Review

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A comprehensive biotechnological and molecular insight into plastic degradation by microbial community

Anshu Priya,[a*] Kasturi Dutta[b] and Achlesh Daverey[c*]

Abstract

Environmental pollution by petrochemical plastic is a matter of serious global concern. Several microbes have the potential to degrade synthetic polymers with the aid of various genes, enzymes, and metabolic pathways. However, the biodegradation of petrochemical plastics by natural microbes is inherently slow. The slow rate of degradation stems from the high molecular weight, strong chemical bonding, and extremely hydrophobic nature of polymer, all of which hinder biodegradability. Nevertheless, the role of genes, enzymes, and interactions between microbes and plastic as a substrate has been inadequately explored. Notably, several biotechnological approaches (such as synthetic biology, metabolic engineering, and bioinformatics tools) have been developed for the efficient biodegradation of synthetic polymers. Further, exploiting the degradative potential of microbes, plastic wastes can be utilized as feedstock for the production of high value compounds. This will not only avert environmental pollution but would also facilitate waste management and circular economy. However, the major limitations to these approaches are lack of experimental validations in real world. In this regard, the present review provides a comprehensive assessment of the biotechnological and molecular advancement in plastic biodegradation to facilitate a better understanding of the role of microbes, genes, enzymes, and biodegradation pathways in plastic mineralization.

Keywords: petrochemical plastics; biodegradation; biotechnological degradation approach; synthetic biology; metabolic engineering

ABBREVIATIONS

ABC	ATP binding cassettes
BHET	Bis (2-hydroxyethyl) terephthalate
EG	Ethylene glycol
HDPE	High density polyethylene
LDPE	Low density polyethylene
MFS	Major facilitor superfamily
MHET	Mono-(2-hydroxyethyl) terephthalate
PA	Polyamides
PAA	Phenylacetic acid
PCA	Protocatechuic acid
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PPE	Personal protective equipment
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
TCA	Tricarboxylic acid
TPA	Terephthalic acid

INTRODUCTION

The production of synthetic polymers from petrochemicals was a major industrial breakthrough that paved the path for the manufacturing of one of the most extensively consumed material on globe: plastic. Numerous attributes, such as strength, water resistance, flexibility, durability, and low-cost, make plastic a superior material compared to its counterparts. The versatile nature of plastic has accelerated its global production to about 20-fold over the last five decades, reaching about 359 million metric tons in 2018.[1] Even in this COVID-19 pandemic health crisis, the use of personal protective equipment (PPE) that is made up of disposable plastic components (such as gloves, face shields, and masks) keeps contributing to the rise of plastic waste. It has been estimated that monthly about 129 billion face masks and 65 billion gloves are being discarded globally, further adding to plastic pollution.[2] The majority of plastics used are polyethylene (PE) and polypropylene (PP), representing a share of 54%, and they are followed by polyvinyl chloride (PVC) with 14%, and then polyethylene terephthalate (PET) with 8%.[3]

* *Correspondence to: A Priya, Amity Institute of Biotechnology, Amity University, Kolkata, West Bengal, 700135, India, E-mail: anshupriya26@gmail.com, apriya@kol.amity.edu; or A Daverey, School of Environment and Natural Resources, Doon University, Dehradun, Uttarakhand, 248012, India. E-mail: ach15may@gmail.com, achlesh.senr@doonuniversity.ac.in*

a *Amity Institute of Biotechnology, Amity University, Kolkata, India*

b *Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, India*

c *School of Environment and Natural Resources, Doon University, Dehradun, India*

About 90% of the plastic generated globally remains in the environment for at least 500 years, causing air, water, and soil pollution.[4] Land disposal of plastics has been revealed to barren the land, while management through burning and incineration releases toxic and hazardous fumes into the atmosphere. The ecological impacts of dumping such a huge amount of plastic into seas and oceans are also detrimental to aquatic animals, as it leads to choking the guts of marine creatures with plastic detritus.[5,6] Further, abiotic degradation of petrochemical plastics leads to slow the fragmentation of large plastics generating microplastic particles that are easily transported to distant places by air and water circulation, making plastic pollution a transboundary issue.[6] There are also concerns related to ecotoxicology due to the leaching of various toxic and carcinogenic chemicals (such as polychlorinated biphenyls, and bisphenol A and S) from plastics.[7–9]

To combat this issue, attempts are being made to utilize plastics as bitumen modifiers for the construction of roads.[10] Further, reuse and recycling of plastic waste are also being promoted to produce value-added products. Thermo-chemical biomass conversion technologies are being employed for liquid fuel production from municipal plastic waste.[11] The general fate of plastic waste in the environment is demonstrated in Fig. 1. Thus, owing to the recalcitrant nature of plastics, their persistence in the environment for several hundreds of years, and the hazards associated, management of plastic waste through an eco-friendly, biological approach is an important concern for waste treatment to avert environmental pollution.

