

Microbial Innovation: Enzymes and Modern Strategies for Accelerated Plastic Upcycling

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ABSTRACT

Over the past few years, plastic polymers with diverse properties have revolutionized many industries, replacing traditional materials such as wood, glass, and metal used in day-to-day life. Despite widespread use of plastic, the properties that make plastics indispensable, such as durability and resistance to degradation, also mean that they persist in natural ecosystems, resulting in alarming levels of plastic waste that have become a significant global problem. In particular, plastic pollution of water bodies has caused severe ecological impacts, including physical damage and chemical toxicity to marine organisms, as well as bioaccumulation of microplastics in the food chain. Traditional methods of managing plastic waste, such as incineration, landfilling, and mechanical recycling, are increasingly viewed as inappropriate and unsustainable due to their environmental and economic costs. As a result, there has been growing interest in using biological systems for the biodegradation of synthetic plastics. Recent studies have focused on the ability of various microorganisms, including bacteria, fungi, and algae, to metabolize plastics through enzymatic pathways and break down plastics into simpler, non-toxic compounds. This review summarizes recent advances in microplastic degradation focusing on the roles and mechanisms of microbial enzymes such as PETase, laccase, and cutinase. This paper also summarizes current trends to improve microbial degradation such as pretreatments consortium as an effective and sustainable solutions for plastic waste management.

Keywords: Biodegradation, Microbial Enzymes, PETase, Manganese Peroxidase, Fungi, Bacteria.

Running Title: Current trends for plastic upcycling

Introduction

Plastics are currently applied in numerous various fields, including technology, medicine, building, and clothing, and have become indispensable in the modern world. Even when plastics are used to save lives with life-saving medical devices or energy-saving modes of transport, their characteristics render them virtually indispensable in a range of uses where they are needed (Magnin *et al.*, 2020). In addition, plastic has conservation applications that could substitute non-renewable resources such as tortoiseshells and ivory. Nevertheless, this reliance on plastics is high in terms of environmental cost (Humbert *et al.*, 2009).

Plastic pollution is one of the most pervasive and enduring problems currently confronting the world. Plastics have contaminated nearly all of the planet, from ocean floor depths to remote mountain ridges. That makes them highly useful for use in industry, but it means that the organisms can last hundreds of years rather than decades within the environment (Ostle *et al.*, 2019). It gets aggravated because they allow plastics to remain in terrestrial and aquatic environments when they are mismanaged, disposed of improperly, or components of transport accidents. They also serve as vectors of toxic substances, aggravating their effects on health and the environment (Alimi *et al.*, 2018a).

Plastic production keeps growing at a rate that is alarming, with higher amounts being made in the last few decades than in the entire twentieth century (Geyer *et al.*, 2017). The rate has surpassed the ability to manage plastic trash effectively. Even biodegradable plastics pose difficulties because of differences in rates of breakdown and environmental degradation requirements (Turner, 2018).

Since there are so many varieties of plastics, such as thermoplastics that can be moulded repeatedly and thermosetting that cannot be reshaped at all or easily, their ecological impact is also very diverse in terms of how they affect living organisms and ecosystems (Alimi *et al.*, 2018b). For instance, microplastics and nano plastics, which are often created as a result of the fragmentation of larger plastic products, can become ingested into aquatic and land animals, with high levels present that are both biological and health hazards up and down the food chain (Cole *et al.*, 2013).

Environmental Degradation of Plastics

All plastics are subject to physicochemical and/or biological degradation. Physicochemical processes include weathering (degradation caused by sunlight, wind, and waves) and

hydrolysis/oxidation. These processes affect all plastics and are the main source of microplastics (Kalogerakis *et al.*, 2017). Plastics that degrade by oxidation or hydrolysis reactions are referred to as oxo- or hydrogenolytic plastics (Scott, 2002). However, biodegradable plastics degrade due to microorganisms (bacteria, fungal enzymes) (Biobased and Biodegradable Polymers, 2014). Biodegradability can be humidity, temperature, and other condition dependent. Ideally, plastics are broken down by aerobic and anaerobic microorganisms into CO₂, methane, water, and edible biomass/compost. Most commercially available biodegradable plastics are converted into compost rather than gaseous products. For plastics to be compostable, the organic matter formed must be harmless to animals and plants. Compost can be made at room temperature using food waste or, more commonly, in temperature-controlled industrial plants (usually at 58°C). This so-called industrial compost requires proper collection and sorting of plastic waste (Filiciotto & Rothenberg, 2021)

