Recent Advances in

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Enzymepathway and Applications

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Recent Advances in Microbial Plastic Degradation: Enzymepathway and Applications

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Abstract

The use of plastic has increased tremendously in all areas of life, including but not limited to, packaging, transportation, healthcare, and many other industries; therefore, no modern society would function without it. Unfortunately, the continued growth of plastic production, consumption, and accumulation across the globe is creating tremendous environmental problems. Globally, the accumulation of plastic waste in landfills, oceans, and on land contributes to greenhouse gases, pollution, and generation of micro- and nano-sized plastics which are unsafe for the environment and threaten the health of animals and humans. Traditional methods for managing plastic waste, including landfilling and burning (incinerating), have limited, negative impacts on our environment and economy. As such, there is an urgent need for ecological and sustainable alternatives for getting rid of plastic waste. Biodegradation by microorganisms is an exciting area of research as a biological solution to the effects of plastics in the environment. Many microorganisms, such as bacteria and fungi, have enzymes that can break down certain types of synthetic polymers, such as polyethylene terephthalate (PET), polyamides (PA), and polyurethane (PUR). However, there remains limited knowledge on how other recalcitrant types of plastics, such as, polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), epoxy resins, and silicones are biodegraded by enzymes and microorganisms. A better understanding of the biochemical pathways and enzymatic systems that biodegrade plastics will be critical for developing and implementing strategies for efficiently biodegrading plastics, developing biodegradable plastic products, and improving methods of managing plastic waste.

1. Introduction

Plastic has been a vital aspect of daily life for many years. Whether used as a packaging material, for transportation purposes, or in healthcare settings, it has been shown that plastic is an essential part of society and has both positive and negative impacts. The accumulation of plastic waste is considered a major global challenge in the 21st century. While many consumers have now realised this challenge, the volume of single-use plastics produced continues to rise each year and thus create problems for both the economy and the environment due to the increasing amounts of plastic waste in our oceans and landfills [1].According to a report, India came out in third place among all countries for being the biggest consumer of plastics, with 21 million tons consumed in 2021[2]. If there is no change in the manner in which plastic waste is disposed of by unorganized waste disposal services and if plastic waste continues to be disposed of without adequate recycling methods, it is anticipated that by the year 2030, global plastic waste could produce an additional 53 million tons of carbon dioxide equivalent in greenhouse gas emissions[3]. Many countries across the globe build landfills throughout their nations without looking at the costs.In addition, most of the methods used to dispose of plastic waste are landfilling or incineration. In 2021 alone, India will require 120 hectares of land for landfilling; however, only 21% of landfills in India are managed properly, due to greenhouse gas emissions, noxious gas emissions, and the risk of fire[4].On the other hand, advanced economies such as Germany, Japan, France and the United States are using thermal waste-to-energy facilities in order for energy retrieval from plastic waste, however progressing nations have yet to do so and have a very large uphill battle ahead of them because it requires such a very large capital investment and concerns about administration hazards from toxic manufacturers (including dioxins/furans, etc) [5] and thus far, one report concluded the combustion of plastic packaging alone has created ~ 16 million metric tonnes of CO2 emissions [6] . Plastics continually break down into small particles, causing microplastic (5mm to 0.1µm) and nanoplastic (0.1µm to <100nm) pollution, when exposed to other environmental elements including (but not limited to) weathering, solar radiation, mechanical forces, and microorganisms [7]. These new pollutants continue to be a nuisance to the environment and people around the globe and are found in freshwater sources, oceans, drinking water, and soil [8,9].

1.1 Types of plastics

Industrial- scale plastic production primarily focuses on manufacturing a wide range of thermoplastics materials because they are widely used in the production of everyday products and commercial good.