The critical features that make plastics resistant to biodegradation are their hydrophobicity, high molecular weight, and long-chain polymer structure lacking a favorable functional group. The high molecular weight of the compound offers an obstacle in easy transportation across the microbial cellular membrane and, therefore, depolymerization of the compound becomes essential.[12,13]

Recent research has revealed that specialized marine microbes (such as bacteria, fungi, and actinomycetes) have the potential to biodegrade petrochemical plastics and to utilize many of them as a carbon source.[14,15] Some of the prominent bacterial taxa involved in plastic biodegradation include *Arthrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Corynebacterium*, *Streptomyces*, and *Nocardia*.[14–16] Several fungi, such as *Fusarium spp*., *Aspergillus spp*., and *Penicillium spp*., have also been found to play a role in plastic biodegradation.[13,17] Biodegradation of plastic is believed to occur following or concomitant with physicochemical degradation, which disrupts the structure and length of polymers, making them susceptible to microbial action.[14,18] By contrast, elucidation of bacteria-plastic interactions is limited. Various enzymes (such as oxygenases, dehydrogenases, lipases, and esterases) have been found to play a role in the oxidation and fragmentation of polymers, facilitating biodegradation.[16,19,20]

As the world is gradually becoming aware of the seriousness of plastic pollution, novel strategies are required both to reduce the flow of recalcitrant plastic into the environment and to eliminate its vast historic build-up in an eco-friendly manner. Therefore, this

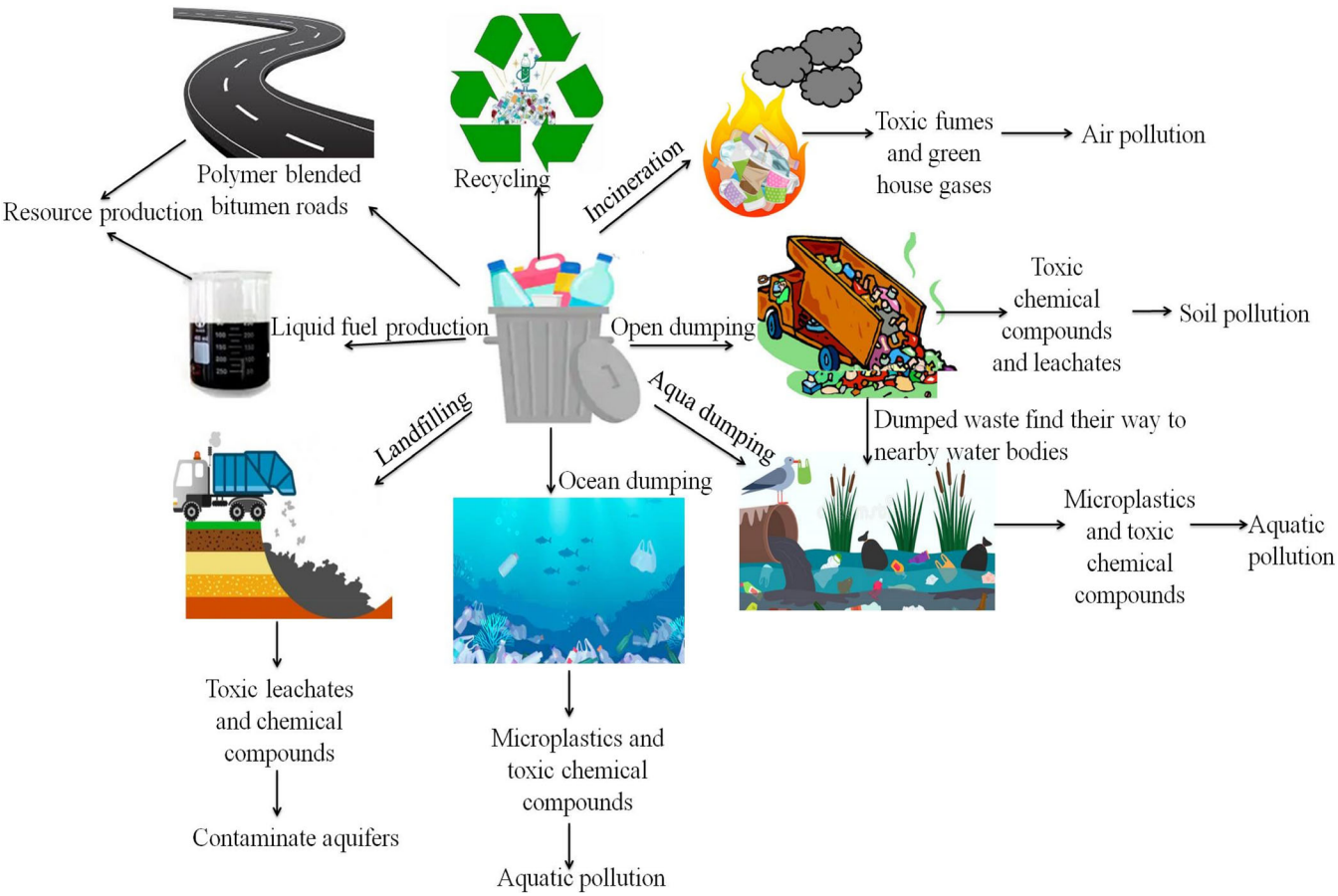

Figure 1. Fate of plastic waste in environment.

review article presents an extensive insight into the various biotechnological and molecular apparatuses involved in microbial plastic degradation, underscores the knowledge gaps, and identifies future challenges, technologies, and key areas where further research would be beneficially directed.

MICROBIAL DEGRADATION OF PETROCHEMICAL PLASTIC VARIANTS

Petrochemical plastics are the conventionally used non-biodegradable products derived from a wide range of synthetic polymers manufactured from hydrocarbon and petrochemical derivatives.[21] These plastics have a high molecular weight and a highly stable configuration due to the presence of extensive repetitive monomeric units.[5] The repetitive monomeric structure makes the polymers recalcitrant to degradation.[18]

Plastic is a synthetic polymer composed of elements like carbon, hydrogen, oxygen, nitrogen, chlorine, and sulfur, all of which polymerize efficiently and give plastic high strength and durability. Some of the major polymers that are widely produced and are vital to the economy are nylon, PE, PET, PVC, PP, polyamides (PA), polystyrene (PS), and polyurethane (PUR). The microorganisms and the identified mechanisms of their plastic variant-specific biodegradation highlight their potential to be developed as a bioremediation tool.