Biodegradation: Strategies for Reducing Plastic Waste

Biodegradation is the process by which organic materials are broken down by living organisms, making it a promising way to reduce the environmental impact of plastic waste. This review aims to provide a comprehensive overview of the current state of research on plastic biodegradation. It describes the mechanisms and factors influencing biodegradation, and the microorganisms involved in these processes. Furthermore, it discusses recent advances, challenges, and future directions in biodegradation technology, and highlights the potential and limitations of biodegradation as a solution to plastic pollution.

Mechanism of Biodegradation

A major part of any degradation process is the breakdown of polymeric materials by mechanical forces, which is typically regarded as the initial phase. The degradation process causes polymers to break up into smaller fragments and lowers the polymer's molecular weight (MW). Microbes break down these low molecular weight substances (Carbery *et al.*, 2018). The mechanism by which anaerobic microorganisms convert polymeric substances into biomass or biogas in the absence of air is referred to as plastic biodegradation (Ali & Sun, 2019). It allows the polymer to be utilized efficiently as a source of carbon for growth (Shah *et al.*, 2008). Enzymes initially degrade polymer chains into low molecular weight molecules such as oligomers, dimers, and monomers, which is the initial process of biodegradation of plastic waste. Enzymes interact with the polymer and catalyse its hydrolytic cleavage, through which this is achieved. The low molecular weight molecules later mineralize to give CO₂ and H₂O

due to this process. Among the effective microorganisms that are capable of degrading polymers such as PE and PU are bacteria, fungus, and algae (Ali *et al.*, 2021).

Microbial Biodegradation

Microbial biodegradation refers to a natural process whereby microorganisms like fungi and bacteria degrade complicated organic compounds into simpler form. This process is significant in the decomposition of organic matter, nutrient recycling, as well as overall ecosystem health.

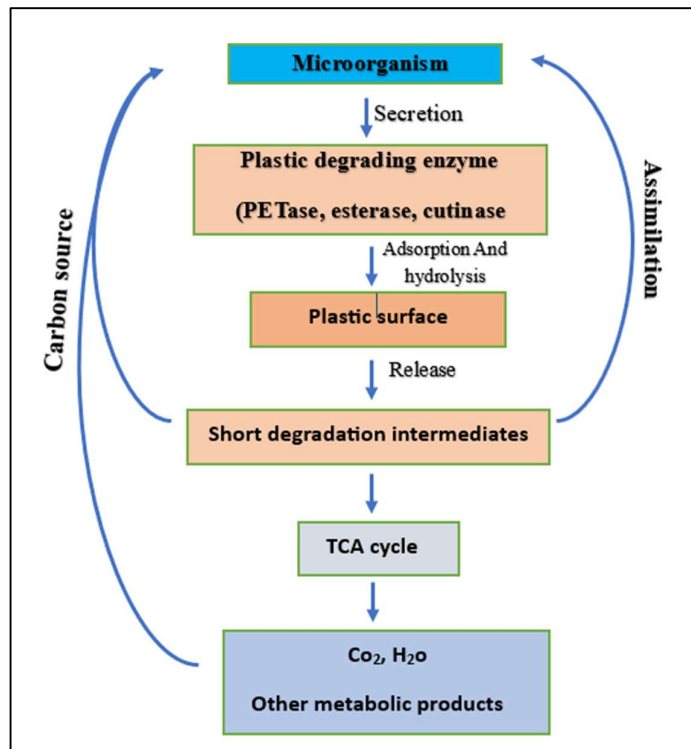


Fig 1: The general mechanism for biological degradation of plastics under aerobic conditions (Mohanani *et al.*, 2020).

