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Biodegradation of Microplastic: A Sustainable Approach

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A B S T R A C T

Annual production of plastic has increased the 390.7 million metric tons in 2021 and plastic's reprocessing has all but its sustainable solution for disposal of plastic waste has been unsuccessful. Plastic materials (fragments) are continuously accumulating in the environment, like, in sea, soil, air, rivers as well as oceans. Microplastic contamination is becoming a major concern worldwide. Nowadays, scientists are developing sustainable idea for the degradation of plastic waste with the help of microorganisms. In biodegradation of microplastics by microorganisms like fungi and bacteria are playing vital role in breaking-downs of the plastic polymers in simpler form and after that plastics are biologically degraded. Microorganisms (*Pseudomonas sp., Rhodococcus sp., Bacillus sp., Zelerion maritimum, Microalgae*) that can degrade the different types of regular used synthetic plastics. The bacterial and fungal species produced Biosurfactants which helps the degradation process rapidly.

Introduction

Microplastics are common in today's our world. Microplastics are the pieces of any kinds of plastic lower than 5 mm (0.20 in) in diameter (Montealegre *et al.*, 2014). Microplastic can enter in natural ecosystem directly through various sources like plastic water bottle, cloths, cosmetics, food packaging materials as well as industrial effluents. Two categories of microplastics are presently considered (primary microplastic and secondary microplastic). Primary microplastics are any plastic pieces that are naturally 5.0 mm (or less than 5mm) in size or less prior entering in the natural environment. These involves microfibers from clothing, plastic pellets from petrochemical chemical companies and microbeads from cosmetics. Secondary microplastics comes from the break-downing of the heavy plastic materials through natural erosion processes after introducing into the ecosystem. The sources of secondary microplastics are the food and water containers,

microwave containers, beer, toys, plastic bags and tea bags. Plastics in marine habitats are a growing source of worry due to their endurance and consequences on seas (Green *et al.,* 2015), potentially humans (Jambeck *et al.,* 2015) and wildlife (Li *et al.,* 2016). Approximately 6300 million metric tons of plastic trash were produced between 1950 and 2015. The majority of this waste about 4900 million metric tons, ended up in landfills and the environment. Researchers predicted that by 2025, there will be 12,000 million metric tons of plastic garbage in landfills and the environment based on patterns from the previous years.

Approximately, a million birds and 10,000 marine species expire annually after plastic ingestion (Green *et al.,* 2015; Webb *et al.,* 2013). There are many different kinds of plastic garbage, A large majority (46.5%) of the tremendous weight of plastic pollution is made up of Polyethylene terephthalate and Polyethylene. A form of semi-aromatic thermoplastic co-polymer resin from the polyester family is polyethylene terephthalate, they have heteroatoms from the aromatic group in their main chain (Koshti *et al.,* 2018). Polyethylene contains a carbon-carbon backbone which is highly resistant to various degradation process, because its non-hydrolysable covalent bonds (Bombelli *et al.,* 2017; Y. Yang *et al.,* 2016). As chemical compounds, Numerous properties of polyethylene and polyethylene terephthalate are non-biodegradable, like its long hydrocarbon chain, low gas permeability, high tensile strength, high molecular weight, low gas permeability and resistance to chemical and physical deterioration (Gewert *et al.,* 2015; Joo *et al.,* 2018)

Suman *et al.,* (2020) stated that the histopology examination showed that chronic and acute exposure to polystyrene microplastics at 1 and 100 mg/respectively resulted in distortion of epithelial cells in the midgut area to polystyrene microplastics. In another study, (Chen *et al.,* 2020) suggested that *Cherax quadricarinatus* (a redclaw crayfish), was subjected to varying concentrations of 200 nm-sized polystyrene microspheres (0, 0.5 and 5 mg/L) for 21 days, the microplastics were dispersed in the intestines and hepatopancreas after ingestion and hampered *Cherax quadricarinatus* development. (Xiao *et al.,* 2018) suggested that 1 mg/L of polystyrene microplastics (PS-MPs) were introduced to freshwater microalgae *Euglena gracilis* for 24 hours. Microalgae had their vacuoles triggered and the pigment content had dramatically decreased ($p<0.05$).

When in the environment, (micro) plastics are subject to biotic and/or abiotic degradation, is mediated by microorganisms and, hence, is defined as a "process which is capable of decomposition of materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism in the enzymatic action of microorganisms, that can be measured by standard tests, in a specified period of time, reflecting available disposal conditions" (ASTM, 2010).