Intriguingly, bioremediation processes are being extensively investigated due to the efficiency of various microorganisms, their enzymes, and the degradation pathways in the degradation of plastic degradation. The rate of degradation through enzymatic interactions has been found to be influenced by factors, such as the carbon content and structural arrangement of the polymer. PE, synthetically produced by polymerization of ethane as either high-density polyethylene (HDPE) or low-density polyethylene (LDPE), is reported to be degraded by a variety of bacteria and fungi.[15] The notable bacterial and fungal genera degraders of PE are *Pseudomonas*, *Staphylococcus*, *Streptomyces*, *Rhodococcus*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhodococcus ruber*, *Brevibacillus borstelensis*, *Streptomyces spp*., *Bacillus cereus*, *Penicillium simplicissimum*, and *Aspergillus niger*. These microbes utilize PE as a sole source of carbon, bringing about its degradation with the aid of extracellular enzymes.[17,19,22,23]

It has been reported that pre-physical (UV treatment or photo-oxidation) and chemical treatments (acid and/or base) of PE increase the effectiveness of the biodegradation process.[18]Chemical treatment with nitric acid and sodium hydroxide has also been reported to accelerate the rate of biodegradation of PE by indigenous and augmented microflora, such as *Bacillus subtilis*, *Aspergillus niger*, and *Pseudomonas spp*.[24] Further, in a study by Thomas *et al*.,[25] *P. fluorescens* was reported to achieve PE degradation of about 8% within a month under laboratory conditions and 16% within a year in field conditions. A study by Muenmee *et al*.[26] demonstrated the role of a bacterial consortium comprising heterotrophs, autotrophs, and methanotrophs in the degradation of HDPE and LDPE. Additionally, a microbial consortium comprising of *Nitrosomonas spp*. AL212, *Nitrobacter winogradkyi*, *Burkholderia spp*., *Methylobactor spp*., *Methylococcus capsulatus*, *Methylocystic spp*., and *Methylocella spp*. was also found to be potential degraders of HDPE and LDPE.[26] Devi *et al*.[27] reported HDPE degradation by *Aspergillus tubingensis* VRKPT1 and *Aspergillus flavus* VRKPT2 through biofilm formation and structural deformation.

Actinomycetes like *Thermobifida* and *Thermomonospora* were reported to have a role in PET biodegradation.[28,29] Several enzymes (such as PET hydrolase and tannase, MHETase, and fungal cutinase) were found to play a role in PET degradation.[28,30] PUR is also reported to be efficiently biodegraded by Gram negative betaproteobacteria, such as *Pseudomonas protegens* strain Pf-5,[31] *Pseudomonas chlororaphis*, *and Pseudomonas putida*.[29,32] Various studies have also identified fungal stains, like *Fusarium solani*, *Cladosporium pseudocladosporioides*, *Aspergillus fumigatus*, *and Penicillium chrysogenum*, to have a role in PUR biodegradation.[33,34]

Synthetic PA extensively used in textile, sportswear, carpets are also reported to be utilized as a sole source of carbon and nitrogen by several bacteria.[12] *Flavobacterium*, *Arthrobacter*, *Achromobacter*, and *Pseudomonas* are some of the dominant bacteria involved in PA and PVC biodegradation.[16,19,21] Similarly, numerous bacterial strains (pure as well as in consortium) have been reported to initiate PS degradation through biofilm formation on the polymer surface.[20,21] PS biodegradation capability is demonstrated by several bacterial genera, such as *Pseudomonas*, *Xanthobacter*, and *Arthrobacter*.[20,35,36] However, plastics generally have low bioavailability (on account of being hydrophobic), and so, they display low accessibility for microorganisms and enzymes, restricting them to the superficial layer of polymers.

MECHANISM OF BIODEGRADATION

Several studies have highlighted the remarkable role of biocatalysts in the form of microorganisms belonging to the groups of bacteria and fungus, enzymes, and metabolites in efficient biodegradation of complex petrochemical plastics.[20] These microorganisms either use petrochemical plastics directly as a source of nutrients or they indirectly act upon plastics with the aid of several enzymes and metabolites.[15,19] Several bacteria and fungi have been reported to produce exoenzymes, which are metabolites to catalytically degrade the polymers into carbon dioxide and water.[16] The degradative mechanism, enzymatic activity, and degradation products, however, depend on the microbe employed.[13,14] Various enzymes (such as proteases, hydrolases, depolymerases, lipases, laccases, esterases, and peroxidases) have been reported to be involved in polymer biodegradation in an efficient manner.[31,37–39]

The microbial biodegradation mechanisms initiate with the attachment of the microbes on the polymer substrate, followed by colonization.[31,36] Next, the microbes act on the polymer and degrade it into low molecular weight oligomers, dimmers, and monomers, all of which are eventually mineralized into carbon dioxide and water.[16,22] Aerobic and anaerobic mechanisms of biodegradation have been demonstrated to be involved in plastic biodegradation[40] (Fig. 2). In aerobic biodegradation, oxygen molecules serve as electron acceptors, yielding carbon dioxide, water, and microbial mass as the final products of polymer degradation. Alternatively, anaerobic degradation utilizes inorganic species (such as sulfate, nitrate, and manganese) as electron acceptors to form the degradation products: methane, carbon dioxide, and water.[40,41] The polymer biodegradation mechanisms are, however, slow and demand extensive research in terms of the identification of novel microbes and enzymes, and of the elucidation of metabolic pathways for efficient and fast degradation.

