Review Article

The Enzymatic action on Plastic Degradation: A Fruitful Approach for reduce the plastic Garbage.

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ABSTRACT

Plastic pollution is a significant issue globally. Plastic pollution in the environment and its entry into the food chain are detrimental to humans, animals, and plants. There are many drawbacks to using traditional approaches to tackle plastic pollution. A proposed solution to this problem is the biodegradation of plastics by microbial enzymes. Enzymes produced by bacteria and fungi have a propensity to completely break down polymers. This review included level and causes of plastic pollution and the conventional ways for reducing plastic pollution, the biodegradation of plastics using plastic-degrading microbial enzymes, which included various sources of bacterial and fungal originated enzymes to breakdown the plastic contents.

Keywords – Plastic, Enzymes, Biodegradation, Polymers, Bacteria, Challenges

1. INTRODUCTION

Plastic pollution has emerged as one of the most pressing environmental challenges on a global scale, largely due to the non-biodegradable nature of plastics. The rapid expansion of plastic manufacturing, usage, and waste output, fuelled by rapid globalization, has resulted in an alarming surge in plastic production over recent decades. In 1950, a modest 1.5 million metric tonnes (MT) of plastic were produced annually; however, by 2019, this figure skyrocketed to a staggering 368 million MT, with China leading in production (Tiseo, 2021). Projections indicate that the annual production of plastics could reach an astounding 2000 million MT by 2050 (Tiseo, 2021). This exponential increase in plastic usage has led to an overwhelming accumulation of plastic waste worldwide, giving rise to what is now commonly referred to as "white pollution". Research by the World-Wide Fund for Nature (WWF) and the University of Newcastle in Australia indicates that individuals may ingest an average of 5 g of plastic every week through food, water, and air, depending on their consumption habits (Molina Velásquez & Darío Giraldo, 2021). The large-scale accumulation of plastics on our planet presents a grave concern. Various methods, such as landfilling, incineration, and recycling, have been employed to address plastic pollution. While each approach has its merits, they also have limitations. In recent years, a method that has gained attention is plastic biodegradation by microorganisms, which proves to be an environmentally favorable approach (Gan & Zhang, 2019). The biodegradation of plastic waste by microbes, using plastic-degrading enzymes, offers a promising pathway to rid the environment of plastic waste.

In this study, we explore the topic of plastics-degrading enzymes and their processes, focusing on the biodegradation of plastics by microorganisms. We also examine various types of these enzymes derived from fungi and bacteria, discussing their potential in tackling the serious issue of plastic pollution. Furthermore, we assess the challenges associated with conventional plastic waste management methods and the urgent need for sustainable solutions to combat plastic pollution effectively.

Plastics pollution and conventional strategies to deal with it

Plastics are synthetic or semi-synthetic materials primarily composed of polymers, created through the process of polymerization. The term "plastics" is derived from the Greek word "plastikos," meaning "able to be molded or sculpted." Their widespread adoption across various industries is due to their lightweight, versatile, and durable nature. Plastics are composed of long, intricately linked chains of molecules, providing them with strength and flexibility. Commonly produced polymers include polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyurethane (PUR), and polyvinyl chloride (PVC) (Clunies-Ross, 2019). The chemical composition and structural makeup make these plastics resistant to natural degradation (Zeenat et al., 2021). Plastics and synthetic polymers like Bakelite existed from the early 20th century, and after World War II plastics usage has been extensively done in non-military applications. Since then, their production and hence accumulation in the environment has grown exponentially (Geyer et al., 2017).

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The introduction of plastics into the environment, regardless of their sizes, forms, or varieties, can be referred to as plastic pollution. This can pose risks to the environment, species, or even human health. The main features of plastic pollution in the environment are: 1. Diversity - because they are present in every sector and can thus infiltrate the ecosystem either directly or indirectly, 2. Persistence - because of their distinctive chemical compositions, they tend to endure in the environment and decomposition is quite difficult, 4. Global issues - due to their stability and durability, they persist in the environment and pose a threat to living things, 4. Combined pollution - meaning they serve as a vehicle for other environmental pollutants to enter and 5. Threats to organisms and human health - may result in physical harm, chemical harm, and biological concerns (Ivar do Sul & Costa, 2014, Wang et al., 2018, Li et al., 2020).

