



# Biodegradation of Polyethylene Microplastics by Bacteria, Fungi, and Algae: Mechanisms, Enzymes, and Microbial Diversity

Dinesh Singh Rauthan<sup>1</sup>  and Arti Rauthan<sup>2</sup> 

<sup>1</sup>Department of Public Health, COER University, Roorkee, Uttarakhand, India

<sup>2</sup>Sai Institute of Paramedical and Allied Science, Dehradun, Uttarakhand, India

Received: Jan 24, 2025/ Revised: Feb 14, 2025/Accepted: Feb 27, 2025

(✉) Corresponding Author: [dineshrrauthan@gmail.com](mailto:dineshrrauthan@gmail.com)

## Abstract

Polyethylene (PE) microplastics, known for their environmental persistence and resistance to degradation, pose a major ecological threat in terrestrial and aquatic systems. This study explores the biodegradation potential of bacteria, fungi, and algae against PE microplastics, highlighting microbial diversity, degradation pathways, and enzymatic mechanisms. The process typically begins with abiotic or enzymatic oxidative pretreatment, forming hydrophilic functional groups that facilitate microbial colonization. Microorganisms such as *Bacillus cereus*, *Pseudomonas*, *Aspergillus niger*, and *Scenedesmus dimorphus* demonstrated significant capacity to degrade low-density PE (LDPE), supported by evidence from SEM, FTIR, GC-MS, and weight loss assays. Bacterial consortia showed synergistic effects, achieving up to 58.21% LDPE degradation, while engineered strains like ARTP-mutagenized *Bacillus safensis* exhibited enhanced laccase gene expression and higher biodegradation efficiency. Fungi such as *Aspergillus fumigatus* and *Trichoderma harzianum* secreted oxidoreductases capable of depolymerizing PE under diverse conditions. Algae and cyanobacteria also facilitated degradation via biofilm formation and PETase expression, particularly in marine and freshwater environments. The proposed mechanism includes oxidative cleavage, enzymatic depolymerization, assimilation of intermediates, and their eventual mineralization via  $\beta$ -oxidation and TCA cycle pathways. Incorporating green nanomaterials, metagenomic approaches, and genetic engineering further enhanced microbial action on PE. The findings underscore the ecological relevance of microbial systems in bioremediating PE pollution and offer promising directions for biotechnological interventions. This comprehensive insight into microbial PE degradation paves the way for scalable, environmentally friendly solutions to the global plastic crisis.

**Keywords:** Polyethylene microplastics, microbial degradation, bacterial consortia, oxidative enzymes, fungal biodegradation, algal degradation.

## 1. Introduction

Plastic pollution, especially from polyethylene (PE), has emerged as one of the most pressing environmental concerns of the 21st century. Polyethylene, including low-density polyethylene (LDPE) and high-density polyethylene (HDPE), is widely used in packaging and consumer products due to its durability, flexibility, and resistance to degradation. However, these very properties contribute to its persistence in the environment, leading to extensive accumulation in terrestrial

and aquatic ecosystems. The breakdown of PE into microplastics (<5 mm in size) exacerbates ecological harm, impacting soil microbiota, marine food chains, and potentially human health through bioaccumulation. Addressing this crisis requires sustainable, eco-friendly strategies, among which microbial biodegradation has gained significant scientific attention.

Recent research has illuminated the potential of various microorganisms—bacteria, fungi, and algae—to degrade

polyethylene microplastics via enzymatic and oxidative pathways (Xiong et al., 2025). These organisms employ a suite of biochemical reactions to colonize, oxidize, and metabolize plastic polymers, converting them into simpler compounds like carbon dioxide, water, and biomass. Notably, the degradation often involves structural changes in the PE, including surface erosion, the formation of carbonyl groups, and reductions in molecular weight, all of which can be tracked through techniques like SEM, FTIR, GC-MS, and XPS.