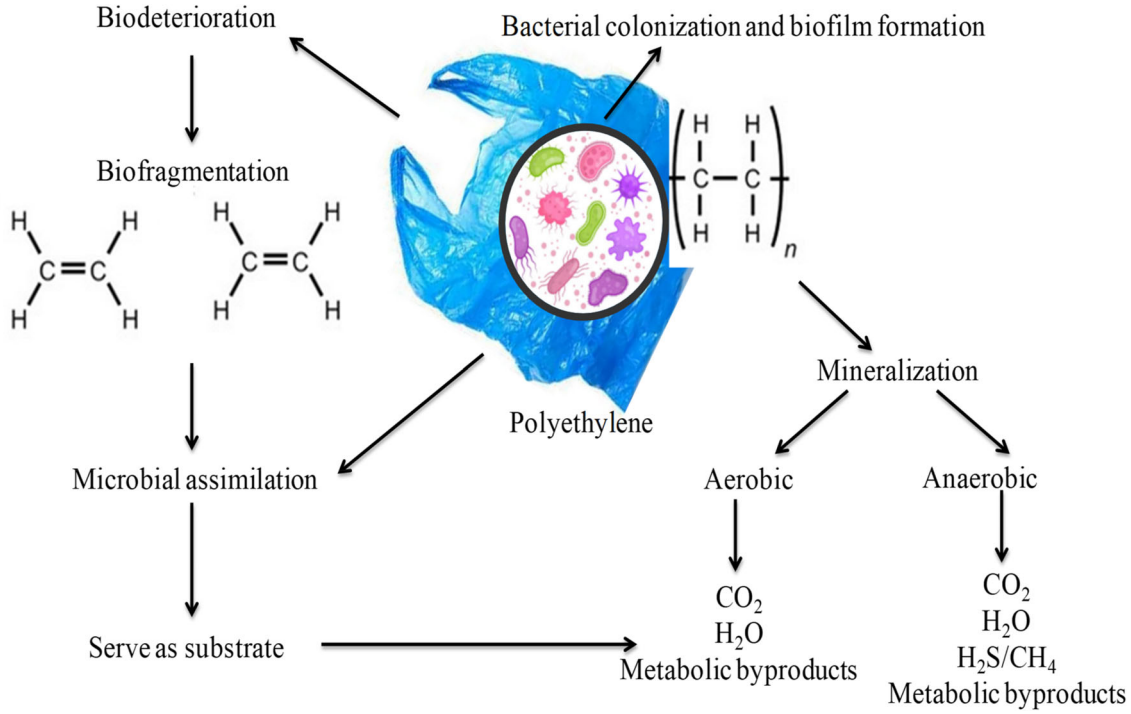

Figure 2. Schematic representation of mechanism involved in biodegradation of plastic.

FACTORS AFFECTING MICROBIAL DEGRADATION

Biodegradation of polymer is an inherently slow process and is governed by several factors. Optimizing the factors governing biodegradation will have a positive role in increasing the degradation efficiency. Several physicochemical and biological factors, such as microbial culture, pH, temperature, and polymer characteristics, play a decisive role in polymer degradation. Therefore, it is important to understand how these process parameters are affecting the biodegradation of plastic. An optimum combination of these process parameters is necessary to enhance the rate of plastic biodegradation. The key process parameters are discussed below.

Microbes

The phenomenon of polymer biodegradation exploits the ability of microbes, their enzymatic machinery, and their metabolic products. Several microbes belonging to the group of bacteria and fungus have been reported to play a significant role in plastic biodegradation.[5,13] The microbial consortium, biofilm formation, enzymatic makeup, adaptability, and tolerance to the substrate are some of the major factors affecting plastic biodegradation.[15] Zhu *et al.*[42] demonstrated that the effects of dissolved organic carbon and chemical leachates (as a major byproduct of plastic degradation) affect the growth and rate of plastic biodegradation. Microbial community, composition, colonization, and biofilm formation have a significant effect on plastic biotransformation and biodegradation.[43]

pH

Bioprocesses are sensitive to the environmental pH and, therefore, it is a key parameter for the biodegradation of plastic. The pH of the substrate has a direct relationship with microbial growth which, in turn, regulates biodegradation.[24] The pH can influence hydrolysis reaction rates by modifying the acidic or basic conditions.[17,44] King *et al.*[44] demonstrated an increase in biopolymer degradation at increased pH.

Temperature

Temperature significantly affects all reactions. Biochemical reactions are extremely sensitive to temperature, so it is a key parameter for the biodegradation of plastic by the microbes or their enzymes. Most mesophiles operate at an optimum temperature range of 25–30 °C.[15] Enzymatic activity is also influenced significantly by the polymer's softening temperature. Polymers demonstrating a high melting point are less susceptible to biodegradation and vice-versa.[18] It has also been reported that potential enzymatic degradability has an inverse relationship with temperature.[22]

Substrate characteristics

Several characteristics of the substrate, such as molecular weight, shape, size, and composition, have a significant role in its degradation. Molecular weight has an inverse relation with degradability.[20] Degradability is reported to decrease with the increase in the molecular weight of the polymer.[16] Similarly, the shape and size of the polymer also play a remarkable role in degradability.[38] A polymer with a large surface area is more prone to biodegradation than its counterpart with a small surface area.[31] Further, the polymer composition (in terms of structural complexity, number of carbon atoms, and degree of polymerization) is coherently linked to biodegradability.[22]