I. **Polyethylene terephthalate (PET)**
Melting point- 245-265^{0}C
Recyclability- commonly recycled

II. **High- density polyethylene (PE-HD**)
Melting point- 130-140^{0}C
Recyclability- commonly recycled

III. **Polyvinyl chloride (PVC**)
Melting point- 100-260^{0}C
Recyclability- sometimes recycled

IV. **Low- density polyethylene (PE-LD**)
Melting point- 85-125^{0}C
Recyclability- occasionally recycled

V. **Polypropylene (PP**)
Melting point- 165-175^{0}C
Recyclability- occasionally recycled

VI. **Polystyrene (PS**)
Melting point- 240^{0}C
Recyclability- commonly recycled but sometime difficult

2. Microbial plastic degradation

2.1 Polyethylene terephthalatePET (Polyethylene Terephthalate) is primarily produced for manufacture of PET bottles, PET film or foil. And also to produce fibers for the textile industry with PET being an aromatic, linear polymer with repeating monomer units of terephthalic acid and ethylene glycol, With the PET monomer being referred to as bis (2-hydroxyethyl) terephthalate (BHET) [10]. PET is a thermoplastic polymer and is somewhat crystalline in nature. In 2017, more than 30 million tons of PET were manufactured. Although currently there are very few bacteria or fungi reported to partially degrade or convert PET into oligomeric or monomeric forms of PET [11].All of the PET degrading bacteria identified so far exhibit low rates of hydrolysis on PET compared to other polymer degradation. Most of the identified bacteria with the ability to degrade PET belong to one of a few select bacterial phyla, with the majority of the isolates being members of the Gram positive phylum Actinobacteria [12]. The most well characterized examples of this ability... are from the genusThermobifida and Thermomonospora [13-14]. The enzymes that have been shown to degrade PET (such as the PET hydrolase, tannase, and MHETase) are all classified as serine hydrolases, which can be further subdivided based upon their substrate specificity: cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3), carboxylesterases (EC 3.1.1.1) belong to this class of enzymes. All of these enzymes have a common hydrolase fold, and possess a catalytic triad containing serine, histidine, and aspartate residue [15-16].

2.2 Polyurethane Polyurethanes (PURs) are formed from a range of different polyol materials which can be polyether or polyester. The polymer formed from the addition of a carbamate structure forms the backbone of PUR.The incorporation of additional, highly branched aromatic ring structures further alters the chemistry and physicochemical performance of the polymer. PUR is one of the most extensively used synthetic polymers, used to manufacture foams, thermal insulation, coatings for textiles, and paints to prevent rust and corrosion [17]. PUR is produced on average, over 27 million metric tons annually, and ranks as the 5th most frequently produced synthetic polymer. Currently, only the bioactivities of microorganisms on ester-based PURs have been documented [18-19]. Fungi and bacteria have been identified to contribute to biodegradation of PUR. Gram-negative Betaproteobacteria identified as belonging to the genus Pseudomonas are among the most commonly identified groups of bacteria that are known to degrade PUR. The discovery of the first PUR-degrading enzyme identified was the PueB lipase from Pseudomonas chlororaphis [20].

2.3 Polyethylene Polyethylene (PE), a polymer made of long chains of ethylene, can be found as either high density (HD-PE) or low density (LD-PE). The ability to manufacture PE chemically through the polymerization of ethylene, creates a highly variable end product, primarily due to the addition of side chains depending on how it was processed. Such side-chain additions can significantly impact both the crystallinity and molecular weight of the polymer. PE is predominantly used in the packaging industry, as one of the primary materials for packaging and over 100 million metric tonnes of PE are manufactured globally on an annual basis [21-22]. The degradation of PE has been related to a surprisingly high number of different genera of bacteria; those types of bacteria are mainly composed of a significant number of Gramnegative people such as Pseudomonas, Ralstonia, and Stenotrophomonas as well as many Gram-positive families such as Rhodococcus, Staphylococcus, Streptomyces, Bacillus and numerous other families [23].

2.4 Polyamide Polyamide (PA) is a polymer composed of an array of amide-linked repeating units of aliphatic and/or semiaromatic or aromatic molecules. Monomer diversity permits many different types of synthetic polyamides, with the two most widely used being nylon and Kevlar. The primary applications of synthetic polyamides include textiles, automotive manufacturing, carpets, and athletic wear [24]. Surprisingly, proteins and natural silk are both polyamide polymers themselves. Therefore, one might expect there to have been some evolutionary process involving enzymes capable of degrading these polymers in nature; however, to our knowledge, there are no microorganisms that can completely metabolize intact, high-molecular-weight polyamide polymers (or use them as their sole carbon source). Conversely, there are multiple reports on the ability of various microorganisms to degrade either linear or cyclic nylon oligomers with relatively low molecular weight, and one of the earliest publications describes bacterial growth on various oligomers produced during the synthesis of nylon [25].In the waste produced by factories that create nylon, 8-caprolactam, 6-aminohexanoic acid, 6-aminohexanoic acid cyclic dimer, and 6-aminohexanoic acid oligomers are created. These compounds act as the functional carbon and nitrogen source for specialized bacteria. One of the first known bacteria that utilized these oligomers was Flavobacterium sp. strain KI72, which went on to become known as Achromobacter guttatus KI72, and most recently was reclassified as an Arthrobacter sp. strain KI72 [25,26]. Bacterial isolates from Arthrobacter that can degrade nylon oligomers have genes encoding for various hydrolases and many different amino-transferases that are needed for the initial degradation and subsequent metabolism of the nylon oligomers. The relevant genes for strain KI72 are located on a secondary plasmid, pOAD2 [27,28].