Bacterial species of the genus *Bacillus*, *Streptomyces* and *Pseudomonas* and fungal species of the genus *Aspergillus* and *Penicillium* can degrade Low-density polyethylene (LDPE) at an appreciable rate. (Equation 1), Under aerobic conditions, the polymers are degraded into monomers and released as water (H_2O) and carbon dioxide (CO_2) in a warm and humid atmosphere.

$$Organic\ matter + S + O_2 \rightarrow NO_2 + SO_2 + CO_2 + H_20 \ldots \quad (1)$$

(Equation 2), Under anaerobic conditions, plastic undergo biodegradation and release gases like methane and carbon dioxide

$$Organic\ matter + H_20 + Nutrients \rightarrow Residual\ matter + CO_2 + CH_4 + NH_3 + H_2S + Heat \ldots \quad (2)$$

The polymeric molecules are oxidized by free radicals, which breaks the chains. The process of light oxidation results in a number of physical and chemical alterations, including the formation of carbonyl groups and a reduction in the molecular weight of polymers. Heat of fusion is decreased by

thermal oxidation because carbonyl groups are produced more quickly and at temperatures higher than the melting point. Because of this, polymers are more vulnerable to microbial breakdown (Manzur *et al.*, 2004). There are a number of factors that contribute to biodegradation and environmental contamination, including erosion, discoloration treatment types, cracking, phase separation, and different types of polymers (Thomas *et al.*, 2015).

Microplastic Toxicity

Microplastics have been shown to affect plants and wildlife because they circulate in soil and aquatic habitats. Microplastic can have both direct and indirect effects on plants. Microplastics have been discovered to indirectly change soil characteristics, such as the number of soil-dwelling microbes and physiochemical properties, in addition to having a direct impact on plants by obstructing nutrient uptake and building up in roots, shoots, and leaves (Khalid *et al.*, 2020). Build-up of microplastic in various locations of plants, according to an investigation utilizing the aquatic plant *Utricularia vulgaris*. Plant's roots, leaves as well as bladders contained microplastics. Through an increment in the plant *Vicia faba*, microplastic accumulations in the plants have been proven to induce oxidative damage. Exposure to microplastics activates a number of oxidative enzymatic processes, such as those brought on by catalase, superoxide dismutase, and peroxidase. The presence of microplastics and other pollutants, such as heavy metals and plasticizers, as well as oxidative stress, which is a condition that can be triggered by these enzymes, has been associated to. Microplastics' ecotoxicity has been prior noted in plants but there is also evidence that it affects animals. Biological models frequently utilized to study the toxicity of microplastics include fish. According to research performed in laboratory environments, microplastic can affect dietary intake by building up in the fish gut. Additionally, it has been shown that microplastics promote organ failure and fish innate immune, growth, and inflammatory responses. Although in terms of microplastic ecotoxicity, data from lab-based environments fails to provide an accurate environment scenario. According to some reports, the environmental microplastics contain co-contaminants like plasticizing agents and metals like mercury and lead. Additionally, pollution caused by microplastic may influence humans. Almost everything may be a source or site of pollution, include water resources, sources of nutrition, as well as the atmosphere. Plastic particles encountered in the soil and water are consumed along with inhaled by people, causing an extensive variety of illnesses. Microplastics have been identified as stimulating the nitrogen-activating protein kinase (PK) pathway, that has been linked to irritation in human beings. They have consistently been found to possess neurological adverse effects by decreasing acetylcholinesterase activity, the inflammatory response that might cause cancers to grow. Also, it was previously proven that the association with microplastics and people alters cell activity at the level of molecular structure. (Chen *et al.*, 2020; Guo *et al.*, 2020; Hwang *et al.*, 2019; Llorca *et al.*, 2020)

Types of degradable plastic

Numerous plastics made from synthetic materials used on a regular basis, including polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) polystyrene (PS) and polyethylene (PE), are capable of being broken down by specific microorganisms (Figure 1). Four different kinds of readily biodegradable plastics may be distinguished: compostable bioplastics, bio-based bioplastics, biodegradable bioplastics and photodegradable bioplastics.