Surfactants

Amphiphilic compounds, such as chemical surfactants or biosurfactants, assist the biodegradation process by increasing the surface area of hydrophobic water-soluble substances, therefore making them more amenable to microbial attack. It has been

reported that the amphiphilic nature of biosurfactants facilitates the attachment of microorganisms on hydrophobic polymer surfaces, thereby enabling them to utilize the polymer as a nutrient source.[45] Albertsson *et al.*[46] incubated LDPE in 0.5% non-ionic surfactant Tween 80 and *P. aeruginosa* for 60 days and they observed significant degradation as compared to the control experiment without surfactant. In a similar recent study by Tadimeti,[47] surfactin production by *B. subtilis* was demonstrated to facilitate HDPE and LDPE degradation to about 6% in freshwater, 5% in brackish water, and 3% in ocean water. Additionally, non-ionic surfactants were reported to increase cell surface hydrophobicity, which further enhances biofilm formation and initiates plastic biodegradation.[48]

GENES, ENZYMES, AND METABOLIC PATHWAYS INVOLVED IN PLASTIC BIODEGRADATION

It is assumed that once plastics are disposed into the environment as waste, they may undergo degradation due to the synergy of biotic and abiotic factors.[4,49] Microbial degradation of plastics has emerged as a safe and effective tool for plastic waste treatment. In this regard, a plethora of genes, enzymes, and metabolic pathways have been characterized to have the potential to transform synthetic plastics and facilitate depolymerization.[31,38,39]

There are more than 5300 grades of synthetic plastic polymers commercially available today and each one of these must be biodegraded with heterogeneous metabolic machinery.[50] Different enzymes (such as lipases, esterases, cutinases, and carboxylesterases) from several bacterial and fungal species have been shown to modify and/or degrade various synthetic polymers.[51] Several oxygenases, like monooxygenases and dioxygenases, are also reported to be present to facilitate the oxidation of the plastic polymer.[9,29] Plastic oxidation plays a vital role in biodegradation. The oxidation of plastics increases the hydrophilicity of the polymer, which assists in further microbial colonization and in the activity of various extracellular enzymes, such as lipases, hydrolases, and esterases.[17,52,53] The copper-binding enzyme (laccase produced by actinomycete *Rhodococcus ruber* and *Aspergillus flavus*) is also reported to have a role in PE biodegradation.[51,54] Another actinomycete, *Rhodococcus rhodochrous*, is reported to degrade and assimilate PE oligomers the size of 600 Da with the help of specialized carrier proteins, such as major facilitator superfamily (MFS) or ATP binding cassettes (ABC).[55,56]

Gravouil *et al.*[56] examined the overexpression of enzymes in bacteria growing in PE-containing media. The metabolism of PE involved a series of events, initiating from the involvement of acetyl coA and succinyl coA in the tricarboxylic acid (TCA) cycle, followed by the production of chemicals in the form of compounds like NADH. The chemical energy generated is utilized in the production of ATP via the respiratory chain, along with CO_2 and H_2O, marking the complete mineralization of PE. The preliminary work on the biodegradation of PE highlighted the role of specialized genes, enzymes, and transporter proteins.[56]

Table 1 shows various specialized bacteria, along with their genes and enzymes, that have been reported to play a role in plastic biodegradation. Specialized genes, such as Alkane hydroxylase (alkB) genes in *Pseudomonas spp*. E4 strain, were found to have a critical role in achieving 28.6% of degradation of the organic carbon of PE in 80 days. To test the efficacy of the gene, alkB gene was expressed in *Escherichia coli* BL21 strain, which then attained a mineralization potential of 19.3% for the organic carbon of the PE.[57]

Several marine and terrestrial bacteria have revealed the role of hydrolases in plastics biodegradation.[58,59] Other than the enzyme, the PET hydrolase *I. sakaiensis* genome has been found to code for another unique enzyme that has been found to have high similarity to enzyme tannases, and be capable of degrading mono-(2-hydroxyethyl) terephthalate (MHET).[60] PETase (hydrolase) and

Table 1. Specialized bacteria and their genes and enzymes involved in plastic biodegradation

Marine bacteria	Genes involved	Enzymes involved	References
Alcanivorax spp. 24	*Cytochrome* P450 genes, *alk*B1 and *alk*B2 genes, *alm*A gene	Alkane monooxygenases *AlkB* and alkane hydroxylase *AlmA*	39
Roseobacter, *Oleiphilus* and *Aestuariibacter*			38
Bacillus spp.			38
Desulfovibrio, *Desulfobacteraceae*, and *Desulfobulbaceae*		Depolymerases, adenylyl sulfate reductases (*aprBA*), and dissimilatory sulfite reductases (*dsrAB*)	37
Comamonas testosteroni		Depolymerase	31, 37, 39
Brevibacillus borstelensis			22
Moritella spp. JT01	*H39* Lipase Gene	Lipases	52
Ideonella sakaiensis	*cut1*	Hydrolases	37
Achromobacter xylosoxidans			19
Pseudomonas spp.		Esterase	29
Thermobifida fusca	*ISF6_4831*	Hydrolases	28
P. chlororaphis		Polyurethanase	19
Alcaligenes faecalis AE122		Depolymerase	23

MHETase are two crucial hydrolases produced by bacteria *I. sakaiensis* 201-F6 upon adherence to the PET substrate. These two enzymes facilitate further degradation of PET to simpler compounds, such as terephthalic acid (TPA), MHET, and bis (2-hydroxyethyl) terephthalate (BHET). MHET is further hydrolyzed by MHETase to TPA and ethylene glycol (EG).[9,53] TPA is then catabolized to protocatechuic acid (PCA).[59] Enzyme PCA 3,4 dioxygenase (PCA34) subsequently cleaves PCA to form 4-carboxy-2-hydroxymuconic, which is then dehydrogenated to form 2-pyrone-4,-6-dicarboxylic acid. The 2-pyrone-4,6-dicarboxylic acid enters the TCA cycle and gets transformed into pyruvate and oxaloacetate, which further gets assimilated as carbon dioxide and water molecules (Fig. 3).