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Plastics are widely employed in all industries due to their many favourable characteristics, notably structural features, chemical makeup, and low cost. Between 2005 and 2017, more plastics were manufactured overall than in the previous 50 years. It was predicted that up to 23 million tonnes of plastics entered the world in 2016, and waste management systems around the world are failing to keep up with the flood of rubbish that has resulted. Even with aggressive reduction goals, it is anticipated that this amount will quadruple by 2030 and grow by 300–400% by 2050. Plastic waste can pollute land, waterways, and coastlines, and lifeforms, especially those that live in marine habitats, may be affected by it. Some significant sources contribute significantly to plastic pollution in both land and water. Freshwater input, domestic and residential activities, tourism, and other economic activities, such as harbour operations, are illustrations of land-based sources and commercial fisheries, navigational activities, waste disposal, and shellfish and fish culture are some ocean-based. Plastics harm the ecosystem by destroying habitat, entangling marine life, aiding in the spread of invasive species, and depositing in sediments, which may influence creatures that inhabit and forage in the benthos. They have negative physical (blockage of the digestive tract and entanglement) and chemical effects (release of toxins) on marine animals when they swathe it. This can migrate to both humans and land animals and lead to serious consequences (Horton, 2022, Rhodes, 2018, Thushari & Senevirathna, 2020). In land animals, plastics can enter due to improper disposal and can cause ingestion or entanglement. Plastics can also enter into organisms through inhalation too due to the presence of minute plastics that is microplastics in the atmosphere. As plastics ascend the food chain, they transport their chemicals, pathogens, or parasites that may have attached themselves to the plastics as well as additives from the production process and can lead to major ailments in humans. Humans can be exposed to these chemicals through the nose, mouth, or skin. Plastic has harmful effects that could prove to be carcinogenic or promote endocrine disruption. At the level of the individual, assemblage, and ecosystem, plastic pollution has a variety of ecological effects. Plastic pollutants mix with other harmful chemical substances, such as heavy metal ions, antibiotics, and POPs, to progressively cause ecotoxicological effects. In addition, soil contamination brought on by inappropriate soil dumping of plastic waste makes soil infertile, which negatively impacts both plants and soil organisms. In addition, the damage to assets caused by plastic pollution influences socio-economic status. (Schmaltz et al., 2020, Chauhan & Wani, 2019, Thushari & Senevirathna, 2020, Chae & An, 2018).

Several methods from the past have been developed to address the issues posed by plastic waste. The three main plastic disposal methods that have been used from the beginning are landfilling, incineration, and recycling, each of which has certain drawbacks. Landfilling results in the overuse of space, the growth of hazardous bacteria, the release of toxins into the environment, a prolonged lifetime, and a slower rate of decomposition. Waste incineration produces a few dangerous compounds, some of which are released into the atmosphere. Recycling is a very expensive and ineffective process since additives and contaminants can make it more difficult to recycle materials and lower the yield and quality of the recovered product (Webb et al., 2012). As there was no reliable method for eliminating plastic waste from the environment, its degradation is necessary. Plastic degradation conventionally can be done via two methods. One method is the abiotic deterioration of plastics utilizing UV radiation, heat, mechanical stress, and chemicals. These methods are found to be non-environmentally friendly, expensive, and less efficient as eventually, plastics sustain as microplastics in the environment. Another way is biotic degradation, which means using organisms to degrade plastics (Zhang et al., 2021). Biodegradation is an intriguing alternative to existing waste disposal practices. it is generally a less expensive procedure that has the potential to be considerably more efficient and does not produce secondary pollutants such as those associated with incineration and landfill (Webb et al., 2012). The impact of plastics on environment and conventional approaches to deal with it can be seen in Figure 1.