Compostable bioplastics

Composting need a particular climate for the ingredients to break down, in contrast to biodegradable products, which break down spontaneously and leave no harmful residue. Meereboer and colleagues, 2020. The rate of decomposition of this plastic is comparable to that of other biodegradable polymers. Plastic is classified

as biodegradable by standard testing that considers its degree of breaking down, ecological toxicity, and overall biodegradable properties.

Bio-based bioplastics

Bio-based plastics are varieties of polymers where all of the carbon is sourced by natural sources, such as woodland and farming products. Cellulose, soyabean, corn and starch are the certain examples of these sources (Getachew & Woldesenbet, 2016; Marichelvam *et al.,* 2019; Maraveas, 2020)

Biodegradable Plastics

Plastics that degrade owing to microbial activity are known as biodegradable materials. Biodegradable substances are those that decompose into biomass and biogases thanks to microbial activity (Jain *et al.,* 2010).

Photodegradable plastics

In this plastic kind, Light-sensitive subunits are linked by the polymeric structure. Ultraviolet (UV) absorption over an extended period of time might cause the structure of polymers to break down. Degradation is impossible when the radiation source is cut off.

Landfills lack sunlight that's why plastics in landfill cannot break down (Ezgi Bezirhan Arikan & Havva Duygu Ozsoy, 2015). The emission of harmful volatile organic compounds (VOCs), that are possibly harmful and connected to the natural deterioration of plastic waste, can result from artificial photo-degradation (Lomonaco *et al.,* 2020)

Biodegradation

This is the term for any chemical and physical transformation of a substance brought on by the activity of microbes. Bacteria, bacteria, and fungus, among other microorganisms, destroy both natural and manmade plastics (Iiyoshi *et al.,* 2014; Ishigaki *et al.,* 2004)

Aerobic biodegradation (aerobic respiration)

In this form of degradation, bacteria use oxygen as an electron acceptor to break down big organic molecules into smaller ones. This method produces carbon dioxide and water as byproducts. (Kawai *et al.,* 2019; Priyanka & Archana, 2011)

Carbon plastic+ Oxygen $\rightarrow$ CO_2 +H_2O+ carbon residual

Anaerobic biodegradation

Oxygen is not required for the breakdown of substances by the activity of microbes during anaerobic biodegradation. The natural attenuation of pollutants at hazardous waste sites depends heavily on oxygen. In place of oxygen, anaerobic bacteria employ the electron acceptors nitrate, iron, sulphate, manganese, and CO2 to break down big organic molecules into smaller ones.

Carbon(plastic) $\rightarrow$methane+carbon dioxide+ water+ carbon residual

All polymers are huge in size and insoluble in water, which prevents them from being immediately carried into the cells of microbes via their cell walls. By producing extracellular enzymes, microbes can exploit these polymers as a source of energy. These enzymes function outside of the bacterial cells to depolymerize polymers. Enzymes play a part in the intracellular and extracellular biodegradation of polymers. The biological deterioration of plastic polymers involves two processes: depolymerization and mineralization.

Exoenzymes, which are enzymes released outside of the cell, disassemble big polymers to create tiny, water-soluble molecules. These compounds are used as an energy source because they can cross semipermeable bacterial membranes. Depolymerization is the process by which big polymers are broken down, whereas mineralization is the process by which the end products are inorganic species like H_2O, CH_4, and CO_2. (Gu,

2003). Only the formation of water, carbon dioxide, and microbial mass was noted in an aerobic environment, however in anaerobic/methanogenic and sulfidogenic circumstances, CH4 and H2S were noted as additional final outcomes generated by the polymer (Shahnawaz *et al.,* 2016)

Mechanism of biodegradation

Polymer biodegradation happens in three stages; (a) microbial adhesion to the polymer's surfaces, (b) using polymers as sources of carbon and (c) polymer break downing. For the purpose to gain energy for their development, microorganisms cling to the surface of polymers and break them down by secreting enzymes (Danso *et al.,* 2018). Large polymers decomposed into low molecular weight monomers and oligomers. Following internal diffusion, certain oligomers may be absorbed in the environment of microbes (Fig 2).