Among bacterial strains, mutagenized variants have shown promise in improving degradation efficiency. Xiong et al. (2025) reported that a bacterium, strain XZ-60S, modified through atmospheric and room temperature plasma (ARTP) mutagenesis, degraded up to 53.65% of PE microplastics after 50 days. Transcriptomic analyses revealed increased expression of laccase genes, suggesting enhanced enzymatic oxidation of PE. These findings demonstrate the potential of genetic tools to boost microbial degradation and pave the way for future strain development.

Similarly, sulfate-reducing bacteria have shown utility in nanomaterial-assisted PE degradation. Cheng et al. (2025) synthesized CuS quantum dots (Bio-CuS) via a green biological route and employed them as photocatalysts to degrade polyethylene films under light exposure. Bio-CuS facilitated 12.8% weight loss of PE in just 14 days—significantly outperforming its chemically synthesized counterpart. The involvement of reactive oxygen species (ROS), such as hydroxyl radicals and holes ( $h^+$ ), in photodegradation underscores the importance of combining microbial systems with advanced material science for effective PE breakdown.

Microbial consortia also offer synergistic degradation capabilities. Zhang et al. (2023) demonstrated that a bacterial consortium containing *Bacillus cereus*, *B. parapsilosis*, and *B. megaterium* degraded 31.3% of PE film over 60 days. The consortium produced a crude enzyme mixture that catalyzed depolymerization of PE chains, generating lower molecular weight by-products. Such consortia replicate real-world microbial interactions more accurately than pure cultures and may therefore offer more effective biodegradation in situ.

In marine ecosystems, the plastisphere—biofilms forming on plastic surfaces—harbors unique microbial communities adapted to plastic degradation. Febria et al. (2024) isolated several marine bacteria from PE films, with *Bacillus paramycoides* IBP-3 showing the highest degradation potential. The strain caused significant structural disintegration of the PE film, supported by SEM and FTIR analysis. Similarly, Rong et al. (2024) identified *Rhodococcus* sp. C-2 from seawater, which degraded LDPE films through enzymatic depolymerization involving glutathione peroxidase. Such findings highlight marine microbes as untapped resources for plastic biodegradation.

Fungal species also play a significant role. Duan et al. (2024) identified *Aspergillus niger* DL-1 as a potent degrader of PE microplastics, achieving a 7.65% degradation rate within 30 days. The strain secreted plastic-degrading enzymes under the induction of PE, leading to visible surface damage and molecular disintegration. Ji et al. (2024) demonstrated that plasma-activated *Bacillus safensis* exhibited higher colonization efficiency and degraded 27.78% of LDPE films—suggesting that physical pretreatments can enhance microbial action.

Rhizosphere and soil microbes are increasingly recognized for their PE-degrading potential. Dhaka et al. (2023) reported that *Bacillus pumilus* and *Priestia aryabhatai*, isolated from plant roots, degraded over 65% of PET, indicating the applicability of rhizobacteria in agro-ecosystems. Likewise, *Brevibacillus brevis* from soil reduced PE microplastic weight by 19.8% within 35 days through laccase secretion and the generation of small metabolic by-products (Tiwari et al., 2023).

In a metagenomic breakthrough, Jahanshahi et al. (2025) predicted 21 novel PET-degrading enzymes from contaminated soils using deep learning models like GANs and ESMs. These enzymes were further validated for active sites and structural compatibility, providing a computational pathway for enzyme discovery. Li et al. (2024) also highlighted the synergy between microbial communities and introduced hydrolases in PET degradation during high-temperature composting. Lastly, large-scale environmental studies provide insight into microbial diversity. Niu et al. (2023) conducted a 1150-day in situ experiment in urban rivers and identified both core (C-bacteria) and resuscitated (R-bacteria) polyethylene/polypropylene-degrading bacteria. The findings emphasized the potential of natural bacterial communities, particularly in sediment-rich eutrophic zones, to participate in microplastic degradation.

Collectively, these advances emphasize a multifaceted approach to PE biodegradation. Genetic engineering, microbial consortia, green nanotechnology, metagenomics, and environmental screening converge to offer sustainable solutions. Understanding microbial diversity, metabolic pathways, and the enzymology of plastic breakdown is crucial for developing scalable strategies to mitigate the global plastic pollution crisis.