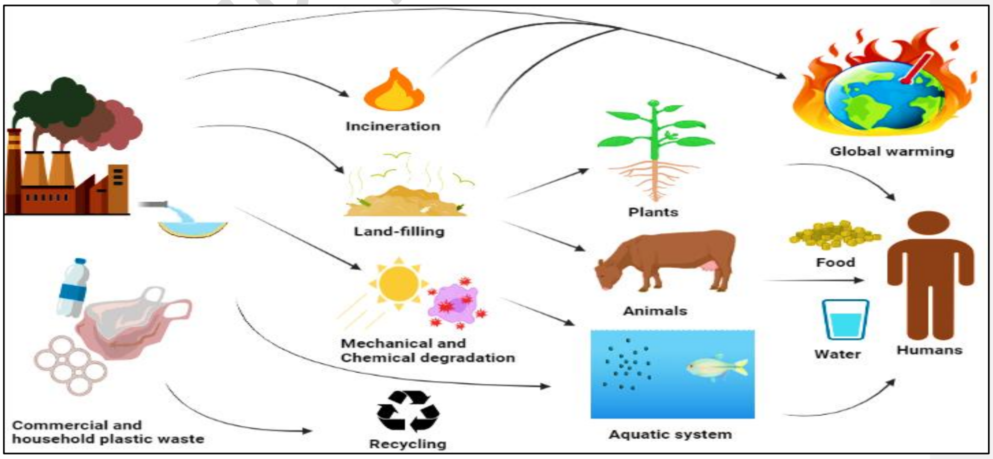

Figure 1: Plastic pollution and Conventional approaches to deal with it.

2. Biodegradation of plastic waste using microbial enzymes

Biodegradation is referred to as the breakdown of organic materials by living organisms. In plastic degradation, microorganisms proved to have a great impact as they breakdown the polymer waste by physical, chemical, or enzymatic action. Both synthetic and natural polymers are subject to degradation and deterioration by microbes (Devi, 2015). The idea of using microbes to clean up waste is widely accepted as they have a remarkable capacity for dissolving complex compounds and advanced polymers, including chitin, lignin, pectin, keratin, and even polythene. They can adapt quickly to every environment on Earth acting as the "natural ecosystem engineer" for habitat restoration. With a variety of catalytic tools present in the microbes, they are highly proficient at breaking down extremely complex carbon-based chemicals into simpler ones (Purohit et al., 2020). Microbes adhere to the surface of polymers and break down these polymers by secreting enzymes to get energy for their growth as seen in Figure 2. (Zeenat et al., 2021). Algae, actinomycetes, bacteria, and fungi are the microbes known to cause the breakdown of plastics' polymers (Urbanek et al., 2020). Bacteria and fungi are the most common source (17 genera of bacteria and 9 genera of fungi). (Raziyafathima et al., 2016) (Refer to Table 1 for a list of bacterial and fungal sources). These microbes secrete extracellular and intracellular enzymes which then are utilized for plastics degradation. Bano et al. explain the general mechanism of plastic degradation via microbial enzymes as an intriguing and environmentally responsible way to get rid of plastics. Due to the actions of high temperature, ambient moisture, and sunlight that condense the discharged product and produce more tenacious lasting residues, this degradation process differs significantly from typical biological degradation, which ends with the disintegration of the polymer. Thus, the term "biodegradation of plastics by enzymes" describes an attack by desirable microorganisms on water-impermeable plastic polymers. Enzymatic activity of certain microbial flora causes a chain fragmentation of polymer into monomers, which is how plastics are broken down. (Bano et al., 2017). The qualities of the polymer, the type of organism, and the method of pre-treatment all play a significant role in how quickly microorganisms can degrade plastics. The degradation of plastics is significantly influenced by the properties of the polymer, including its mobility, crystallinity, molecular weight, kind of functional groups and substituents present in its structure, as well as any plasticizers or additives added to the polymer. Abiotic elements including temperature, moisture, pH, and UV rays as well as biotic factors, mainly enzymes, and their interactions, are crucial in the microbial breakdown of plastics (Rajendran et al., 2016).