2.5 Polystyrene The polymer, polystyrene, is composed of styrene monomer units. Due to their widespread usage in the packaging industry, polystyrenes are also used to construct many everyday items (i.e. compact disc cases, disposable cutlery, petri dishes, etc.) [29]. Unfortunately, there are currently no known enzymes that can degrade high molecular weight polystyrene. However, researchers including Krueger have recently identified several strains of brown rot fungi as capable of degrading polystyrene via hydroquinones (via Fenton's reaction). These studies revealed that the brown rot fungus Gloeophyllum striatum DSM 9592 and Gloeophyllumtrabeum DSM 1398 significantly depolymerized polystyrene after 20 days of incubation. The most aggressive Gloeophyllum strains produced reductions of nearly 50% in molecular weight [30].The previous inquiry demonstrated that coincubation was associated with the breakdown of polystyrene by white rotting fungi (Pleurotus ostreatus, Phanerochaetechrysosporium, and Trametes versicolor) and brown rotting fungi (Gloeophyllumtrabeum) [31]. These preliminary, yet significant, findings indicate that high-molecular-weight polymers can undergo chemical transformations by degradation processes; however, more research is needed to identify what types of enzymes (if any) are responsible for the chemical breakdown. Additionally, as mentioned previously, it is possible that the weight reduction is also due to the loss of chemical additives.

2.6 Polyvinylchloride and polypropylenePVC (polyvinylchloride) and polypropylene (PP) are major polymers produced in larger amounts than has been described for other polymers previously. PVC ranks as the third most popular polymer by production volume, after polyethylene (PE) and PP. PVC consists of repetitive chloroethyl units, while PP is made up of repetitive propane-1,2-diyl units [32,33]. To date, there is little published information related to microbial degradation of either polymer despite the large amount of this type of polymer that has been produced globally. There have been only a few publications describing the loss of mass of PVC and PP due to microbial degradation in mixed microbial communities [34,35]. However, a significant number of the references have probably attributed microbial degradation of PVC and PP attributed to the degradation of chemical additives and not to the degradation of the polymers. To date, we do not know of any defined enzymes and/or pathways that are responsible for the microbial degradation of either of these two high molecular weight polymers.

3. Enzymatic Mechanisms of Biodegradation

3.1 Depolymerase- Enzymatic degradation of biodegradable plastics occurs mostly through enzymes produced by microbial populations. Microbial populations release enzymes that catalyze the breakdown of complex polymers into individual monomer units through degradation; therefore, the breakdown includes the polyethylene or polyhydroxyalkanoate depolymerizing enzymes of microorganisms. Various forms of enzyme activity exist for degrading polyhydroxyalkanoates, and their applications are well-researched with regards to their ability to cleave the polymer into oligomers or monomers. Most extensively studied are the PHA depolymerases which display a high level of specificity for their respective substrates. As a result of which microorganisms have the ability to produce extracellular, cell surface-bound and/or intracellular polyhydroxyalkanoate depolymerases based on the species type [36].

3.2 Esterases and Lipase-Esterases and lipases are enzymes that hydrolyze ester bonds in various synthetic biodegradable plastics, such as PLA and PCL. They are found in many different types of bacteria and fungi and can work well in multiple environments. Lipases will primarily act on the amorphous sections of the polymer, and thus begin to erode the surface of the material [37].

3.3 Cutinases- Cutinases are a class of serine hydrolases that were first described as enzymes that help degrade plant cuticles. In addition to being highly stable and having very high rates of catalytic reaction, cutinases can also work on a wide variety of substrates, thus making them extremely effective at breaking down aliphatic polyesters. This combination of qualities makes cutinases of particular interest to researchers looking for alternative solutions for recycling bioplastics and for treating waste [38].

Table 1. Major Enzyme involved in microbial degradation of biodegradable plastics

Enzyme class	Target polymers	Produced	Degradation stage
PHA depolymerase	PHA, PHB	Bacteria, fungi	Initial depolymerization
Esterases	PLA, PBS, PBAT	Bacteria, fungi	Surface erosion
Lipase	PCL, PLA	Bacteria, fungi	Amorphous region
Cutinases	PLA, PCL	Mainly, fungi	Rapid chain scission

4. Factors Affecting Microbial Degradation Efficiency

4.1 Environment Microbial growth and enzyme activity are greatly affected by four factors; temperature, moisture, oxygen availability, and pH. The controlled conditions of industrial composting systems allow for rapid degradation of organic material, but natural soils and marine environments usually exhibit much lower degradation rates [39].

