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BIOLOGICAL DEGRADATION OF SYNTHESISED POLYETHLENE PLASTIC BY MICROORGANISMS AND INVERTEBRATES

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Abstract:
Plastic waste management and recycling have become important global issues because they damage living creatures in all environments. Although plastics make our lives easier, their uncontrolled use and thoughtless disposal pose a persistent threat to the ecosystem since they do not dissolve naturally even after many years and interfere with a variety of natural and artificial processes. Plastics' inability or poor biodegradation has resulted in their accumulating in the environment, creating widespread contamination and damaging both marine and terrestrial life forms. There has been little or no systematic analysis on polyethylene degradation; this review focuses on biological polyethylene degradation, with a special emphasis on bacteria, fungus, and algae involved in the polyethylene degradation process. Furthermore, invertebrates and microbial enzymes engaged in the process were highlighted, while the mechanism of biodegradation was not overlooked.

Keywords: Polyethylene, Biodegradation, Bacteria, Fungi, Algae and Invertebrates

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1. Introduction

Due to its lightness, durability, inertness, and low cost, plastic have become a highly important aspect of human society [1]. A synthetic polymer is plastic. Carbon, hydrogen, silicon, oxygen, chloride, and nitrogen make up this compound. It is obtained from a variety of sources, including oil, coal, and natural gas [2]. Because of their strength and durability, plastics are widely used. Polyethylene (PE), Poly Ethylene Terephthalate (PET), Nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) are a few examples of plastics [2].

Although plastics make our lives easier, their uncontrolled use and thoughtless disposal pose a persistent threat to the ecosystem since they do not dissolve naturally even after many years and interfere with a variety of natural and artificial processes [3]. Plastics' non-or sluggish biodegradation has resulted in their accumulating in the environment, creating widespread contamination and damaging both marine and terrestrial life forms [4]. Plastics not only create flooding by clogging the drainage system, but they also enter the food chain of animals and harm their digestive systems [5]. Long-term buildup of plastics in soil alters the microbial community structure [6].

Polyethylene (PE) accounts for 64% of total synthetic plastic since it is widely utilized in the production of bottles, carrier bags, disposable products, rubbish containers, margarine tubs, milk jugs, and water pipes [7]. Every year, between 0.5 and 1 trillion polyethylene bags are used worldwide [8]. Because of their longevity, light weight, and processability, these polymers can persist in nature for generations and end up in landfills and natural water

resources, posing a serious threat to the environment and its ecosystems [8, 9]. Polyethylene consumption worldwide is quickly increasing by approximately 320 million tons per year and appears to be doubled by 2034 [10].

Polyethylene littering in the environment is a prevalent concern in most African cities because the bulk of their garbage is not recycled [11]. PE bags have been observed to kill terrestrial animals such as cows [12]. Most manmade plastics degrade in nature over thousands of years as a result of synergistic action between environmental conditions and microorganisms [13].

Faced with an expanding global polyethylene waste problem and the limitations of traditional non-biological technologies, biological approaches to depolymerizing waste or converting it into valuable products are increasingly being studied. Biodegradation is carried out by a group of microorganisms, some of which break down the polymer into smaller parts, while others use the monomers and produce less toxic and recalcitrant byproducts that serve as an energy source for other microbial groups [14].

Polyethylene biodegradation refers to the processes through which living organisms (mostly invertebrates, algae, bacteria, fungi, and others) depolymerize polyethylene and utilize it as a food source. Biodegradation, as compared to typical degradation technologies, has the advantages of low cost and high operability [14]. It also does not emit poisonous fumes or dangerous substances into the environment, making it an appealing, ecologically and environmentally friendly polyethylene degrading process.

Microorganisms capable of colonizing polyethylene were found in the 1970s. Since then, the microbe has been actively researched for application as a biological mechanism of degrading polyethylene. Microorganisms such as bacteria, fungi, and algae are engaged in the biodegradation of materials [15]. Microorganisms like as bacteria and fungi have been linked to PE biodegradation [1]. Several researchers investigated polyethylene degrading bacteria such as *Bacillus spp., Pseudomonas spp., Acinetobacter spp., Brevibacillus sp., Flavobacterium spp., Ralstonia spp., Micrococcus spp., Microbacterium sp*., and *Nocardia sp*.