Many of the fungal species (such as *Humicola insolens*, *Penicillium citrinum*, and *Fusarium oxysporum*) are potent PET degraders with enzymes such as polyesterase cutinase and hydrolase.[49,58,60] The most outstanding ones are *Fusarium* and *Humicola* cutinases. *Humicola* cutinases activity is limited to the accumulation of intermediate MHET formation and, hence, is often used synergistically with the enzyme lipase CalB from *Candida antarctica*, which completely transforms MHET to TPA.[61] Apart from PET hydrolases, PET esterases (such as the metagenome-derived esterases MGS0156, GEN0105) have also been reported to mediate the hydrolysis of compounds like bis(benzoyloxyethyl)-terephthalate and polycaprolactone.[62]

In a study on *P. putida* F1, PS composed of styrene monomers have also been found to be oxidized by styrene epoxide with the aid of enzymes styrene monooxygenase. The second step oxidation further oxidizes styrene epoxide to phenylacetaldehyde, which is then catabolized into phenylacetic acid (PAA). Later in the lower pathway of styrene metabolism, PAA is transformed to phenylacetyl-CoA which, after a series of enzymatic reactions, enters TCA cycle in the form of acetyl-Co A and succinyl-CoA.[63] *P. putida* CA-3 has demonstrated the presence of a specialized pathway Phenylacetyl-CoA catabolon. This pathway utilizes the activity of catabolic operon, which assists the growth of the *P. putida* CA-3 on styrene, facilitating the biodegradation of PS to medium-chain length polyhydroxyalkanoates.[64]

Another petrochemical variant, synthetic polymer PUR, has also been found to be degraded by the activity of bacteria like *P. chlororaphis*, *P. protegens*, and *P. putida*.[65,66] *Comamonas acidovorans* TB-35 is also reported to degrade PUR with the aid of an

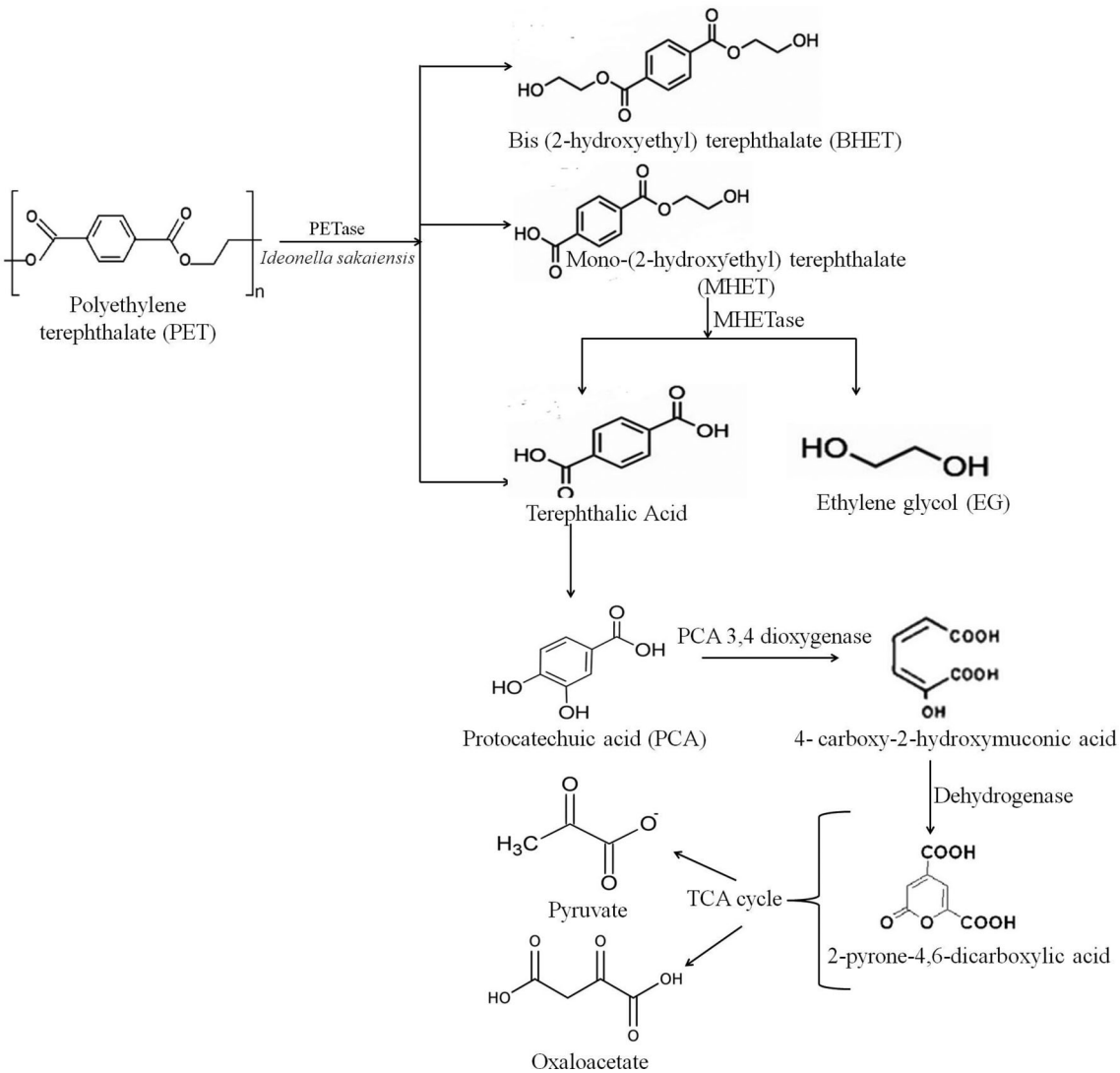

Figure 3. Enzymatic reactions involved in biodegradation of PET.