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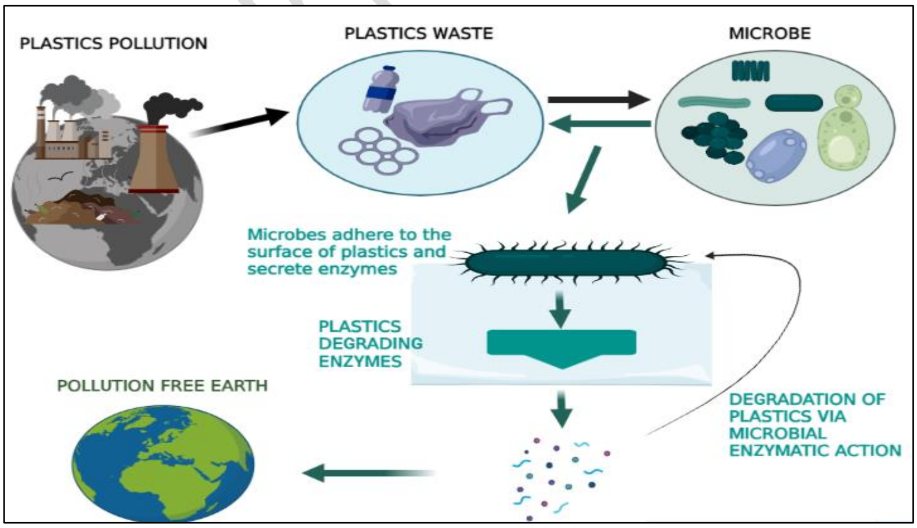

Figure 2: Microbial degradation of plastics.

3. Plastic degrading enzymes (PDE's)

Enzymes are biocatalysts that take part in reactions, act on certain substrates, and enhance the transformation of those substrates into useful products. Enzymes, rather the plastic degrading enzymes found in microbial cells—both extracellular and intracellular—help with degradation by breaking down polymer chains through cellular absorption and generating metabolic by-products such as CO2, H2O, CH4, and N2, which are then utilized in the environment (Amobonye et al., 2021). The class hydrolase enzymes including esterase, cutinase, depolymerase, lipase, and the class oxidoreductase enzymes comprising laccase, and peroxidase are some of the enzymes that are primarily in charge of plastic degradation (Temporiti et al., 2022). The class "Hydrolases" participates in a catalytic reaction that, in the presence of water, results in the dissociation of the chemical bonds of the substrate. Many hydrolases from various fungi and bacteria have been discovered and tested for activity against various aliphatic polyesters (PHA, PBS, PBSA, PCL, PLA), aromatic polyesters (PET, PBT, PMT), and co-polyesters (PBST, PBAT, PBSTIL). The esterase enzyme from the yeast Pseudozyma antarctica has recently been found to expedite the disintegration process of plastic waste films kept in laborious circumstances.