Microbial biodegradation of plastics is the process of using particular microbial strains capable of metabolizing the polymers found in plastic materials. The microorganisms generate enzymes that start the degradation of plastic polymers into smaller molecules by secreting enzymes such as PETase, esterase, and cutinase, which adsorb onto the plastic surface and degrade it into short degradation intermediates. These intermediates further become part of the TCA cycle, which produces CO₂, H₂O, and other metabolic byproducts. The plastic that has degraded is a source of carbon, which the microorganism takes up for development and existence and thus

the process becomes very vital in the case of plastic biodegradation as well as environment sustainability., which are subsequently broken down and incorporated into microbial cells as explained in Fig:1. Knowing the mechanisms and the factors that govern the microbial biodegradation of plastics is vital in the creation of effective methods to control plastic waste and limit its contribution to the environment. Microorganisms, such as fungi, have the ability to biochemically degrade, assimilate, and metabolize recalcitrant organic compounds, xenobiotics, and refractory compounds in order to generate energy (Amobonye *et al.*, 2021) and (Harms *et al.*, 2011)). Fungi are involved in the degradation of polymeric materials. Fungal mycelium effectively invades the surface of polymeric materials and penetrates into their bulk to a great extent, degrading the highest amount of this substrate (Sánchez, 2020). Moreover, fungal mycelia release extracellular enzymes (e.g., depolymerases) that hydrolyse polymeric substrates to oligomers, dimers, and monomers; low molecular weight fragments are degraded (Ameen *et al.*, 2015). Application of filamentous fungi in the bioremediation of plastics is able to resolve this issue. Indeed, filamentous fungi show a classical hyphal apical growth pattern, through which they can elongate their network of hyphae into diverse kinds of material (Daccò *et al.*, 2020) . Major enzyme classes with their mode of action are mentioned in Fig:2.