## 2. Degradation of Polyethylene

Polyethylene (PE) microplastics are among the most persistent environmental pollutants due to their high stability and resistance to natural degradation processes. However, recent advancements in microbial biotechnology suggest that bacteria, fungi, and microalgae have the potential to biologically break down PE, offering sustainable strategies for mitigating plastic pollution. The microbial degradation of PE typically proceeds through two primary stages: enzymatic fragmentation, where polymer chains are cleaved into smaller oligomers, and subsequent bioassimilation, during which

microorganisms metabolize these low-molecular-weight fragments—generally below 500 g/mol (Bonhomme et al., 2003; Montazer et al., 2019).

Several microbes capable of degrading PE also utilize long-chain hydrocarbons such as linear n-alkanes (e.g., para-n, C44H90, Mw = 618), which some species can completely mineralize within 20 days (El-Shafei et al., 1998; Haines, 1975). The degradation pathway often begins with the hydroxylation of carbon-carbon bonds, forming primary alcohols. These intermediates are then oxidized into aldehydes or ketones and finally into carboxylic acids, which enter the microbial  $\beta$ -oxidation pathway for complete mineralization (Yoon et al., 2012).

Crucial enzymes in this process include alkane hydroxylases (AlkB), whose gene variants differ significantly among microbial species. For instance, *Pseudomonas aeruginosa* possesses two AlkB genes (alkB1 and alkB2), whereas *Rhodococcus* sp. TMP2 expresses five distinct forms (alkB1 to alkB5) (Stempfle et al., 2016). Enzymes from strains like *Pseudomonas putida* GPo1 and *P. aeruginosa* E7 are especially effective in oxidizing terminal carbon atoms of low molecular weight PE (LMWPE), converting them into CO<sub>2</sub> through metabolic processes (Yoon et al., 2012; Rojo, 2009). Additionally, laccase enzymes secreted by organisms such as *Rhodococcus ruber* have been shown to enhance the degradation of PE (Yoon et al., 2012). Although research into the genetic regulation of PE-degrading enzymes is still developing, studies reveal that certain cloned AlkB

enzymes—such as those in *Pseudomonas* sp. E4—can function independently of auxiliary proteins like rubredoxin and rubredoxin reductase (Yoon et al., 2012). These findings highlight the potential of genetically optimized strains for accelerating PE biodegradation and call for deeper investigation into their enzymatic pathways and regulatory mechanisms.

### 3. Bacterial Degradation of Polyethylene

A wide range of bacterial species have demonstrated the ability to degrade polyethylene (PE), particularly its low-density form (LDPE). Among these, *Paenibacillus* sp. has shown discernible biodegradation activity, as confirmed by morphological and structural analyses using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) (Bardaji et al., 2019). In another study, *Acinetobacter baumannii* exhibited robust growth when incubated at 37°C in the presence of untreated PE, with GC-MS and FTIR techniques confirming the degradation process (Pramila et al., 2015).

Similarly, *Bacillus cereus* strain A5 was reported to induce a 35.72% reduction in LDPE weight, validated through gravimetric methods and FTIR spectral analysis (Muhonja et al., 2018). Hydrocarbon-degrading bacteria such as *Pseudomonas* and *Cellulosimicrobium* were found to facilitate PE breakdown by introducing functional groups like aldehydes, ethers, and carboxylic acids into the polymer matrix (Muhonja et al., 2018).