Most commercially used plastics, such as polyethylene (PE) (low density, i.e., LDPE, and high density, or HDPE), are biodegradable [16]. Their biodegradability is generally hampered by several factors, including their inability to enter the microbial cell due to high molecular weight; improved chemical structure stability; the absence of functional groups that microbial enzymes can attack; and high hydrophobicity and degree of crystallinity due to their large carbon backbone [17]. Plastics' environmental toxicity, large-scale accumulation, and persistence necessitate immediate action on the development of efficient and environmentally acceptable technologies for their destruction, as well as research into microbial catabolic capacity for plastic biodegradation [16].

Low density polyethylene can also be degraded by the laccase enzyme (copper-binding bacterial enzyme) (LDPE). This enzyme decreases the molecular weight of the low density polyethylene (LDPE) polymer (40,000 daltons) while increasing the keto-carbonyl index [18]. Extracellular depolymerase enzymes disassemble complicated synthetic polymers into monomers and dimmers [19]. Fungi have a hydrolytic enzyme system that produces hydrolases that aid in polysaccharide degradation [20]. The current review includes not only bacteria, fungus, algae, and invertebrates involved in polyethylene biological degradation but also microbial enzymes and biodegradation mechanisms.

2. Bacteria Involved in the Degradation of Polyethylene

The ability of bacteria to degrade various materials such as petroleum, compounds, metals, and polymers has been demonstrated. A large variety of bacterial species from the genera Bacillus, Lysinibacillus, and Marinobacter have demonstrated the ability to breakdown polyethylene polymer [21]. These polyethylene-degrading bacteria are found in a variety of ecological settings, including forest soil, landfills [22], the marine environment [23] and insect guts [24].

The biodegradation of polyethylene by *Lysinibacillus* isolated from forest soil was reported by Jeon *et al*., [21]. During the process, a reduction in weight of about 9% was recorded for a period of more than twenty-six days, while through Scanning Electron Microscope (SEM) imaging, an increase in rough surfaces was observed. Also, the detection of various CH_2 group-containing oxidation products was reported in the same study [21]. The degradation ability of *Pseudomonas aeruginosa* toward polyethylene and how it was impacted by environmental factors was assessed by Tamnou *et al*., [25]. They found that the degradation products of polyethylene seemed to have an inhibitory effect on the growth of *P. aeruginosa*.

Another work published by Park and Kim [26] showed the potential of a mixed bacterial population of Bacillus and Paenibacillus to reduce HDPE weight and particle size. The consortium isolates were isolated from a landfill and cultivated in aqueous medium with polyethylene microplastics as the sole carbon source; weight and average particle size were reduced by 14.7% and 22.8%, respectively. SEM revealed colonization of these bacterial isolates on the surface of the microplastics, while GC-MS analysis confirmed the production of metabolites (2-dodecanol, 1,8-nonanediol, and 1-dencene).

In another study, Gao and Sun described a bacterial community isolated from a maritime environment that was exclusively focused on polyethylene breakdown, consisting primarily of Idiomarina (50%), Marinobacter (28%), Exiguobacterium (18%), and others (4%) [27], this community colonized, decomposed, and used polymer