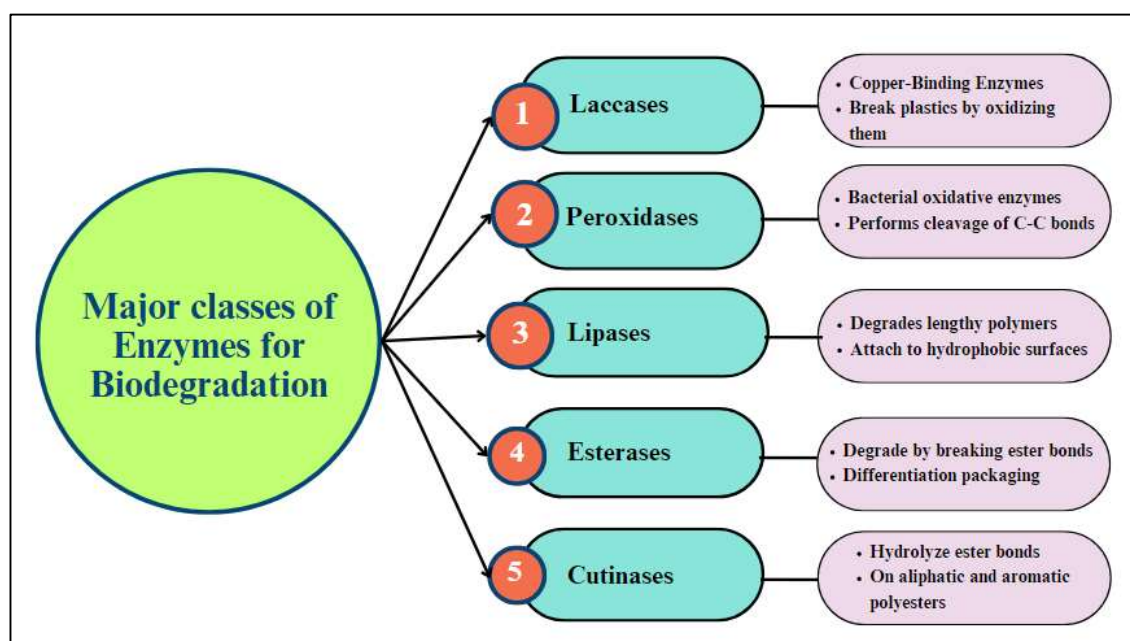


Fig: 2 Major Classes of enzymes which performs plastic biodegradation

Biodegradation by Bacteria

From literature review, it was found that several bacterial strains perform biodegradation by secretion various enzymes such as esterases, lipases, PETases, cutinases etc. and can degrade different types of plastic under different environmental conditions. These species are highly specific towards the biodegradation of several kinds of synthetic polymers. Some of them are mentioned in Table 1

Table:1 Bacterial species performing biodegradation

Bacterial Species	Enzyme(s)	Plastic Degraded	References
<i>Alcaligenes faecalis</i>	Polyhydroxybutyrate depolymerase	Poly(3-hydroxybutyrate)	(Romen <i>et al.</i> , 2004)
<i>Bacillus subtilis</i>	Laccase, Esterase	Polyethylene (PE), Polystyrene (PS)	(Yao <i>et al.</i> , 2022)
<i>Brevibacillus borstelensis</i>	Esterase	Polyethylene (PE)	(Hadad <i>et al.</i> , 2005)
<i>Clostridium botulinum</i>	Lipase	Polycaprolactone (PCL)	(Abou-Zeid <i>et al.</i> , 2001)
<i>Ideonella sakaiensis</i>	PETase, MHETase	Polyethylene terephthalate (PET)	(Yoshida <i>et al.</i> , 2021)
<i>Klebsiella pneumoniae</i>	Oxidase, Peroxidase	Polystyrene (PS)	8
<i>Pseudomonas aeruginosa</i>	Esterase, Lipase	Polyurethane (PU)	(Mahajan & Gupta, 2015)
<i>Pseudomonas indica K2</i>	Esterase	Poly(3-hydroxybutyrate-co-3-mercaptopropionate)	(Ganesh Kumar <i>et al.</i> , 2021)
<i>Pseudomonas putida</i>	Cutinase, Esterase, Lipase	Polyurethane (PU), PET, Polyvinyl chloride (PVC)	(Wilkes & Aristilde, 2017)
<i>Rhodococcus ruber</i>	Laccase, Alkaline hydroxylase	Polyethylene (PE)	(Santo <i>et al.</i> , 2013)
<i>Schlegelella thermodepolymerans</i>	Esterase	Poly(3-hydroxybutyrate-co-3-mercaptopropionate)	(Elbanna <i>et al.</i> , 2004)
<i>Streptomyces spp.</i>	Laccase, Peroxidase	Polystyrene (PS)	(Zhang <i>et al.</i> , 2022)
<i>Thermobifida fusca</i>	Cutinase	PET	(Yan <i>et al.</i> , 2020.)

Fungal-Mediated Biodegradation of Plastics

A variety of fungal species have been extensively studied for their capacity to biodegrade different types of plastics, offering promising solutions to plastic waste management. Among

these, *Aspergillus fischeri* from the Deuteromycota genus has been identified as a key player in the degradation of polycaprolactone (PCL), a synthetic polyester that is widely used due to its biodegradable properties (Benedict *et al.*, 1983). Another fungus, *C. globosum* QM459, belonging to the Ascomycota genus, has demonstrated the ability to degrade both polyethylene adipate (PEA) and polyphthalamide (PPA), indicating its potential in managing complex plastic waste mixtures (Darby & Kaplan, 1968). Similarly, *Curvularia senegalensis* from Deuteromycota is involved in breaking down polyethylene (PE), one of the most commonly used plastics in consumer goods, which poses significant environmental challenges due to its slow degradation rate (Howard, 2002).