**Table 1. Bacterial Degradation of Polyethylene**

Microorganism	Biodegradation Potential	Method Used	References
<i>Paenibacillus</i> sp.	LDPE weight loss observed	SEM, FTIR	Bardaji et al. (2019)
<i>Acinetobacter baumannii</i>	Biomass increase; PE degradation at 37°C	GC-MS, FTIR	Pramila et al. (2015)
<i>Bacillus cereus</i> strain A5	35.72 ± 4.01% LDPE weight reduction	Weight loss, FTIR, GC-MS	Muhonja et al. (2018)
<i>Brevibacillus borstelensis</i> strain B2,2	20.28 ± 2.30% LDPE weight reduction	Weight loss, FTIR, GC-MS	Muhonja et al. (2018)
<i>Pseudomonas</i> , <i>Cellulosimicrobium</i> , <i>Lysinibacillus</i>	Degradation via hydrocarbon metabolism; functional groups formed	16S/18S rDNA sequencing, FTIR	Muhonja et al. (2018)
<i>Actinobacteria</i> & <i>Firmicutes</i> ( <i>Lumbricus terrestris</i> gut)	LDPE-MP size reduction; long-chain alkanes formed	16S rRNA sequencing, TEM	Lwanga et al. (2018)
<i>Aneurinibacillus aneurinilyticus</i> , <i>Brevibacillus</i> spp.	Max 58.21% weight loss of LDPE/HDPE/PP	FTIR, SEM, AFM, EDS, NMR, GC-MS	Skariyachan et al. (2018)
<i>Alcanivorax borkumensis</i>	Biofilm formation and LDPE degradation	16S rRNA microbiome profiling	Delacuvellerie et al. (2019)

Further enhancement in PE degradation was observed with bacterial consortia. A notable example includes a consortium led by *Aneurinibacillus aneurinilyticus* bDSCE01, which achieved up to 58.21% reduction in LDPE weight across both sheet and pellet forms. The biodegradation was characterized using advanced tools such as FTIR, SEM, and GC-MS (Skariyachan et al., 2018).

Additionally, *Alcanivorax borkumensis* contributed to PE degradation through biofilm formation on LDPE surfaces, facilitating surface erosion and microbial colonization—key steps in the degradation process of petroleum-derived plastics (Delacuvellerie et al., 2019).

#### 4. Fungal Degradation of Polyethylene Microplastics

Fungal species have emerged as effective agents in the biodegradation of polyethylene (PE), owing to their ability to produce extracellular enzymes and adapt to diverse environments. One of the most notable examples is *Aspergillus fumigatus*, which demonstrated rapid degradation

of both polyethylene and polyhydroxybutyrate (PHB) at an incubation temperature of 45°C, achieving near-complete breakdown within just 17 hours (Jung et al., 2018).

Extended degradation capabilities have been observed in *Penicillium* species such as *Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10, which degraded low-density (LDPE) and high-density polyethylene (HDPE) films over a 90-day incubation period. Structural and morphological changes in the plastic surface were monitored using field emission scanning electron microscopy (FE-SEM) and atomic force microscopy (AFM) (Ojha et al., 2017).

In aqueous conditions, *Aspergillus clavatus* significantly degraded PE, as evidenced by substantial weight loss and increased CO<sub>2</sub> release during the degradation process (Gajendiran et al., 2016). Another efficient degrader, *Trichoderma harzianum*, achieved approximately 40% degradation of treated PE, with confirmation through SEM imaging, FTIR spectroscopy, and nuclear magnetic resonance (NMR) analysis (Sowmya et al., 2014).

Furthermore, *Aspergillus flavus* was found to reduce the molecular weight of PE by 16%, with visible degradation features confirmed by SEM (Deepika & Madhuri, 2015). *Aspergillus terreus* also contributed significantly, demonstrating up to 21.8% weight reduction in PE samples, as validated by FTIR and SEM observations (Alshehrei, 2017).