polyethylene in the marine environment successfully. In the same group, the bacteria were researched further on the polyethylene degradation processes, followed by characterization of the polyethylene degradation products [27]. A transcriptome approach was used to characterize the intricacies of polyethylene degradation in this bacterial population, indicating the potential steps of biodegradation. Within a few days, the reconstituted bacterial community significantly destroyed polyethylene and achieved nearly total destruction within two weeks.
Acinetobacter and *Bacillus* isolated from the intestinal tract of *Tenebrio molitor* larvae were co-cultured with polyethylene as the sole carbon source [28]. These bacteria were observed to remove about 18% of the polyethylene mass after 30 days of cultivation and form a dense biofilm on the surface of the polyethylene film. ATR-FTIR-based observation of-C-C stretching and-O-H stretching showed that polyethylene was oxidized after exposure to the flora [28].
Similarly, Tarafdar *et al*. [29] discovered that Bacillus siamensis interacts with Low Density Polyethylene (LDPE) as the sole carbon source to build a biofilm on the surface of microplastics. The same bacterium might use LDPE to produce unsaturated hydrocarbons, polyketides, terpenoids, aliphatic/peptides, dicarboxylic acids, lipid molecules, and other products [29]. As previously said, a variety of external factors can either enhance or impede the rate of microbial degradation of polyethylene, and the favorable conditions have been the focus of research to promote bacterial degradation of polyethylene. UV pretreatment of polyethylene is one such strategy, since polyethylene treated with UV radiation [30] and thermally treated [31] has been demonstrated to be more rapidly destroyed by bacteria.
Both culture-based and culture-independent metagenomic studies have highlighted the PE (LDPE or HDPE) biodegradation abilities of several bacterial taxa, viz. *Pseudomonas* [5]; *Alcanivorax, Ideonella, Marinobacter, Arenibacter* [32]; Aneurinibacillus [3]; *Comamonas, Stenotrophomonas* and *Delftia* [33]. Besides, several members of soil-inhabiting actinobacteria (Rhodococcus sp., Streptomyces coelicoflavus, Streptomyces KU1, KU5, KU6, KU8, Streptomyces werraensis, Streptomyces humidus, Streptomyces parvullus, Streptomyces aburaviensis, Amycolatopsis sp. HT-32, Nocardia sp. Saccharothrix wayandensis, etc.) have shown either weight reduction or partial degradation of PE films [34, 35].
Skariyachan *et al*. [36] found that Bacillus vallismortis bt-dsce01 could degrade LDPE up to 75% after 120 days of incubation. Maroof *et al*. [37] identified a new bacterial strain, *B. siamensis*, which can breakdown 8.46% LDPE after 90 days of incubation.

3. Polyethylene Degradation by Fungi

The ability of fungal species to breakdown various polyethylenes has also garnered increased attention in recent years, and their ability to utilize polymers as their primary or sole carbon source has been widely demonstrated and published. As an example, Gajendiran *et al*. [38] investigated the degradation of LDPE using fungal strains recovered from landfills. *Aspergillus clavatus* destroyed over 35% of LDPE in 90 days. FTIR investigation found signals related to C-O production, N-O bending, and C-O stretching at 1735 cm^{-1}, 1365 cm^{-1}, and 1217 cm^{-1}. Kunlere *et al*. [39] revealed that Aspergillus flavus strains MCP5 and MMP10 exploited LDPE as a carbon source without additions.
Members of the fungus species Aspergillus [5], Penicillium, and Zalerion [3] are well known for biodegrading low density polyethylene (LDPEs) and high density polyethylene (HDPEs) Proteases, lipases, cutinases, laccases, manganese peroxidases, lignin peroxidases, and alkane hydroxylases have been discovered as significant microbial enzymes in the biodegradation of PE (40). Similarly, Muhonja et al. [5] reported that after 112 days of incubation, both Aspergillus oryzae strain A5 and B. cereus strain A5 were able to degrade LDPE at 36.4 and 35.72%, respectively. Taghavi et al. [41] claimed that the fungus Aspergillus flavus could degrade 5.5% of HDPE in 100 days.
Polyethylene breakdown has been recorded by *Aspergillus, Cladosporium, Fusarium, Penicillium*, and *Phanerochaete* [16, 42]. *Phanerochaete chrysosporium* is a fungus that degrades high molecular weight polyethylene in nitrogen- and carbon-limited environments [43]. Pencillium has been found to have additive-free Polyethylene breakdown. *Aspergillus niger, Aspergillus japonicas*, and *Fusarium sp.* [44]. *Penicillium chrysogenum* NS10 (KU559907) and *Penicillium oxalicum* NS4 (KU559906) have been identified as HDPE and LDPE degraders [45].

4. Polyethylene Degradation by Algae

Algae biodegradation characteristics for the environment have been widely established in industrial applications. They can lower various organic and inorganic contaminants in the environment to a somewhat safe level by accumulation, adsorption, and/or metabolism [46]. However, much recent research has concentrated on the use of algae to generate green polymers.
Relatively few studies have examined the potential of algae to degrade synthetic polyethylene. That said, some research groups have shown that Anabaena spiroides, Navicula pupula, and Scenedesmus dimorphus can degrade HDPE and LDPE [47, 48]. The strains formed biofilms on the surface of HDPE and LDPE. Moreover, a genomic

analysis predicted the existence of key enzymes responsible for polyethylene degradation, including laccase, esterase, lipase, thioesterase, and peroxidase [48].
However, when compared to typical bacteria, algae's breakdown efficiency is inadequate. This is related to the fact that they get their energy from sunlight and consume carbon dioxide in the air as their primary carbon source [49]. Although algae can colonize and assimilate microplastics on polyethylene surfaces, their metabolic processes do not seem to mineralize polyethylene. This metabolic mechanism allows polyethylene to bio-accumulate and enters the food chain [50].