Biologically upcycling conception for plastic wastes

The primary current techniques for getting rid of plastic garbage include landfilling, burning, mechanical and chemical recycling. (Peng *et al.,* 2018). Due to its practicality and affordability, dumping is the primary technique for disposing of plastic trash in most developed and developing nations. Although the accumulating plastic trash has taken up a lot of space. The incineration of plastic trash can minimize the need for dumpsters to produce thermal energy, but it also needs to limit the environmental consequences of secondary pollutants such dioxins, CO_2, NO, and other byproducts of the procedure of incineration. Despite the fact that mechanical recycling has taken over as the main recycling technique and is used to reuse thermoplastic wastes, the qualities of most recovered materials are considerably impaired after many processing cycles, and the resultant economic values are thus constrained. An alternate method is the recycling of chemicals, which may salvage monomers and other compounds from plastic trash, but its effectiveness depends on how affordable the procedures are and how effective the catalysts are (Rahimi & Garciá, 2017). According to current reports, just 9 to 12% of the world's plastic garbage gets recycled or burned, while up to 79% is dumped into landfills or the environment. This shows that there is an urgent need to research creative recycling techniques for getting rid of plastic waste (Geyer *et al.,* 2017; Garcia & Robertson, 2017).

Recent research has shown that a variety of microbes and biocatalysts are able of breaking down manmade polymers. Despite the fact that there have been a lot of evaluations and opinions on the subject of plastic biological degradation, most of them have primarily concentrated on the biodegradation of a specific kind of plastic, such polyethylene (Restrepo-Flórez *et al.,* 2014), Polystyrene (Ho *et al.,* 2018), Polypropylene (Arutchelvi *et al.,* 2008), Polyurethane (Cregut *et al.,* 2013; Magnin *et al.,* 2020; Peng *et al.,* 2018), and Polyethylene terephthalate (Kawai *et al.,* 2019; Taniguchi *et al.,* 2019; Wei & Zimmermann, 2017). It is crucial to conduct a comprehensive investigation of how all significant forms of plastic deteriorate (Wei & Zimmermann, 2017). A review that emphasizes both biological upcycling and biological degradation of plastic trash is much more appealing (Salvador *et al.,* 2019; Wei *et al.,* 2020; Wierckx *et al.,* 2015). We have outlined the microbes and enzymes that have been demonstrated to be able to break down plastics like polyethylene and polyethylene terephthalate in this review. We have also discussed the microbial metabolic pathways of the products of plastic depolymerization and the current efforts to use these products as feedstocks for microbial valorization. By creating a metabolic connection between the biodegradation of plastic wastes and the manufacture of useful compounds in microorganisms, we have sought to construct a biologically upcycling notion for plastic wastes based on the aforementioned understandings.

Biodegradation of synthetic plastic

Several microorganisms that can break down polyolefins (PE, PS, and PP), PVC, PUR, and PET

have been isolated from the open environment, including soil contaminated by crude oil, landfill sludge, marine water and soil from plastic-dumping sites. Identification of the depolymerases and other important enzymes involved in plastic degradation depends on the screening of microorganisms that degrade plastic. In this review we only discussed about PE and PET only.

Polyethylene (PE)

Even before in the 1970s, Albertsson conducted research on the microbiological breakdown of ^{14}C-labeled PE (Molecular weight of 300,000 Da) by inoculating three various soil microbes (Albertsson, 1978). Regarding the emission of $^{14}CO_2$, after two years, it was estimated that the microbial breakdown rate of PE was between 0.36 to 0.39 %. (Albertsson, 1978). The microbial breakdown rate decreased to 0.16% when the ^{14}C-labeled PE was extracted with cyclohexane to remove its low molecular weight components (average weight molecular weight of 1,000 Da) (Albertsson & Bánhidi, 1980). The release of $^{14}CO2$ was accordingly shown to be mostly caused by the microbial breakdown of the low molecular weight PE portion, which was comparable to that of straight-chain n-alkanes (Albertsson & Bánhidi, 1980). Then, following the outcomes of a numerical simulation, (Kawai *et al.,* 1995) asserted that the maximum molecular weight required for PE breakdown by microbes was around 2,000 Da (Kawai *et al.,* 2019; Kawai *et al.,* 1995)

Although PE's large weight in molecules was expected to be a major barrier to microbial decomposition, physical and chemical pretreatments, such as ultraviolet (UV) radiation, have been found to be effective (Albertsson, 1978; Albertsson & Bánhidi, 1980; Karlsson *et al.,* 1988; Karlsson & Albertsson, 1995), chemical oxidizing agents (Brown *et al.,* 1974), and thermo-oxidation (Lee *et al.,* 1991), Given that both pretreatments caused long-chain PE to depolymerize and create low molecular weight products, they could aid in the microbial breakdown of long-chain PE (Albertsson *et al.,* 1995, 1998; Hakkarainen & Albertsson, 2004). Therefore, it was believed that photo- or thermo-oxidation and the biological processes of microbes may work together to degrade long-chain PE in the natural world (Hakkarainen & Albertsson, 2004).