**Table 2. Fungal Degradation of Polyethylene**

Microorganism	Biodegradation Potential	Method Used	References
<i>Aspergillus fumigatus</i>	Complete PHB degradation in 17h at 45°C	LC-MS/MS	Jung et al. (2018)
<i>Penicillium oxalicum</i> , <i>P. chrysogenum</i>	LDPE/HDPE degradation over 90 days	RSM, FE-SEM, AFM, FTIR	Ojha et al. (2017)
<i>Zalerion maritimum</i>	PE pellet degradation	FTIR-ATR, NMR, mass quantification	Paço et al. (2017)
<i>Aspergillus clavatus</i>	LDPE weight loss and CO <sub>2</sub> emission	SEM, AFM, FTIR, Strum, rRNA	Gajendiran et al. (2016)
<i>Trichoderma harzianum</i>	40% polyethylene degradation	SEM, FTIR, NMR	Sowmya et al. (2014)
<i>Aspergillus flavus</i>	FTIR changes, gene upregulation	HT-GPC, Halotest, FTIR	Zhang et al. (2020)
<i>Aspergillus terreus</i>	6–21.8% LDPE weight loss	FTIR, SEM, protein content	Sangeetha Devi et al. (2015); Alshehrei (2017)
<i>Alternaria alternata</i>	Biomass increase, enzyme production	Enzyme assay, CO <sub>2</sub> quantification	Ameen et al. (2015)
<i>Aspergillus niger</i> , <i>A. sydowii</i> , <i>A. caespitosus</i>	LDPE weight loss (up to 37.9%), cracks	SEM, FTIR, tensile strength	Deepika & Madhuri (2015); Sangale et al. (2019)
<i>Aspergillus oryzae</i> strain A5,1	36.4 ± 5.53% LDPE weight reduction	FTIR, GC-MS	Muhonja et al. (2018)

## 5. Algal Degradation of Polyethylene Microplastics

Microalgae and cyanobacteria have also shown considerable promise in the biodegradation of polyethylene (PE), offering environmentally sustainable strategies for mitigating plastic pollution. One such species, *Scenedesmus dimorphus*, a freshwater green alga, was observed to colonize and degrade low-density polyethylene (LDPE) films. Its growth on the plastic surface led to clear signs of erosion and deterioration (Kumar et al., 2017).

Among cyanobacteria, *Anabaena spiroides* demonstrated the highest LDPE degradation efficiency, achieving a breakdown rate of 8.18%. This was confirmed by scanning electron microscopy (SEM) and transverse section (T.S.) analysis, which revealed surface disruption and structural changes in the plastic matrix (Kumar et al., 2017).

In marine settings, *Phaeodactylum tricorutum*—a diatom microalga—has been genetically modified to express PETase, an enzyme that breaks down polyethylene terephthalate (PET). This innovation enabled its use in saltwater environments, where it facilitated the enzymatic degradation of PET and related plastics (Moog et al., 2019).

Other cyanobacterial strains, including *Phormidium lucidum* and *Oscillatoria subbrevis*, were also found to be effective PE degraders. These species induced substantial physical and chemical alterations in LDPE, as evidenced by advanced analytical techniques, indicating their strong potential in plastic biodegradation (Sarmah & Rout, 2018).

**Table 3. Algal Degradation of Polyethylene**

Microorganism	Biodegradation Potential	Method Used	References
<i>Scenedesmus dimorphus</i>	Surface colonization and LDPE erosion	SEM	Kumar et al. (2017)
<i>Anabaena spiroides</i>	8.18% LDPE degradation	SEM, Transverse Section	Kumar et al. (2017)
<i>Navicula pupula</i>	Mild degradation on polyethylene	SEM	Kumar et al. (2017)
<i>Phaeodactylum tricorutum</i>	Engineered to express PETase enzyme	Synthetic biology, enzyme assays	Moog et al. (2019)
<i>Phormidium lucidum</i> , <i>Oscillatoria subbrevis</i>	Structural/chemical degradation of LDPE	FTIR, SEM, NMR, CHN, enzyme assay	Sarmah & Rout (2018)
<i>Chlamydomonas reinhardtii</i>	PET degradation to terephthalic acid (TPA)	HPLC, SEM	Kim et al. (2020)

## 6. Proposed Mechanism for the Biodegradation of Polyethylene (PE)

Polyethylene (PE), one of the most persistent synthetic polymers, presents significant environmental challenges due to its high molecular weight, hydrophobicity, and chemical inertness. The proposed mechanisms of microbial degradation of PE typically follow a multi-phase process involving abiotic and biotic steps, ultimately leading to its mineralization.