5. Invertebrates Responsible for Polyethylene Degradation
Recent research has revealed that invertebrates can play an essential role in the breakdown of polyethylene. Some research groups have debated whether different insects can genuinely breakdown polyethylene and the importance of symbiotic microbes in their intestines [51, 52, 53]. For example, investigations into polyethylene decomposition utilizing mealworms, superworms, larger waxworms, and mealybugs have been published [54; 52]. However, whether polyethylene breakdown is dependent on stomach commensal microorganisms in these creatures is still debated.
Cassone *et al*. [54] nassessed the biodegradation of polyethylene by G. mellonella larvae and found that a diet of LDPE alone enabled the subsistence of G. mellonella larvae but was insufficient for growth. These results suggest real biodegradation did not occur during passing of the PE through the gut's larva, but the first biodeterioration or minor oxidation may have taken place, resulting in changes in physical properties rather than chemical ones. They demonstrated that a reduction in the gut microbiome significantly hinders the ability of *G. mellonella* to metabolize polyethylene.
The chewing and ingesting of polyethylene by T. molitor larvae created holes and reduced the size of polyethylene films, but digestion was not explicitly confirmed [55]. They suggested that the intestinal microbiomes of these insect larvae play a key role in the initial short-term biodegradation process, which occurs rapidly within the larval insect gut. However, the insect gut microbiome alone is not sufficient for this initial rapid biodegradation of PE. Rather, both the insect digestive system and the larval gut microbiome are required to achieve accelerated biodegradation of the PE polymers [56].
Acinetobacter sp. strain NyZ450 and Bacillus sp. strain NyZ451 were recovered from the intestines of T. molitor larvae [28]. Both strains' cells can depolymerize LDPE but cannot grow on it. Over 30 days, their co-culture grew on LDPE and eliminated 18% of the LDPE mulching films. This implies that numerous microorganisms are required for LDPE biodegradation.
According to a recent study, gut bacteria of T. molitor larvae and Tenebrio obscurus (dark mealworms) larvae can still execute LDPE depolymerization when gut microbes are suppressed by antibiotics, but it is less effective than when the microbes are not suppressed [57].. Tenebrio molitor, yellow mealworms, can digest polyethylene on their own and have intestinal microorganisms that influence decomposition [58]. In another work, Li *et al*. [51] found that despite antibiotic inhibition of gut microorganisms, T. molitor may still undertake LDPE depolymerization, implying that LDPE biodegradation is less dependent or independent of gut microbes [51].

Similarly, the mealybug *Planococcus citri* and the symbiotic bacteria in its body also use polyethylene as a carbon source cooperatively [55]. Peng and coworkers found that *Z. atratus* larvae defragmented ingested LDPE and PS foam into microplastics but not into nanoplastics during biodegradation [52].
A study published in 2017 indicated the rapid biodegradation of polyethylene by larger waxworms, larvae of the wax moth Galleria mellonella, which chew and potentially destroy the polymer at a high pace [59]. A later study found that bigger waxworm salivary glands were engaged in polyethylene breakdown, although only to a limited amount [60, 52]. The researchers did not address the independence between the ability of bigger waxworms to degrade polyethylene and the microbial ecology in the stomach in the aforementioned article.
In another study, however, polyethylene breakdown was still seen after antibiotics reduced the gut bacteria of larger waxworms [24]. According to current research findings, larger waxworms exhibit LDPE degradation capacity, and LDPE-degrading bacteria were recovered from their intestines. The interaction between the larval host and plastic-degraders should be studied further.
Besides mealworms, superworms, greater waxworms, and mealybugs, other invertebrates tested in plastic degradation studies (or less studied on PE), include snails, other darkling beetle's larvae, and earthworms. The ability of terrestrial snail *Achatina fulica* to degrade PET and PS was demonstrated [61]. However, there are few reports on the degradation ability of polyethylene by snails, and further research is needed.
Although the snail Cantareus aspersus was able to consume LDPE, ingestion and digestion along the snail's digestive tract did not result in appreciable fragmentation of LDPE particles, according to Colpaert et al., [62]. Earthworms, as the most well-known decomposers, have long played an important role in the natural environment. Although there is no evidence that earthworms can directly decompose polyethylene, studies have demonstrated that PE particles of various sizes in the environment have little or no effect on earthworm growth [63]. Given the above-mentioned research findings indicating symbiotic bacteria in the bodies of insects can breakdown