It was interesting to determine whether the long-chain PE (molecular weight > 2,000 Da) natural microbial species could breakdown it. Several strains that can break down untreated PE have been found in a range of settings, such as mulch films, water from the ocean, sewage waste, garbage dumps, and soils polluted with petroleum products (Arutchelvi *et al.,* 2008; Balasubramanian *et al.,* 2010; Delacuvellerie *et al.,* 2019; Harshvardhan & Jha, 2013; Paço *et al.,* 2017; Restrepo-Flórez *et al.,* 2014; Sarmah & Rout, 2018; Tribedi & Sil, 2014; Yang *et al.,* 2014). Depending on the descriptions of biofilm development on PE films, weight loss of PE materials, surface degradation, and variations in the thermal and mechanical properties of PE, several of these strains demonstrated the potential to use un-pretreated PE as sources of carbon. For instance, it was noted that after 70 days of the incubation process a strain of *Serratia marcescens* decomposed untreated PE at an average weight loss of 36 percent(Azeko *et al.,* 2015). Additionally, during a 42-day period, two cyanobacteria, *Phormidium lucidum* and *Oscillatoria subbrevis*, had the potential to degrade 30% of the original weight of the tested PE (Sarmah & Rout, 2018). These encouraging reports of long-chain PE disintegration based on weight loss, however, are less compelling because there isn't any more proof that the degradation of the long-chain PE is what is causing the weight loss in addition to the low molecular weight PE components.

Notably, a few studies claimed that waxworms could chew and consume PE films in addition to beeswax, given their innate capacity to consume and digest beeswax. Notably, a few studies claimed that waxworms could chew and consume PE films in addition to beeswax, given their innate capacity to consume and digest beeswax (Bombelli *et al.,* 2017; Chalup *et al.,* 2018; Kundungal *et al.,* 2019; Yang *et*

al., 2014). Interaction to the homogenization of the waxworm *Galleria mellonella* has revealed the natural breakdown of PE (Bombelli *et al.,* 2017) or after transit via *Achroia grisella*, a smaller waxworm (Kundungal *et al.,* 2019), according to the changes in chemical compositions characterized by the analyses of Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). In order to ascertain if PE has been depolymerized in the waxworm stomach, however, more research is required.

Since it has been established that intestine microbial symbionts are essential for the digestion of insects (Engel & Moran, 2013), We have proposed that an essential role in the breakdown of PE is also played by the microbial symbionts found in the waxworm stomach (J. Yang *et al.,* 2014). Within a constrained period of incubation of 60 days, the PE-degrading capacity of two bacterial strains, *Enterobacter asburiae* YT1 and *Bacillus sp.* YP1, was documented based upon characterizations of biofilm formation, changes in the PE's physical properties (tensile strength and surface topography), chemical structure (hydrophobicity and appearance of carbonyl groups), molecular weight (accompanied by the format), and molecular weight. These results suggested that waxworm bacteria can be a useful source for future screening of PE-degrading microorganisms (Yang *et al.,* 2014; Yang *et al.,* 2015).

A wide variety of PE-degrading microorganisms have been identified, however only four microbial enzymes have been identified as being essential for PE breakdown (Iiyoshi *et al.,* 1998) discovered that the lignin-degrading fungus *Phanerochaete chrysosporium's* manganese peroxidase (MnP) may reduce the tensile strength and average molecular weight of PE film. (Zhao *et al.,* 2004) Moreover, it was discovered that combining hydrogen peroxide with soybean peroxidase (SBP) might oxidize the PE film's surface and reduce its hydrophobicity. (Santo *et al.,* 2013) shown that the PE-degrading bacteria *Rhodococcus ruber* C208's extracellular laccase was capable of oxidizing the PE films to produce carbonyl groups and reduce their molecular weight. Although the aforementioned peroxidase and laccase were shown in these earlier investigations to be capable of catalyzing the breakdown of PE, their catalytic processes in the microbial degradation of PE remained unknown. Additionally, *Escherichia coli* was used to clone three alkane hydroxylase genes, alkB, alkB1, and alkB2, and the resulting recombinant strains were discovered to be capable of destroying low molecular-weight PE (Gyung Yoon *et al.,* 2012; Jeon & Kim, 2015). According to these findings, alkB, alkB1, or alkB2 were crucial in the breakdown of low molecular-weight PE. Additionally, a recent research based on calculations in the field of quantum mechanics revealed that it would be feasible for oxidases or oxygenases to enzymatically cleave the carbon-carbon bonds of polyolefins (PE and PS) (Xu *et al.,* 2019). To describe the biochemical roles that oxidases or oxygenases, such as the enzymes produced by the genes alkB, alkB1, or alkB2, play in the biodegradation of PE, further work will be needed in the future.