esterase named pudA.[31] Additionally, fungi like *Fusarium solani*, *Candida rugosa*, *Aspergillus fumigates*, *Candida ethanolica*, and *Penicillium chrysogenum* have also been identified to accelerate PUR degradation with the aid of enzymes like lipase, hydrolases, and esterases.[41,66,67] Synthetic PA extensively used in textiles, carpets, and sportswear have also been reported to be degraded by several microbes like *Bacillus cereus*, *Vibrio furnisii*, *Bacillus sphaericus*, *Anoxybacillus rupiensis*, *Bacillus subtilis*, and *Brevundimonas vesicularis*.[41,68,69] Nylon oligomer-degrading *Arthrobacter* isolates have been reported to code several hydrolases and aminotransferases that assist synthetic polymer degradation. *Arthrobacter spp*. KI72 genome is reported to comprise nylD1 and nylE1 genes, which encode for enzymes 6-aminohexanoate aminotransferase and adipate semialdehyde dehydrogenase that metabolize 6-aminohexanoate to adipate semialdehyde and, subsequently, adipate semialdehyde to adipate.[31,70] Figure 4 presents the enzymes and microbes generally involved in the degradation of different petrochemical variants.

BIOTECHNOLOGICAL DEVELOPMENTS IN PLASTIC BIODEGRADATION AND FUTURE PERSPECTIVE

Microbial activities capable of mineralizing synthetic polymers are emerging as an alternative to chemical and physical depolymerization methods. Synthetic polymers are being used as biotechnological feedstocks for microbial activities because of the metabolic versatility of the microorganisms. The thermo-chemical depolymerization of plastic wastes is being subjected to biodegradation to improve the degradation products.[71] The advances in synthetic biology and metabolic engineering have facilitated the development of engineered microbial strains for safe, eco-friendly plastic wastes degradation and recycling.

Genetic engineering approaches serve as powerful tools to manipulate the genetic make-up of microorganisms and improve their ability to degrade plastics.[72] By genetic engineering, a native marine bacterium was transformed into a hydrocarbon degrader through the transfer of genes from hydrocarbon degrader *P. putida*.[73] Similar genetic engineering approaches can be applied to strategically unite the functions of genes and enzymes that can facilitate plastic degradation. Further, the systems biology utilizes different omic approaches, such as genomics, transcriptomics, proteomics, metabolomics, and fluxomics, for promoting the bioremediation of environmental pollutants.[74]

Several metabolic engineering strategies have also been implemented either independently or in combinations to construct engineered microbial degraders to degrade recalcitrant plastics. Degradation approaches involved genome manipulation, recombinant gene expression, and protein engineering. Systems metabolic engineering (SysME) has emerged as a potential technology that can facilitate the development of microbial constructs with optimized cellular performance to achieve plastic degradation.[75] The evolution of gene editing tools and approaches (such as Zinc finger proteins, TALENs, and the CRISPR/Cas9) can also be explored for their ability to enhance the microbial potential for bioremediation. These strategies can be applied for the incorporation of genes encoding enzymes, such as esterase, PETase, depolymerase, and laccase, for plastic degradation in a non-degrading microbe.[76]

Beyond the conventional recycling technologies, novel advancement is needed to harness the biotransformation potential of microorganisms for plastic waste management. Several studies have highlighted that degradation of plastics by the