Further research has revealed that *Cryptococcus laurentii*, a Basidiomycota species, also plays a role in PCL degradation, contributing to the growing body of evidence that different fungal groups are capable of attacking synthetic polymers (Benedict, Cameron, *et al.*, 1983). Additionally, *F. moniliforme*, another Deuteromycota species, not only degrades PCL but also cutin, a natural polyester found in plant cuticles, suggesting that fungi adept at breaking down natural polymers may also be effective in synthetic polymer degradation (Murphy *et al.*, 1996). The Ascomycota genus is further represented by *Debaryomyces hansenii*, (Gonda *et al.*, 2000) which has been identified for its role in the biodegradation of polyhydroxybutyrate (PHB), a biodegradable plastic used in various applications.

Notably, fungi from the Zygomycota group, such as *Mucor sp.*, are also involved in PHB degradation (Matavulj & Molitoris, 1992). Another Basidiomycota species, *Rhodotorium sphaerocarpum*, has been found to degrade PHB, reinforcing the idea that different fungal species can complement each other in the degradation process (Gonda *et al.*, 2000). The diverse range of fungal species across different genera and fungal groups highlights the potential of harnessing fungi for large-scale plastic biodegradation, which could significantly reduce the environmental impact of plastic waste. Some fungal species with the known plastic degrading enzymes are mentioned in Table 2.

Table 2: Fungal species performing biodegradation

Fungal Species	Enzyme(s)	Plastic Degraded	References
Aspergillus flavus	Peroxidase, Laccase	Polyethylene (PE), Polystyrene (PS)	(Şimşek Uygun & Malkoç, 2024)
Aspergillus fumigatus	esterase	Polycaprolactone (PCL)	(Novotný <i>et al.</i> , 2015)
Aspergillus terreus	Cutinase, Esterase	Polyurethane (PU), PET	(Temporiti <i>et al.</i> , 2022)
Chaetomium globosum QM459	Unknown	Polyethylene adipate (PEA), Polycaprolactone (PPA)	(Ekanayaka <i>et al.</i> , 2022)
Fusarium solani	Cutinase, Esterase	PET	(Ahmaditabatabaei <i>et al.</i> , 2021)
Fusarium sp.	Cutinase	Polycaprolactone (PCL), Cutin	(Ahmaditabatabaei <i>et al.</i> , 2021)
Penicillium chrysogenum	Lipase, Esterase	Polyurethane (PU)	(Álvarez-Barragán <i>et al.</i> , 2016)
Penicillium simplicissimum	Laccase	Polyurethane (PU)	(Sowmya <i>et al.</i> , 2014)
Trichoderma harzianum	Lignin peroxidase	Polystyrene (PS)	(Carlier, 2017)

Advancements in Plastic Biodegradation

Plastic biodegradation understanding is necessitated by an integrative strategy that closes the gap between molecular interactions and environmental observations. Figure 3 illustrates this holistic approach outlining steps from discovery of enzymes to product analysis. It outlines the intricate mechanisms of plastic degradation following a multi-disciplinary approach with the initial extensive description of biochemical pathways. This is achieved through the identification of novel enzymes through DNA metagenomics, where environmental DNA is searched for genes that code for putative plastic-degrading enzymes. The enzymes are subsequently optimized in function using techniques like site-directed mutagenesis and enzyme engineering. The degradation process is then extensively defined by a wide range of analytical methods. High-Performance Liquid Chromatography (HPLC) is used to separate and analyse the different degradation products, giving the overall picture of degradation. Scanning Electron Microscopy (SEM) can be used for imaging plastic surface morphology alteration, reflecting the physical impact of enzymatic activity. The study detects and quantifies volatile degradation products using gas chromatography-mass spectrometry (GC-MS), which gives data on chemical transformation in degradation.

Enzyme tests, which provide valuable kinetic data, are used to assess enzyme activity and substrate specificity. Additionally, bioinformatics application in delving into microbial protein functional potential has been extensively applicable, ranging from pathogen research like the case of *Naegleria fowleri* (Sehgal P, 2024), to the discovery of enzymes that can break down synthetic polymers.