### Initial Oxidative Pretreatment (Abiotic/Biotic Trigger):

The degradation process begins with oxidative cleavage of the PE polymer chains. This initial activation can be triggered by physical (UV, heat), chemical (oxidants), or biological (oxidative enzymes) means. Abiotic factors such as UV irradiation or heat induce the formation of free radicals and hydroperoxides on the polymer backbone, resulting in chain scission and the formation of carbonyl, hydroxyl, and carboxyl functional groups (Restrepo-Flórez et al., 2014). These modifications enhance hydrophilicity, enabling microbial colonization. In some studies, low-temperature plasma and chemical pretreatment have been used to enhance microbial access by increasing surface oxidation (Ji et al., 2024; Xiong et al., 2025). For instance, plasma-activated *Bacillus safensis* exhibited enhanced colonization and oxidative attack on

LDPE films due to increased carbonyl index and surface hydrophilicity.

### Biofilm Formation and Colonization

Following surface modification, microbial colonization begins, often with biofilm formation. Biofilms create a localized microenvironment enriched in extracellular enzymes and acidic conditions that facilitate further polymer breakdown (Zhang et al., 2023). Bacterial and fungal strains such as *Bacillus cereus*, *Aspergillus niger*, and *Rhodococcus sp.* have shown efficient colonization and biofilm development on PE substrates (Rong et al., 2024; Duan et al., 2024).

### Enzymatic Depolymerization

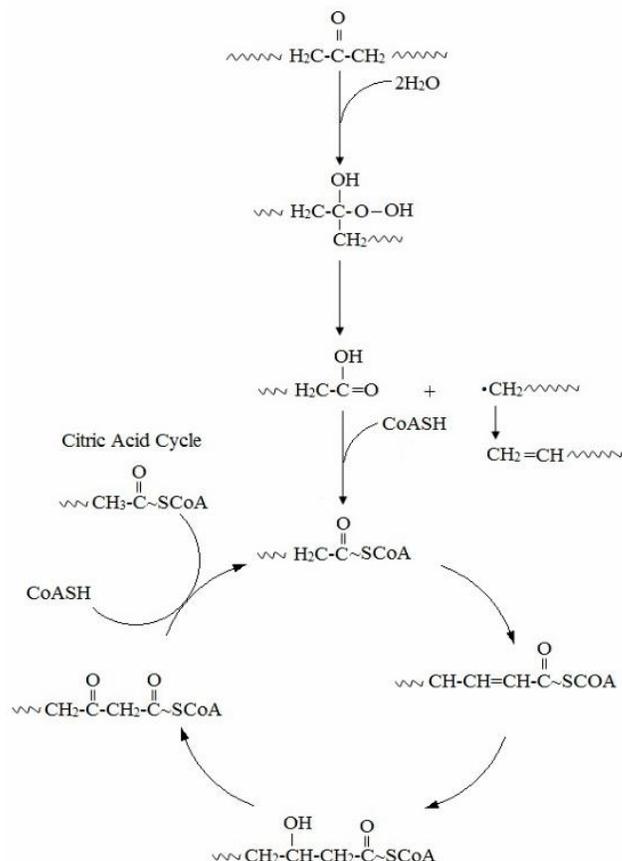
Enzymatic cleavage is central to PE biodegradation. Although PE is a carbon-carbon backbone polymer without readily hydrolysable bonds, microbial enzymes such as laccases, peroxidases, oxygenases, and hydrolases are implicated in initiating and propagating depolymerization (Tiwari et al., 2023). For example, laccase release by *Brevibacillus brevis* led to a significant reduction in microplastic weight, along with structural changes observed through SEM and FTIR (Tiwari et al., 2023). Likewise, glutathione peroxidase (GPx) and superoxide radicals in *Rhodococcus sp.* were reported to

facilitate oxidative depolymerization of LDPE (Rong et al., 2024).

### Assimilation and Mineralization

Once the polymer chains are cleaved into oligomers and monomers, these smaller molecules can be transported across the microbial cell membrane and utilized as carbon sources. Inside the cell, these fragments are further metabolized

through  $\beta$ -oxidation and enter central metabolic pathways like the tricarboxylic acid (TCA) cycle (Restrepo-Flórez et al., 2014; Polymers, 2020). For instance, intermediates such as alcohols, ketones, and carboxylic acids are detected as degradation products, highlighting oxidative metabolism pathways (Cheng et al., 2025).