polyethylene synergistically, more research is needed to investigate the potential PE-degrading capabilities of earthworms and their intestinal microbes.
Despite high rates of PE biodegradation by living macroorganisms, there are a number of limitations that may limit the use of insect larvae as a waste management approach for petro-plastics such as polyethylene. These constraints include: (i) the need to sustain insect cultures in order to produce the larvae that feed on PE; (ii) the potentially high cost of maintaining these cultures; and (iii) the generation of microplastics, which may contribute to environmental problems due to incomplete degradation and a lack of mineralization. Finding new isolates of bacteria and/or fungi that can digest PE, as well as understanding the exact mechanics of biodegradation routes, may be more efficient in developing new means of managing PE waste [56, 30].

6. Microbial Enzymes for Polyethylene Decomposition

Enzymes involved in polyethylene breakdown are classified as extracellular enzymes or intracellular enzymes based on their reaction locations. Because of their vast range of applications, extracellular enzymes have received substantial research [42]. Peroxidases, laccases, hydroxylases, and reductases have been found as biocatalysts for the breakdown of polyethylene from algal, bacterial, actinomycete, and fungal sources [64].
They are primarily involved in the depolymerization of polyethylene's long carbon chains into oligomers, dimers, and sometimes monomer mixes. Because they reside in the liquid phase and act on big molecules at the surface of solid polyethylene, these enzymes are thought to be engaged in heterogeneous processes that occur at the solid-liquid interface [65]. Other enzyme groups are involved in the modification of polyethylene surface characteristics, as well as the breakdown of polyethylene metabolic intermediates into monomers and the final mineralization of the monomers [66]. Santo *et al*. [67] discovered that the copper-binding enzyme laccase, generated by the actinomycete Rhodococcus ruber, had a significant role in polyethylene biodegradation. Moreover, the laccases from *Aspergillus flavus* and *P. ostreatus* also exhibited significant PE-degrading activity [68].
The enzyme system of P. aeruginosa, which included alkane hydroxylase and reductase, decomposed low molecular weight polyethylene. Polyethylene breakdown utilizing a bacterial and fungal mix has been documented in some circumstances [69, 70]. The importance of fungal enzymes, particularly lignocellulolytic and depolymerizing enzymes, has been highlighted in studies. The broad specificity of diverse fungal enzymes offers them an advantage in degrading various polyethylenes [70].
On the surfaces of paper towels and polyethylene polymers, mycelial growth was detected on the surfaces of Pleurotus ostreatus. The addition of this paper towel promotes fungal development and the creation of lignocellulolytic enzymes, which eventually destroy the paper and polyethylene polymer. The great penetrating ability of hyphae is thought to be crucial in fungus colonizing HDPE surfaces [71].

6.1. Polyethylene Biodegradation by Fungal Enzymes

The main fungal enzymes involved in polyethylene biodegradation are the lignolitic enzymes laccases (Lac, EC 1.10.3.2.) and peroxidases (EC 1.11.1.7) [72]. The effect of these enzymes on PE has been extensively studied in Basidiomycota, but they are also present in Ascomycota.
When active in PE biodegradation, the ascomycete Trichoderma harzianum can create laccase (Mw 88 kDa) as well as peroxidase (Mw 55 kDa) [73]. After 10 days of incubation, the treatment of PE with 0.01071 IU/mL of its laccase resulted in a 0.5% loss of mass, while the treatment of PE with 0.01080 IU/mL of its peroxidase resulted in a 0.6% loss of mass. Carboxylic acids, aldehydes, aromatics, alcohols, esters, ethers, and alkyl halide groups were generated as a result of the enzymatic treatment and were detected using Fourier-transform infrared spectroscopy (FTIR) [73].
Aspergillus flavus PEDX3, which was isolated from the intestine of the wax moth Galleria mellonella, is a particularly interesting ascomycete involved in HDPE microplastic biodegradation (density 0.955 g/cm3, size below 200 m) [74]. After 28 days of incubation, this strain was able to depolymerize HDPE long chains and generate lower molecular weight fragments. The ability of A. flavus PEDX3 to manufacture laccases and laccase-like multicopper oxidases may explain its activity (LMCOs). RT-PCR gene sequencing identified two genes (AFLA 006190 and AFLA 053930) that may encode potential LMCO degraders [74].
In the Basidiomycota, partially purified manganese peroxidase (MnP, EC 1.11.1.13) from Phanerochaete chrysosporium ME-446 induced considerable polyethylene degradation when 0.1% Tween 80 was included in the growing medium, lowering tensile strength and elongation [73]. Furthermore, after the addition of 0.1 mM manganese sulfate (MnSO4), the PE molecular weight (Mw) reduced from 716,000 to 89,500 Daltons, and the relative elongation changed from 100% to 0%. Although exogenous H_2O_2 is not required for polyethylene breakdown, it is required for the MnP reaction system [73].
Fujisawa *et al*. [75] evaluated the effects of a laccase-mediator system (LMS) from Trametes versicolor IFO 6482 on polyethylene biodegradation. LMS (500 nkat) was able to limit PE elongation by 20% in 3 days, whereas adding 0.2 mM 1-hydroxybenzotriazole (HBT) to the medium resulted in no elongation and a 60% decrease in