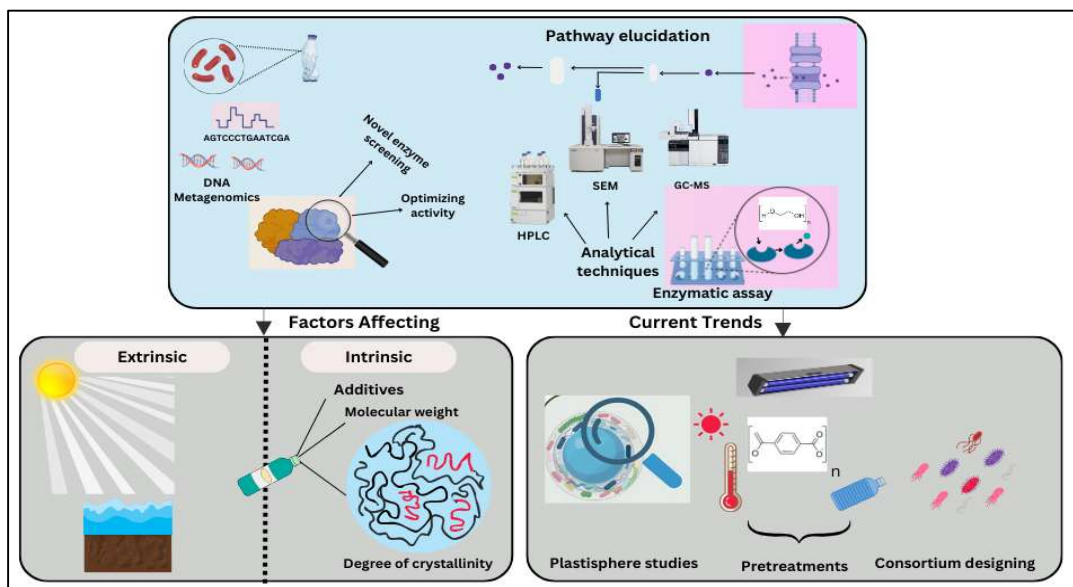


Fig:3 Biodegradation of Plastics: Pathway Elucidation, Influencing Factors, and Emerging Trends [Referred from (Dhali *et al.*, 2024)]

Extrinsic factors such as pH, temperature, and UV exposure are also examined. Some recent advancements in the area are also brought into light in the review, such as the utilization of plastispheres for exploring microbial communities colonizing plastic debris and their role in degrading the same, pretreatment strategies for facilitating access to plastic substrates for enzymatic degradation, and the creation of strategic microbial consortia for advancing synergistic metabolic processes to improve plastic degradation. The integration of these diverse schools of thought and approaches produced an entire paradigm for the construction of bioremediation solutions that effectively respond to the widespread threat of plastic pollution, illustrating the need for interdisciplinarity in response to this environmental issue globally.

Factors Affecting Biodegradation of Plastics:

According to (Ali *et al.*, 2021b) and (Magnin *et al.*, 2020b), a polymer's biodegradability is mostly dictated by the existence of a number of physical and chemical characteristics. There are some major biodegradation controlling factors mentioned in Fig:4 Hydrophilic functional

groups in polymers break down more quickly than hydrophobic ones. Low density and molecular weight polymers break down more quickly. Polymer morphology, i.e., the proportion of crystalline to amorphous regions, plays a role, with the amorphous regions degrading faster. Structural complexity, including branching, may affect the rate of degradation, and polymers with labile bonds, including ester or amide linkages, will degrade more readily. Their polymer molecular structure, nature of polymer, state (film, pellet, powder, etc.), and hardness (more biodegradable if they are soft compared to hard polymers) are among the main determinants of evaluating their biodegradability as well.