4.2**Polymer Properties** Susceptibility of polymers to microbial attack is determined by their crystallinity, molecular weight, surface area, and chemical composition. Rapid degradation is seen with amorphous polymers with lower molecular weights compared to highly crystalline polymers [40].

4.3 Microbial Community Interactions When microbial consortia interact, the total degradation rate is increased due to the production of synergistic enzymes and the cooperation of metabolic processes. Sequential degradation by multiple groups of microorganisms improves the complete mineralization of a polymer [41].

5. Applications of microbial degradation

- The Microorganisms Enzymes to Degrade Plastics Microorganisms have the ability to produce enzymes that can degrade different types of plastics including polyethylene (PE), polyethylene terephthalate (PET), polyurethane (PU) and polystyrene (PS). The degradation of plastics helps reduce the accumulation of plastics in landfills and aquatic environments.

- Microbial Bioremediation of Polluted Environments Microorganisms can degrade a variety of pollutants including petroleum hydrocarbons, pesticides, dyes and industrial chemicals.

Microorganisms can be utilised in the removal of contaminants from soils, water and oil spills, through bioremediation. Microbial Treatment of Wastewater Microorganisms such as bacteria and fungi are able to degrade organic material found within sewage and industrial effluents. Microorganisms can also improve the quality of the water, while decreasing environmental pollution. Composting & Organic Waste Recycling Microorganisms are also able to decompose many types of agricultural residues, food scraps and animal manure into compost with high nutrient value. Microbial compost can improve soil fertility and promote sustainable agriculture. Biofuels Microbial degradation of lignocellulosic biomass (plant material) allows conversion to fermentable sugars. Fermentable sugars can be used to produce bioethanol, biogas and other renewable fuel sources. Agricultural Benefits Microbial degradation of crop residues can contribute to improved nutrient cycling and soil health. Beneficial microbes can assist in maintaining soil fertility and supporting plant growth [42].

- Industrial Biotechnology Microorganisms produce enzymes used in industrial biotechnology applications such as textiles, paper, detergents, food and pharmaceuticals. Microbial degradation processes are environmentally sound and cost effective. Medical and Pharmaceutical Applications

Microbial degradation is one method of treating biomedical waste. Microbial degradation is also used to biotransform or detoxify certain pharmaceuticals.

6. Microbial Metabolic Pathways of Polymer Assimilation

Microbial cells will take up soluble products resulting from the enzyme-induced breakdown of PLA, and they will metabolize them as part of their normal respiration activity. Lactate released during PLA degradation is metabolized to pyruvate and subsequently enters into the tricarboxylic acid (TCA) cycle where it contributes to creating energy as well as biomass growth. In the same manner, the hydroxyalkanoate monomers produced from the degradation of PHAs are converted to either acetyl CoA or related intermediates, i.e. the same intermediary compounds that enter the TCA cycle previously mentioned. Intermediates produced following the degradation of biodegradable plastics allow microorganisms to use these types of plastics solely as carbon and energy sources, thus completely mineralizing these types of materials when environmental conditions are favourable [43].

Table 2. Major microbial pathway

Polymer	Degradation products	Key enzyme	Metabolic entry point	Final fate
PLA	Lactic acid	Esterase, lipase	Pyruvate	CO_2 Biomass
PHB	3- hydroxybutyrate	PHA depolymerase	Acetyl- CoA	CO_2 Biomass
PCL	6- hydroxycaproic acid	Lipase, cutinase	Beta-oxidation	CO_2 Biomass
Starch based plastics	Glucose	Amylase	Glycolysis	CO_2 Biomass
Cellulose based plastic	Cellobiose, glucose	Cellulose	Glycolysis	CO_2 Biomass