relative tensile strength. Furthermore, Mw decreased from 242,000 to 28,300 daltons following 3 days of LMS with HBT mediator treatment at 30ºC.

Another Basidiomycete involved in polyethylene biodegradation is Pleurotus ostreatus, which can hydrolyse C–C bonds by producing extracellular ligninolytic enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases (Lac). During growth on semisolid Radha modified medium in the presence of LDPE sheets, high enzyme production was detected. After 30 days and 90 days, the highest Lac and LiP activities were 2.817 U/g and 70.755 U/g, respectively, while the highest MnP production was observed at day 120 (1.097 U/g) [76].

A recent study published computational molecular simulations of polyethylene (dodecane, 170.3 daltons) and various enzymes known to breakdown it. Santacruz-Juárez *et al*. [72] investigated the interactions between MnP (manganese peroxidase from Phanerochaete chrysosprium), LiP (lignin peroxidase from Trametes cervine), Lac (laccase from Trametes versicolor), UnP (unspecific peroxygenase from Agrocybe aegerita) or Cut (cutinase They assessed binding affinity, which is the strength of the binding relationship between the enzyme and its ligand (polyethylene), and discovered that UnP (34.34 µM) > Lac (40.11 µM) > LiP (66.93 µM) > MnP (82.16 µM) > Cut (5590 µM)

The high interaction with PE was attributed to the UnP catalytic cavity's high area (659.920 Å2), volume (367.243 Å3), and hydrophobicity [77]. The presence of phenylalanine residues in the UnP active site causes hydrophobicity (Santacruz-Juárez *et al*. 2021). The binding affinity paralleled the binding energy scores, which were -6.09, -6.00, -5.69, -5.57, and -3.07 Kcal/mol for UnP-PE, Lac-PE, LiP-PE, MnP-PE, and Cut-PE complexes, respectively. The lower the necessary binding energy, the easier it is to form bonds.

These computational observations demonstrate that peroxidases can play an important role in PE biodegradation and that non-specific UnP enzymes can be used in practical applications due to their distinctive cavities composed of Val244, Phe121, Phe191, Phe199, Phe274, Ala77, Thr192, Gly195, Glu196, Ser123, Cys33, haem propionate, 1H-imidazol-5-yl methanol (Mzo354) and two hypothetical biodegradation processes using ligninolytic enzymes (Lac, LiP, and MnP) and polyethylene have been postulated, with a fungal hydrophobin from class II serving as the biosurfactant [72].

In a computational study, Sánchez [71] proposed using MnP from Phanerochaete chrysosporium. In order to perform their degradative activity, both MnP and LiP require the addition of H_2O_2 to the culture medium [72] and acidic environments (Sánchez, 2020). The involvement of H_2O_2 is to act as an electron-accepting co-substrate in the oxidation-reduction reactions promoted by MnP and LiP [71]. Alternatively, lac causes the transfer of electrons from organic substrates to molecular oxygen. Therefore, the main difference in PE biodegradation pathways between laccases and haem peroxidases (LiP and MnP) is based on the different methods of electron transfer [71].