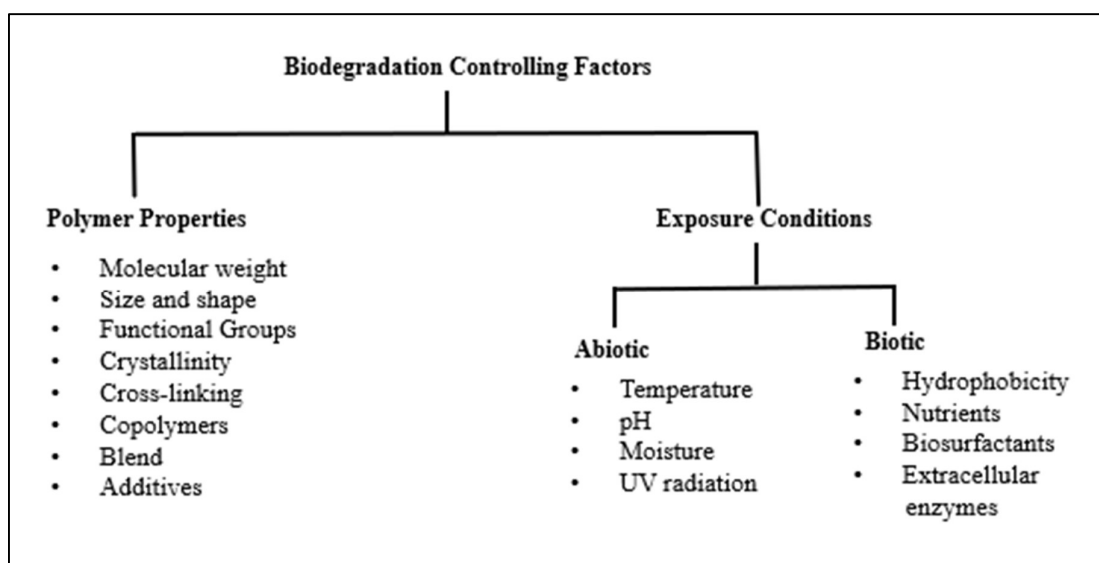


Fig: 4 Key Factors Influencing the Biodegradation of Plastics: Polymer Properties and Environmental Exposure Condition (Kijchavengkul & Auras, 2008)

Results and Discussion

This review consolidates findings on diverse microbial communities, including bacteria and fungi, capable of degrading recalcitrant plastics like polyethylene, PET, PVC and many more. The efficacy of biodegradation varies significantly based on plastic type, environmental conditions, and microbial strain and the type of enzymes produced by such strains. The integrated methodology from metagenomic enzyme discovery through to sophisticated product analysis through HPLC and GC-MS, highlights the synergy in applying molecular biology coupled with analytical chemistry. Multidisciplinary strategies are paramount to creating effective plastic biodegradation processes and unravelling the biochemical pathways, as well as understanding the functional consequences of environmental pressures and emerging

pretreatment practices. This review uniquely integrates metagenomic enzyme discovery with analytical characterization, presenting a comprehensive image of plastic biodegradation. It highlights recent trends such as plastisphere analysis and synergistic microbial consortia, critically examining their applicability in real-world applications. Unlike previous studies, it prioritizes the intricate interaction of environmental factors and research translation into large-scale waste management. Future research should prioritize optimizing these methods for large-scale application and addressing the challenges of microplastic degradation.

Conclusion and Future Prospects

This paper highlights the potential role of different microbes useful in the biodegradation of plastics, which includes both bacteria and fungi. Enzymes secreted by such microbes as a part of their metabolism also have devastating effects on degrading diverse plastic polymers. Most microbes are found in the natural environment indicating their easy access to perform degradation process. However, their efficacy towards plastic biodegradation is not as efficient as it is required. Either they have very slow biodegradation rate or catalysis process is very slow. Bacterial and fungal strains can be modified by their genetic engineering to have additional genes for biodegradation. Existing enzymatic activity can also be increased by genetic modifications. Two or more strains can be combined to have a new strain which can perform biodegradation of more than one type of plastic polymers with different enzymes within that one strain. Additionally, the production of biodegradable plastics can also be enhanced that provide less energy and resource expenditure than degradation of conventional plastics.

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