7. Future challenges in microbial plastic degradation research

Currently, there is a small amount of enzyme and microbial diversity that is able to actively degrade synthetic polymers. Therefore, it is essential that researchers identify the organisms that are capable of degrading the most abundant synthetic polymers. The primary challenge to degrading these types of materials is the initial breakdown of the high molecular weight, thus strong, crystalline structures of the polymers. The other major challenge for future microbiologists will be using enzymes effectively to help degrade plastics that have been introduced into various contaminated ecosystems. There is currently little data available on highly active enzymes for most commercial plastics because there is currently no technology available that has reproduced conditions that allow the cultivation of high actvity enzymes for the majority of synthetic plastics from microflora. The use of extensive non-cultivated microorganisms (Metagenome) and associated so-called dark matter proteins (proteins produced by non-cultivated microorganisms) offer promise as potential biocatalysts for degrading synthetic plastics. Facilitating the further development of advanced algorithms for metagenomic analysis will be a valuable means for finding potential biocatalysts in metagenomes. Also, reliable functional assays need to be developed and utilized to detect high molecular weight polymer active enzymes. Commercially produced polymers and plastic films often include a variety of additives, plasticizers, and biodegradable impurities (such as phthalates), which can degrade much more easily than the actual polymer backbone.

As a result, there is disruption to the data generated and a high tendency to report false positives. In addition, the methodology around how microbial plastic degradation is evaluated still needs to be standardised and optimized further.

Furthermore, a good way to evaluate potential avenues for the deconstruction of rigid and crystalline fibres, such as celulosmic-like structures (i.e."plastosomes") can also be accomplished through the creation of highly active enzymes for the textile industry, since this would help to begin to directly address the growing plastic pollution issue we are experiencing. Furthermore, via the use of synthetic biology tools to developed microorganisms to create high-value products from plastic waste is an exciting objective and would help towards a more circular approach to plastic use. Oligomers and/or monomers created after plastic degradation may be used to produce higher-value end products or alternative (biodegradable) polymers. Furthermore, obtaining plastic-active type enzymes and employing them in developing true biopolymers is an exceptionally beneficial research opportunity and would have a dramatic impact on the global plastic problem. This paper has presented information from a number of recent major studies on microbes that degrade plastics made from various types of polymers using a variety of microbes (bacteria, archaea, and fungi). Significant achievements have been made in the discovery of enzymes that degrade certain synthetic polymers such as PET, ester-based PURs and nylon (PA) oligomers. However, other than a few exceptions, little is known about the ability to identify and utilize enzymes that would degrade the majority of other synthetic polymer types (e.g., PE, PP, PS, PVC, etc.). Therefore, additional research is needed to investigate and identify the genes and pathways contributing to microbial and or Enzyme based degradation of these very resistant and extremely robust polymers by examining the microbiological and biogeochemical diversity of our planet. Identifying enzymes or species of microbial communities that can effectively degrade these synthetic polymers will be of enormous benefit to our society. Therefore, identifying an effective dehalogenase enzyme or organism that can accelerate the microbial degradation of PVC or other per- and poly-halogenated materials (i.e., PFAS) would represent a major advancement in discovering and developing new concepts to support the microbial and enzymatic degradation of synthetic polymers. In addition, identifying highly efficient laccase, peroxygenase, or peroxidase enzymes involved in the initial steps of the microbial oxygen activation of polyolefins and other highly durable polymers (see above) would also represent another major advancement.

8. Conclusion

Approximately four out of every five (80%) plastic products manufactured globally are disposed of through natural processes. Plastics that are released into the environment do not biodegrade, thus they potentially can be in the environment indefinitely.This accumulation of large amounts of plastic poses serious dangers both ecologically, by disrupting cycles of nutrients throughout the world, and socially, by harming both human and animals. As a consequence of plastics accumulating and being distributed to all parts of the environment (land, water, and air), scientists have begun searching for living microorganisms that can decompose plastic, especially from sources of fossil fuels. Though there have been known microorganisms capable of decomposing numerous amounts of different polymers, such as PET, oligomeric PA and ester based PUR, there is limited knowledge regarding the microbial and viral enzymes necessary for the decomposition of ether or poly-ether based polymers (e.g., PUR, PA, PC, PE, PP, PVC, PS), epoxy resins and silicones. Addressing the challenges surrounding the disposal of plastics and finding methods or solutions for their decomposition will be one of the significant goals of future microbiologists. The degradation of materials by microorganisms is fundamental to the long-term sustainability of biodegradable plastics. Microorganisms facilitate degradation through various biochemical pathways or enzyme systems allowing biodegradable polymers to be converted into non-toxic products. Therefore, it is important to develop a detailed understanding of the biochemical pathway or enzyme system that microorganisms use to degrade plastics because this information will allow researchers to better design biodegradable polymers, develop waste management methods, and aid in protecting the environment. Continued interdisciplinary research will help solve the global plastic pollution problem.

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