Polyethylene terephthalate (PET)

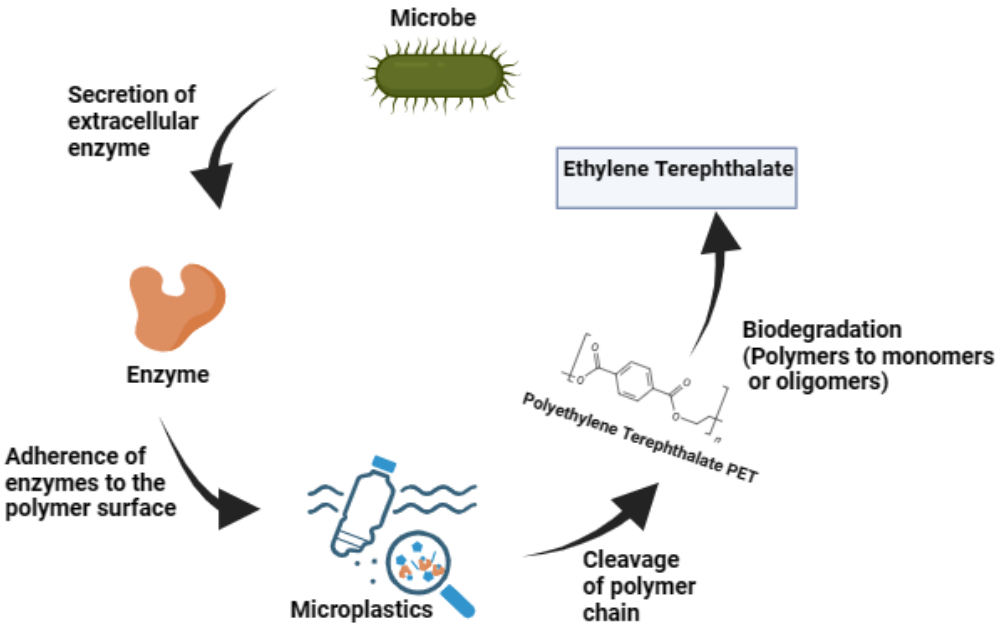
The primary goal of research into hydrolases that can hydrolyze PET was to alter the surface wettability of PET textiles (Alisch *et al.,* 2004; Cavaco-Paulo & Gübitz, 2003; Hsieh & Cram, 1998; O'Neill & Cavaco-Paulo, 2004; Vertommen *et al.,* 2005; Zhang *et al.,* 2004). Ester linkages on the surface of PET were hydrolyzed by an enzymatic surface modification method to yield polar hydroxyl and carboxylic groups, while PET's interior bulk was not damaged.

In a recent analysis that concentrated on the enzymatic breakdown of PET, (Kawai *et al.,* 2019). PET surface-modifying enzymes are hydrolases, which having a modest surface-hydrolyzing capacity (Kawai *et al.,* 2019). In contrast, PET hydrolases were defined as hydrolases having a sufficient capacity to hydrolyze the inner bulk of PET (resulting in at least 10% weight loss) (Kawai *et al.,* 2019)

Fig.1 Types of degradable plastics with their abbreviation, symbol, example

Polyethylene terephthalate	**PET**	1	(Water Bottles,Jars,Caps)
High-Density Polyethylene	**HDPE**	2	(Hand Wash Container,Shampoo Bottles)
Polyvinyl Chloride	**PVC**	3	(PCV Cards,Plumbing Pipes)
Low-Density Polyethylene	**LDPE**	4	(Bread Bags,Plastic Films)
Polypropylene	**PP**	5	(Yogurt Cups,Hangers)
Polystyrene	**PS**	6	(Packaging materials)
Other	**Other**	7	(Baby Bottles,Nylon,CD)