**Figure 1.** Proposed metabolic pathway for polyethylene (PE) biodegradation. The process involves oxidative cleavage of the polymer backbone, followed by enzymatic conversion to low-molecular-weight compounds such as short-chain fatty acids and CoA derivatives. These intermediates are eventually funneled into the citric acid cycle for complete mineralization

### 7. Role of Microbial Consortia and Genetic Enhancement

PE degradation is often enhanced by synergistic interactions within microbial consortia. Mixed cultures produce diverse enzyme sets and metabolize intermediates cooperatively (Zhang et al., 2023). Advanced strategies such as atmospheric and room temperature plasma (ARTP) mutagenesis and genetic engineering have also improved PE-degrading efficiency by increasing expression of laccase and hydrolase genes (Xiong et al., 2025; Jahanshahi et al., 2025). In conclusion, while PE degradation is inherently slow due to its stable structure, a combination of oxidative pretreatment, microbial colonization, extracellular enzymatic activity, and

intracellular assimilation processes contributes to its stepwise biodegradation. Emerging biotechnological tools promise to further enhance this eco-friendly strategy for mitigating plastic pollution.

### 8. Conclusion

The widespread use and improper disposal of polyethylene (PE), especially in its microplastic form, have created a pressing environmental challenge. This review consolidates current insights into the biological degradation of polyethylene by diverse microbial agents—bacteria, fungi, and algae—and emphasizes their mechanisms, enzymatic involvement, and ecological potential. Evidence from numerous studies

demonstrates that microorganisms can colonize, adhere to, and degrade polyethylene surfaces through enzymatic oxidation and depolymerization. Bacterial genera such as *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Acinetobacter* have shown remarkable capabilities in PE degradation via the production of enzymes like laccases, peroxidases, and monooxygenases. Similarly, fungal strains, particularly *Aspergillus* and *Penicillium* species, contribute to degradation by secreting ligninolytic enzymes that break down complex polymer chains into smaller, biodegradable fragments. Microalgae and cyanobacteria also contribute significantly to polyethylene degradation, not only by secreting oxidative enzymes but also through biofilm formation, which facilitates physical and chemical alterations in the polymer matrix. Genetic engineering approaches, such as atmospheric and room temperature plasma (ARTP) mutagenesis and synthetic biology, have further enhanced microbial degradation rates, making them promising tools for biotechnological applications. While these findings are promising, the efficiency and scalability of microbial degradation under natural environmental conditions remain a challenge. Factors such as temperature, pH, nutrient availability, and co-contaminants influence degradation efficiency. Future research should focus on optimizing microbial consortia, identifying novel degradation genes through metagenomic approaches, and developing bioreactor-based systems for large-scale application. In conclusion, microbial degradation offers a sustainable, eco-friendly solution to polyethylene microplastic pollution. A better understanding of microbial pathways, enzyme systems, and synergistic microbial interactions will be key to unlocking their full potential for environmental remediation.

### Author Contributions

Each author has significantly contributed to the study's conception, design, data collection, analysis, and interpretation. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accept full responsibility for the content and integrity of the work.

### Financial Support

The authors declare that no external funding was received for this study.

### Data Availability

All relevant data generated or analyzed during this study are presented within this published article.

### Conflicts of Interest

The authors state that there are no financial, personal, or professional conflicts of interest associated with this research.

### Ethical Approvals

This research did not involve the use of human participants or animal subjects and therefore did not require ethical clearance.

### Publisher's Note

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#### How to cite this article

Rauthan, D.S. and Rauthan, A.(2025) Biodegradation of Polyethylene Microplastics by Bacteria, Fungi, and Algae: Mechanisms, Enzymes, and Microbial Diversity. *Chemical and Environmental Science Archives*, Vol. 5 (1),1-8. <https://doi.org/10.47587/CESA.2025.5101>

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