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In the hydrolysis of polybutylene succinate (PBS) and polybutylene succinate-co-adipate (PBSA), a lipase enzyme isolated from the yeast Cryptococcus sp. MTCC 5455 cultured on residual agricultural waste demonstrated a very good potential. Cutinase from Fusarium solani has also been expressed in Pichia pastoris for overexpression of the enzyme to dissolve PBS plastic. A fusion of cutinase and lipase proved to be suitable for the biological degradation of the plastic material poly Ɛ-caprolactone (PCL) too. (Kaushal et al., 2021) (Urbanek et al., 2020). Esterase was reported as an active enzyme that enhanced the degradation of polyester polyurethane in Aspergillus flavus (Ma & Wong, 2013). A microbial consortium was created in a study in Bangalore to break down plastic trash. A 40% reduction in plastic was noticed, and it was discovered that the two bacteria responsible for garbage decomposition were Pseudomonas spp., and the key enzyme responsible was lipase. (Skariyachan et al., 2014). Cutinases from Thermobifida, Pseudomonasand Streptomyces can degrade PET, polycaprolactone (PCL), polybutylene succinate (PBS) and other synthetic polyesters (Xu, 2020). Pseudomonas aeruginosa strain S3 demonstrated high esterolytic activity against polylactic acid (PLA) at three different ambient temperatures (30°C) (Noor et al., 2020). Oxidoreductases (EC-1) stimulate the exchange of electrons between the donor and acceptor molecules, in reactions Involves tiny electron transfer, proton/hydrogen extraction, hydride transfer, oxygen insertion, or other important steps. The laccases and peroxidases which are popular in plastic degradation are from this class of enzymes. In an *Insilico* study of the binding affinity of microbial enzymes against plastic compounds polyamide (PA), polyvinyl chloride (PVC), polycarbonate (PC), polyethylene terephthalate (PET), polymethyl methacrylate (PMM) and polyurethane (PUR), it was found that Manganese peroxidase enzyme showed the best degradation for all studied plastics (Enyoh et al., 2022). In an experiment to find a way to degrade the polymer, lignin peroxidase from Phanerocheate chrysosporium was used for the degradation of PVC films, and a significant reduction in the weight of about 31% was observed at a temperature of 25°C and pH 5 (Khatoon et al., 2018). The ability of laccase from *Cochliobolus* sp., to degrade low molecular weight polyvinyl chloride (PVC) was depicted in one of the f studies, proving fungal treatment technology as an effective way to degrade plastics (Sumathi et al., 2016). A fungal consortium was built comprising Curvularia lunata, Alternaria alternata, Penicillium simplicissimum, and Fusarium sp. for polyethylene degradation. The enzymes secreted were laccase and peroxidase. Results showed that this is a better alternative for degradation as the activity of the enzymes together was relatively high (Sowmya et al., 2015). In an investigation, low-density polyethylene (LDPE) bags, which account for a significant portion of the plastic waste problem, were depolymerized with Penicillium sp. [OM760513], Aspergillus terreus [OM760511], and Bacillus sp. [OM760515], resulting in a 24% weight loss. Laccase and peroxidase from Aspergillus terreus and Pencillium sp. strains were the key enzymes involved in the degradation (Mohy Eldin et al., 2022). PDEs have been identified utilizing culture-based procedures in which microorganisms are cultivated from an environmental sample and screened for plastic-degrading activity. They are discovered utilizing a combination of biochemical and molecular biology techniques. Proteins, for example, can be isolated from grown microbes and evaluated for plastic-degrading activity. Once isolated, PDE sequence information can be retrieved using mass spectrometry (MS), and biochemical experiments can be utilized to characterize optimal enzyme conditions and substrate selectivity. Alternatively, after a microbe has been discovered, whole genome sequencing and annotation can be used to assist PDE gene prediction.

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Predicted PDEs can be tested for activity by cloning them into a heterologous host. Aside from that, PDEs can be identified using in *silico* techniques too, in which based on similarity searching, one can identify these enzymes (Viljakainen & Hug, 2021). Buchholz et al., 2021 describe the Plastics-Active Enzymes Database (PAZy) as a collection of known and experimentally validated enzymes that act on polymers derived from fossil fuels. Profile-hidden Markov models were used to identify nearly 3000 homologs of PET-active enzymes. BLAST discovered almost 2000 homologs of PUR-active enzymes. Multiple sequence alignments were used to identify the most conserved amino acids, and sequence motifs for PET- and PUR-active enzymes were developed (Buchholz et al., 2021).

Enzymatic degradation of plastics by plastic-degrading enzymes (PDEs) has several benefits, including the ability to transform waste plastic into useful alkane products, lowering plastic pollution, and acting as a promising process for changing waste petro-plastics into short polymer intermediates that can be consumed by microbial cells. Compared to chemical recycling, this method is more effective and energy-saving, and it also offers the chance for upcycling. PDEs' efficiency can be increased by protein engineering. Numerous microbial PDEs have been identified to date, and there is rising interest in discovering novel enzymes with desired characteristics and abilities. (Kaushal et al., 2021, Tournier et al., 2023 & Zhu et al., 2022).

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<u>Table 1: List of Plastic degrading enzymes, their source, and type of plastics.</u>

