surface, establishing a vital interaction necessary for the degradation process. The hydrolysis of ester bonds, primarily present in polyurethane foams and coatings, is catalyzed by esterase enzymes, which facilitate bond cleavage through the utilization of water molecules. This hydrolysis event is crucial in the breakdown of the PU polymer into smaller fragments. Following the cleavage of ester bonds, the

PU polymer undergoes fragmentation into smaller molecules, generating oligomers and monomers that are significantly smaller in size than the original polymer. The resulting smaller PU fragments are released from the polymer matrix, rendering them more susceptible to further enzymatic degradation. Subsequently, enzymes preferentially target the ester bonds in aliphatic and aromatic polyesters, releasing carboxylic acids and alcohols. (Temporiti et al., 2022b)

## II. Conclusion

In conclusion, the process of enzymatic degradation of microplastics represents a promising pathway to address the urgent environmental issues associated with plastic pollution. This review has explored the complex mechanisms involved in the enzymatic breakdown of microplastics by different organisms and enzymes, emphasizing the opportunities and obstacles in this area of study. The escalating problem of microplastic pollution has prompted the investigation of novel and efficient remediation approaches. Among these strategies, enzymatic degradation of microplastics emerges as a hopeful option, utilizing the precision and effectiveness of enzymes to facilitate the decomposition of these persistent contaminants. The review concludes the chemical composition of commonly found microplastics, such as polyethylene terephthalate (PET), polyurethane (PU), polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), and polylactic acid (PLA), along with the corresponding enzymes capable of breaking them down.

The mechanisms through which enzymes act on microplastics have been clarified, emphasizing the functions of PETase and cutinase in breaking down PET, esterases and laccases in addressing PE, and the collaborative actions of cutinase, alkane hydroxylases, and cytochrome P450 in managing PP degradation. Likewise, the degradation of PS is linked to the activities of cytochromes P450, monooxygenase, and alkane hydroxylase, while cutinase is identified as effective in PLA degradation. The degradation pathways of PVC and PU have been demonstrated to involve lignin peroxidase and a combination of lipase and esterase, respectively.

Enzymatic degradation of microplastics emerges as a beacon of hope in the battle against plastic pollution. Despite challenges like enzyme stability, functionality in environmental conditions, and the complete breakdown of degradation byproducts, the advancements in this field are promising. Continuous research and innovation are vital to enhance these biological tools, paving the way for practical and sustainable resolutions. It is crucial to integrate these discoveries with broader environmental management strategies to ensure a cleaner and safer ecosystem for future generations.

Although there have been promising advancements, obstacles persist in scaling up enzymatic degradation procedures for widespread use. Issues related to enzyme stability, efficiency, and cost-effectiveness necessitate further investigation and development. Enhancing enzyme performance across various environmental conditions and refining enzyme production processes are crucial steps in realizing the full potential of enzymatic degradation technologies.

In conclusion, enzymatic degradation offers a promising and ecologically sound approach to combat microplastic pollution. Ongoing research and advancements in enzyme technology are crucial for creating efficient, cost-effective, and scalable solutions to address the intricate challenges posed by microplastics in the environment. Collaborative efforts among scientists, policymakers, industry representatives, and the public are essential to drive advancements in this critical field and alleviate the negative impacts of plastic pollution on ecosystems and human health.

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