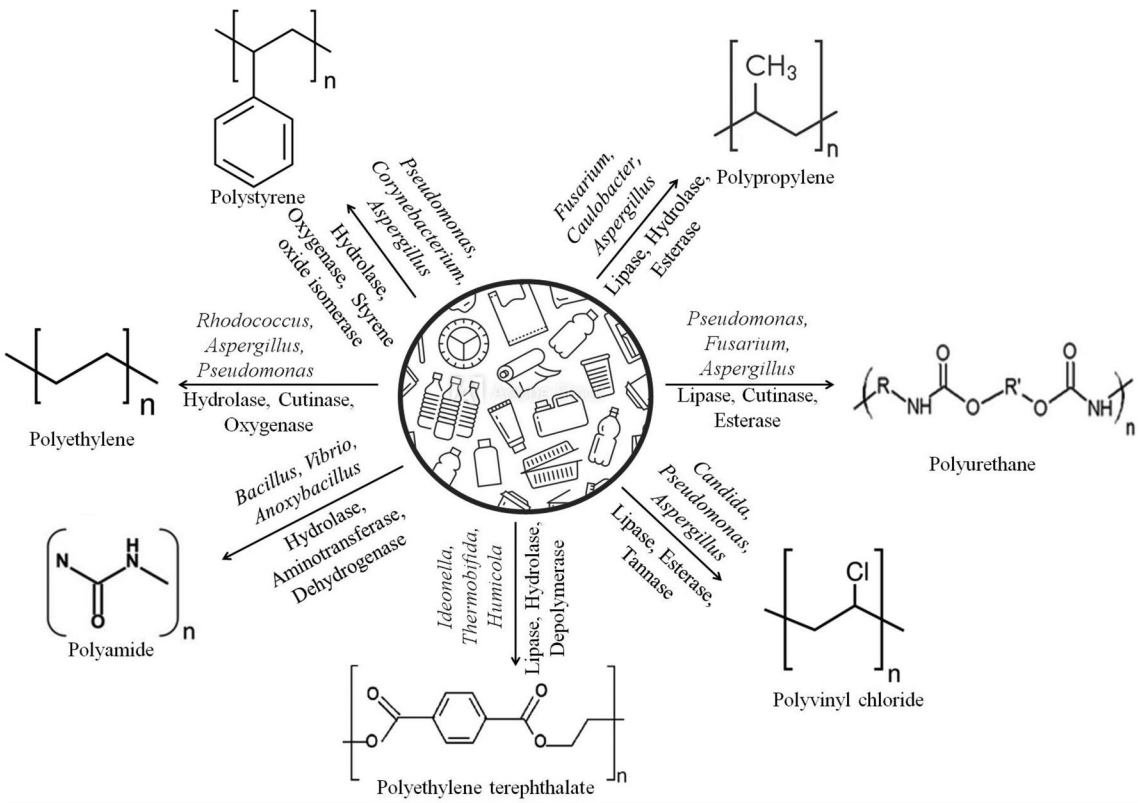

Figure 4. Major microbes and enzymes involved in degradation of petrochemical plastic variants.

natural microbes are intrinsically slow in contrast to the engineered strains.[77] Engineered enzyme cutinase was found to act more efficiently, thereby reducing PUR degradation time from 41.8 to 6.2 h, as compared to wild-type cutinase.[78] Genetic engineering has now unlocked novel avenues to enhance plastic degradation. Syranidou *et al*.[79] have demonstrated the improved plastic degradation potential of tailored marine consortia. The tailored plastic degrading strains can be used for plastic depolymerization.

Despite several successes related to the genetic manipulation of microbes in laboratory conditions, most of these microbes have shown disappointing results in the field. The biodegradation of plastics extensively depends on the chemical structure of the polymer and on the metabolic and enzymatic system of the degrading microbe. The complete knowledge of these two components can comprehensively enhance the biodegradation process. In this regard, bioinformatics has emerged as a productive tool for prediction of biodegradation. Several databases (such as biodegradation pathway prediction systems and chemical toxicity prediction systems) have been developed for the assessment of biodegradation. Some of the well-recognized databases include UM-BBD, MetaCyc, BioCyc, all of which generate useful information related to metabolic pathways, genes, microbes, enzymes, and multistep enzymatic reactions facilitating biodegradation.[59]

These computational techniques are particularly useful, not only in analyzing and identifying the degrading enzymes, but also in predicting the degradation pathways of toxicants for which no pathway has been elucidated in the literature. This bioinformatics approach would help create a path for synthetic biology and metabolic engineering for the development of a novel strategy for plastic bioremediation. However, the major drawback linked to the pathway prediction system is the unavailability of experimental validation. Experimental validation of the computation-derived degradative pathways is an essential part of the future perspective of the bioinformatics study.

Synthetic biology can help overcome major challenges through genetic engineering and genome editing. Synthetic biology (especially the 'omic' studies together with next-generation sequencing and computational algorithm) has coherently contributed to better insight into microbial interactions with the plastic substrate and into the dynamics of polymer biodegradation.[80] Further, tailoring of the metabolic pathway in the context of the biodegradative pathway of the synthetic polymers is also an important aspect to be considered for improved degradation. However, the gaps in knowledge regarding the diversity of synthetic polymer degrading microbes and enzymes are prevalent. Therefore, future studies must focus on the characterization and identification of novel polymer degraders with high degradation potential. Extensive research is required at the level of microbiology, environmental biotechnology, gene expression, and protein engineering to eliminate the constrictions of the biodegradation. In the future, a mutualistic approach (utilizing an amalgamation of bioinformatics, metabolic engineering, system biology, and genetic and molecular techniques) may provide innovative insight into plastic biodegradation.

CONCLUSIONS

Petrochemical plastics pose a significant threat to the environment due to their toxicity and persistence in the environment. Conventional waste disposal methods, like landfilling and incineration, release several toxic byproducts that damage the environment. Thermal and catalytic pyrolysis converting plastics to oil and fuel have emerged as promising techniques aiming at decreasing the global dependence on fossil fuels and promoting waste management. The fuels synthesized from waste plastics are green, clean, and similar in properties to fossil fuels. However, despite all potential advantages, there are many drawbacks, such as high energy demand, high production cost, limitations related to catalyst regeneration, and reuse, all of which reduce the overall process sustainability. Several microorganisms have the potential to degrade the petrochemical plastic under appropriate conditions, and several microorganisms belonging to the bacterial, fungal, and actinomycetes domain have been identified as potential plastic degraders. Microbiological activities capable of catalyzing plastic degradation with the aid of enzymes (such as hydrolases, esterases, lipases, and tannases) are emerging as an eco-friendly alternative to physicochemical depolymerization methods. However, the major drawback of biodegradation is its inherently slow rate. Based on critical assessment of biotechnological approaches, it can be inferred that the applications of genetic manipulation, synthetic biology, metabolic engineering, and bioinformatics have the potential to accelerate the rate of plastic biodegradation. The biotransformation of plastic wastes and their reuse can promote a circular economy, contributing to a significant reduction in environmental pollution along with economic development. However, a critical review of the available literature also points out the knowledge gap that exists regarding the interactions between the microbes and the plastic substrate and the diversity of synthetic polymer degrading microbes and enzymes. Additionally, the dynamics of polymer biodegradation confirmed by the bioinformatics prediction systems need to be experimentally validated to determine the exact fate of plastic pollutants in the natural environment. Thus, further research efforts are needed to fill the knowledge gaps for efficient and effective plastic waste biodegradation employing biotechnological tools and approaches.

CONFLICT OF INTEREST

The authors declare that there is no potential source of conflict of interest with respect to this manuscript.

AUTHOR CONTRIBUTIONS

AP, KD, and AD contributed to writing the original draft, review, and editing. AD conceptualized the work.

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