Fig.2 Mechanism of enzymatic biodegradation of polymers (Alshehrei, 2017)



Nearly 2005, (Müller *et al.*, 2005) a cutinase-like hydrolase TfH from the actinomycete *Thermobifida fusca* has been shown to efficiently break down low-crystallinity PET (lcPET, 9%) at 55 °C for three weeks. This is the initial study on the enzymatic destruction of the PET film's inner bulk, which pave the way for future enzymatic PET recycling (Mueller, 2006). Then, using lcPET films (7%) and high-crystallinity biaxially oriented PET films (hcPET, 35%) as substrates, Ronkvist *et al.*, compared the PET-hydrolyzing activities of three cutinases from distinct microorganisms: *Humicola insolens* (HiC, now called *Thermomyces insolens*), *Pseudomonas mendocina* (PmC), (Ronkvist *et al.*, 2009). The results revealed that HiC, as opposed to PmC or FsC, produced a 97% weight loss of lcPET film (7%) at 70°C after 96 hours. HiC can therefore be referred to as a PET hydrolase, whereas PmC and FsC are PET surface-modifying enzymes. However, only 35% of the hcPET films could be hydrolyzed by the three cutinases.

Then, (Sulaiman *et al.*, 2012) discovered that an LC-cutinase can effectively hydrolyze low-crystallinity PET package film (lcPET-P, 8.4%) at 50°C and cause up to 50% reduction in weight during 7 days. A single gene from a metagenomic library of leaf-branch compost is responsible for encoding this enzyme. Furthermore, Kawai *et al.*, discovered that a cutinase from *Saccharomonospora viridis* AHK190 may hydrolyze the lcPET (7%) and lcPET-P (8.4%) at 63°C, causing a weight loss of 13.5 and 27.0% for lcPET and lcPET-P, respectively, over 3 days (Kawai *et al.*, 2014). Recently, it was demonstrated that two low-crystallinity PET samples from post-consumer packages (AP-PET, 5%; CP-PET, 6%) with maximum weight losses of 50.5 and 56.6%, respectively, could be degraded by the recombinant *Thermobifida fusca* cutinase TfCut2 expressed by *B. subtilis* within 120 hours at 70°C (Wei *et al.*, 2019)

Remarkably, (Yang *et al.*, 2016) discovered a bacterial species, *Ideonella sakaiensis* 201-F6, potential of breakdown lcPET films (1.9%) at room temperature, and they characterized as a PET-hydrolyzing enzyme, termed as *Is*PETase, from this bacterial species (Yang *et al.*, 2016). At a mesophilic temperature of 30°C, the IsPETase showed more PET breakdown efficiency than the previously described PET hydrolases and was heat-labile (20–45°C) (Taniguchi *et al.*, 2019; Yang *et al.*, 2016). However, IsPETase only degraded lcPET film by 1% (weight loss) over an incubation period of 24 hours at 30°C, which is significantly less than the breakdown rates of the previously described PET hydrolases at thermophilic temperatures (50–70°C) (Wei *et al.*, 2019). Additionally, IsPETase's hydrolytic activity against lcPET films (1.9%) was clearly greater than that against hcPET films (30–40%) (Yang *et al.*, 2016).

As a whole, the lcPET (10%) but not the hcPET is more likely to be degraded by the previously known PET hydrolases (Ronkvist *et al.*, 2009; Vertommen *et al.*, 2005; Wei *et al.*, 2019; Yang *et al.*, 2016). The differences in the macromolecular aggregation structures of the polymer might be used to explain how varying crystallinity affects enzymatic breakdown. In general, the packing of polymer molecules is not homogeneous, with both ordered (crystalline-like) and disordered (amorphous) domains present.

The lcPET, which has a large percentage of amorphous domains, is more sensitive to enzymatic degradation because the polymer chains in amorphous domains are less tightly packed than those in crystalline domains. However, the high-crystallinity PET (30∼40%) represents the most prevalent forms of postconsumer plastic, and strategies for reducing the crystallinity of PET to increase the enzymatic breakdown are highly desired.