Name of Enzyme	Source Organism	Type of plastics	Reference

Cutinase	*Thermobifida alba*	Polyethylene	(Mrigwani et al., 2022),
	Thermobifida cellulosilytica	Polyethylene terephthalate	(Zhang et al., 2022)
	Thermobifida alba	Polylactic acid	(Kitadokoro et al., 2019)
	Vibrio gazogenes	Polyethylene terephthalate	(Danso et al., 2018)
	Saccharomonospora viridis	Polyethylene terephthalate	(Numoto et al., 2018)
	Fusarium oxysporum	Polyethylene terephthalate	(Dimarogona et al., 2015)
	Fusarium solani	Polybutylene succinate	(Hu et al., 2016)
	Humicola insolens	Polyethylene terephthalate	(Carniel et al., 2017), (Di Bisceglie et al., 2022)
	Aspergillus oryzae	PBSA: Polybutylene succinate-co-adipate Polylactic acid Polybutylene succinate	(Maeda et al., 2005)
Esterase	*Pseudomonas sp.*	Polyester, Polyethylene terephthalate Polyethylene terephthalate Polyurethane	(Kim et al., 2020), (Tribedi et al., 2011), (Shah et al., 2008), (Roberts et al., 2020),
	Bacillus sp.	Polyethylene terephthalate Polyurethane	(Roberts et al., 2020), (Shah et al., 2008)
	Ideonella sakaiensis	Polyethylene terephthalate	(Palm et al., 2019)
	Brevibacillus borstelensis	Low-density polyethylene	(Ndahebwa Muhonja et al., 2018)
	Acinetobacter baumannii	Polyurethane	(Howard et al., 2012)
	Rhodococcus sp.	Polyurethane Polybutylene adipate terephthalate , PHB: Polyhydroxybutyrate Polybutylene adipate terephthalate	(Zampolli et al., 2022)
	Aspergillus flavus	Polyurethane	(Mathur & Prasad, 2012)
	Moesziomyces antarcticus	Polycaprolactone Polybutylene succinate Polybutylene succinate-co-adipate. Polylactic acids	(Saika et al., 2019)
	Purpureocillium lilacinum	Low-density polyethylene	(Spina et al., 2021)
LIPASE	*Pseudomonas sp.*	Polyurethane Polyvinyl chloride Polyurethane	(Spina et al., 2021)
	Alcaligenes faecalis	Polycaprolactone	(Khatiwala et al., 2008)

	Aspergillus niger	Polycaprolactone Polylactic acid	(Nakajima-Kambe et al., 2012)
	Thermomyces lanuginosus	Polybutylene adipate terephthalate	(Kim et al., 2020)
DEPOLYMERASE	*Rhodospirillum rubrum*	Polyhydroxybutyrate	(Sznajder & Jendrossek, 2011)
	Streptomyces ascomycinicus	Polyhydroxybutyrate	(García-Hidalgo et al., 2013)
	Pseudomonas mendocina	Polyhydroxyalkanoates Polycaprolactone Polybutylene succinate	(Mao et al., 2013)
	Actinomadura sp. S14	Polybutylene succinate-co-adipate.	(Sriyapai et al., 2018)
LACCASE	*Trametes versicolor*	Polyurethane	(Magnin et al., 2021)
	Pleurotus ostreatus	pro-oxidant additive to polyethylene.	(da Luz et al., 2013)
	Aspergillus flavus	Polyethylene	(Priya et al., 2021)
	Aspergillus terreus	Low-density polyethylene Polyethylene	(Mohy Eldin et al., 2022)
PEROXIDASE	*Aspergillus flavus*	Polyethylene	(Zhang et al., 2020)
	Phanerochaete chrysosporium	Polyethylene	(Mukherjee & Kundu, 2014)

Conclusion

Plastic pollution has a detrimental impact on the ecosystem that extends to all facets of ecology, including people, plants, animals, and the atmosphere. Traditional approaches to managing plastic trash, such as landfilling and incineration, are not environmentally beneficial, and recycling is a time- and money-consuming process that fails to eliminate the plastics from the earth's surface. With microbial enzymes, plastic biodegradation is proven to be a promising and environmentally friendly method for eliminating plastic pollution. Plastics degrading enzymes are the biocatalyst produced by the microbes when they adhere to the surface of plastics. These enzymes can be broadly classified into two types: hydrolases (including subtypes such as esterases, cutinases, lipases, and depolymerase) and oxidoreductases (including subtypes such as laccases and peroxidases). These enzymes, sourced from various bacterial and fungal sources, are capable of efficiently degrading various types of plastics. A future, free of the build-up of plastic waste in the environment, is promised by the biodegradation of plastic waste by microbial enzymes.

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