7. Biodegradation Mechanisms of Polyethylene

Despite the above reports, nothing is known about the biochemical activities and structural properties of polyethylene-degradation enzymes. As previously stated, the purpose of polyethylene biodegradation is to transform polyethylene trash that cannot be naturally decomposed into non-toxic low-molecular-weight chemicals that can re-enter the natural environment's chemical cycle. Bio-fragmentation, biodegradation, assimilation, and mineralization are the distinct biochemical breakdown mechanisms involved in polyethylene biodegradation. All of these stages are carried out by numerous active enzymes [78]. The colonization of microorganisms by microorganisms is the first step in the biodegradation of polyethylene. Through chemical and physical mechanisms, microorganisms or invertebrates modify their chemical, mechanical, and physical qualities [79]. Numerous studies have found that biofilm formation significantly improves the interaction between the polyethylene surface and bacteria [80]. Biofilm-forming bacteria, such as R. ruber, have been found to cling more securely and destroy low-density polyethylene more effectively than bacteria that cannot form biofilms [67]. Fungal hyphae may securely connect to the surface of polyethylene; indeed, they can attach to the surfaces of nearly all types of objects [71]. Once the microorganisms adhere to the polyethylene surface, they use the polymer as their sole carbon source to continue to proliferate.

The second stage is depolymerization, which occurs when extracellular enzymes and bacteria produce free radicals, which, in conjunction with enzymatic catalysis, disintegrate the polyethylene into smaller pieces [81]. So far, the most commonly observed depolymerization pattern during PE biodegradation has been extensive depolymerization, which is defined as a decrease in the number-average molecular weight (Mn) and weight-average molecular weight (Mw) of PE, as well as a decrease in the molecular weight distribution (MWD) towards lower molecular weights [82]. For example, Zhang and colleagues, for example, observed that the molecular weight of polyethylene samples treated with the laccase-producing A. flavus strain PEDX3 was much lower than the control samples: Mw of 132 KD and Mn of 29 KD were reduced [68].

Peroxidase also operates in this depolymerization method, and the Mw of peroxidase-treated LDPE samples is dramatically lowered [83] As the molecular weight or crystallinity of the substrate drops, the rate of depolymerization normally increases in an essentially linear relationship [84]. A microorganism must be able to further lower the molecular weight of the polymer and oxidize it into small molecular components to do this. The

primary degradation involves enzymes found in microbial cells (extracellular/intracellular), which cause polymer chains to break down [85]. In the presence of water, these enzymatic processes disrupt the chemical bonds of the substrate, generating oligomers or monomers [78].
The third stage is assimilation, in which the low-molecular-weight chemicals created during the fragmentation process must be delivered to the cytoplasm of the bacteria. Pseudomonas may absorb octadecane, a polymer degradation product, according to Shahnawaz et al. [86]. Another study discovered that membrane-bound enzymes found in olefin-assisting bacteria work on the first oxidation of olefins to accelerate polymer breakdown and metabolite release [87].
The fourth step is the mineralization process. When polyethylene breakdown products enter the cell, they undergo a variety of complicated enzymatic processes. The enzymes involved use intracellular assimilation to break down polymer chains and release metabolites like CO_2, H_2O, CH_4, and N_2.
Finally, polyethylene can be treated or reused without harm. Yang et al. used techniques such as isotope tracing and CO_2 emission quantification to demonstrate the complete mineralization of polyethylene (Yang *et al*., 2020). Whether the mineralization process is conducted aerobically or anaerobically, it necessitates the activity of several enzymes, including peroxidase, lipase, esterase, cutinase, and laccase [89].

8. Conclusion

This review discusses the biological breakdown of polyethylene via the diverse activities of microorganisms such as bacteria, fungus, and algae. This work also enumerates the functions of invertebrates in polyethylene breakdown, as well as microbial enzymes involved in degradation processes, and the method of degradation was comprehensively listed. The most recent advancements in polyethylene biodegradation are also discussed. Biodegradation research on polyethylene waste opens up new avenues for addressing the existing problem of plastic pollution.
Finally, we outline the existing gaps in polyethylene research in terms of environmental toxicology and industrial use of breakdown technology, as well as briefly suggest future research directions. There is a need to provide tangible and credible proof for biodegradation of PE in order to reduce artifacts created from additive degradation rather than PE breakdown. As a result, further studies should be conducted with additive-free PE. Further research into the process of enzymatic degradation will reveal the molecular pathway for efficient PE biodegradation.

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