Furthermore, the PET enzymatic hydrolytic processes tend to occur at temperatures close to the PET glass transition temperature (Tg, 65–75°C). The polymer chains in the amorphous PET domains can become sufficiently mobile at such a thermophilic temperature to reach the active PET hydrolase sites (Kawai *et al.*, 2019; Ronkvist *et al.*,

2009; Wei & Zimmermann, 2017). Therefore, it implies that thermostable PET depolymerases are necessary for effective enzymatic breakdown of PET. Thermostability of these PET hydrolases has been increased through the use of glycosylation (Shirke *et al.,* 2018) and rational protein engineering techniques, such as surface salt bridge optimization (Shirke *et al.,* 2016), mutation of Ca2+ and Mg2+ binding sites (Then *et al.,* 2015), introduction of a disulfide bridge (Then *et al.,* 2016), stabilization of a 6–7 connecting loop, and extension. However, it is still possible to extend the half-life of PET hydrolases over 65°C.

Enzymatic degradations of plastics

Plastic breakdown by microbial enzymes is highly challenging because the carbon-carbon backbone lacks hydrolysable groups. Lowering molecular weight is the initial stage that is performed by both biotic and abiotic agents. When exposed to UV light, the polymer's carbonyl group may be swiftly degraded by microbial enzymes (Leja & Lewandowicz, 2010; Novotný *et al.,* 2018). To break down polymers, many enzymes are used, including laccase, manganese-dependent enzymes (enzymes that break down lignin), urease, lipase, and protease. The thermostable laccase may degrade polyethylene (PE) in 48 hours at 37 °C (Jaiswal *et al.,* 2019)

Characterization of plastic biodegradation

Although not all polymers are soluble in water, those that are quickly broken down and transformed into alcohols, ketones, and acids. There are a few ways to keep track of how plastics degrade (Bhardwaj *et al.,* 2013)

- Altered plastic surface characteristics.
- The plastic's physical and mechanical characteristics are altered.
- Rate of oxygen consumption.
- Biomass production demonstrates how microbes use plastics as a source of carbon for growth.

Factors affecting biodegradation

Physical and chemical factors can help determine a plastic's biodegradability. The following factors influence how quickly bacteria degrade plastic.

- Functional groups that are available and improve hydrophobicity (Wang *et al.,* 2021)
- Structure complexity, including linearity and branching (Tokiwa *et al.,* 2009)
- Bond types, such as amide and ester bonds, are easily breakable. (Ester > Ether > Amide > Urethane) Chain coupling) (Shams *et al.,* 2020)
- Composition based on molecular structure (Shams *et al.,* 2020)
- Example of a polymer's nature and physical attributes: pellets, films, and powder (Kawai *et al.,* 1995)
- The molecular weight and density of the polymer (Tokiwa *et al.,* 2009)
- Amount of crystalline and amorphous regions in the TM morphology (Wang *et al.,* 2021)
- Soft toughness polymers deteriorate more quickly than hard or tough ones (Swift, 1993)

Microorganisms have a lower capacity for degradation when polymer solubility is lowered. Plastics' resistance to microbial assault is increased by reducing their solubility. Their cell membrane allows them to adapt to microorganisms (Siracusa *et al.,* 2008). Polymers with an amorphous structure are more susceptible to assault by microbial enzymes than those with crystalline structures. As a result, an increase in crystallinity slows the breakdown of polymers (Slor *et al.,* 2018). Plastics can limit microbial activity in a hydrophobic environment by preventing water absorption.

Plastics are polymers made from petroleum and have a variety of uses. PE bags are widely utilized all over the world. Due to biodegradation, thermo-oxidative degradation, photodegradation, thermal, and hydrolysis processes in the ecosystem, the availability of micro- and nanoplastics in aquatic environments has increased significantly. This poses a serious threat to aquatic life (fresh and marine), as

well as human life through the food web. To remove these polymers from the ecosystem, suitable biodegradable techniques must be used. The hydrophobic and inert characteristic of polymers makes them difficult to remove or breakdown. Microbes have demonstrated promising potential to degrade these polymers in addition to physical and chemical techniques.

Using wastewater that was originally polluted with polymers, it is necessary to further assess the possible usage of microorganisms for polymers removal. There are still issues to be resolved about the elimination of microplastics and nanoplastics, their toxicity, and the use of microorganisms. There are still questions concerning the removal of microplastics and nanoplastics, their toxicity, and the employment of microorganisms, and it is important to further investigate the probable utilization of microbes for polymers removal using wastewater that was initially contaminated with polymers.

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