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Review

Microplastic Accumulation and Degradation in Environment via Biotechnological Approaches

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Abstract: The extensive use of plastics in daily life has led to the generation of huge amounts of plastic waste, which causes an enormous burden on the environment. More than half of the plastic waste ends up in the landfill, and about one-fifth of waste is managed by incineration. Only about one-tenth of plastic waste is recycled, and the rest, about one-fifth of mismanaged plastic waste, ends up in the terrestrial and aquatic environment. Here, we review how the deterioration of plastics leads to the formation of microplastics and nanoplastics, which are now found abundantly and are contaminating aquatic life and water bodies. It observed that increasing experimental evidence provides data about the presence of these microplastics in food items, terrestrial environment, and even the human body. The harmful effects of microplastics on human health still need to be substantiated with more precise experimental studies. However, measures can be taken to reduce the production of microplastics by improving the methods used for plastic degradation. This review focuses on the use of genetic engineering, genome editing, synthetic biology, and system biology approaches to increase the potential of microorganisms to degrade plastics.

Keywords: biodegradation; genetically modified organism; microplastics; polyethylene terephthalate; system biology

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1. Introduction

Plastics are produced from different natural products following a polymerization or polycondensation process. Plastics are therefore composed of natural and organic materials. For example, cellulose, coal, natural gas, salt, crude oil, etc., act as the raw materials for the production of plastics. These are the organic polymers that are derived from various hydrocarbon and petroleum materials extracted from coal, oil, and natural gas. They consist of a long chain molecule, connected by strong chemical bonds, arranged in repeating units, and exhibiting desirable properties such as strength, flexibility, and a lightweight feature [1,2]. The first synthetic plastic, Bakelite, was produced in 1907 by Leo Baekeland by mixing phenol and formaldehyde and subjecting it to heat and pressure. He also coined the term plastics after the Greek word "plastikos", which means moldable [3]. The development of new polymers with improved qualities such as transparency and color-holding ability resulted in a drastic increase in the commercial production of plastics from the 1930s to the 1950s. Plastic products were extensively used during World War II, and after the war the commercial production of plastic increased by almost 1000-fold [4].

Certain properties of plastic such as high flexibility, resistance to corrosion, high strength-to-weight ratios, and low costs in carrying out fabrication work have resulted in the high usage of plastics in packaging, in the field of construction and transportation, and in the production of electrical, medical, and electronic devices [5]. The global annual production of plastics reached 359 million tonnes by the end of the year 2018 [6]. With the current rate of plastics production and utilization, it is estimated that the number of plastics produced annually will double within the next 20 years [7–9].

Plastics can be classified, according to their size, into three groups. All plastic materials > 200 mm are considered to be macroplastics. Microplastics have a variety of shapes such as spheres, fragments, and fibers [10], and have a size range of 1 µm–5 mm and are present in large quantities in cleaning and cosmetic products, such as facewash/scrubs and toothpaste, and the laundering of synthetic clothes and abrasion of tires through driving serve as primary sources of microplastics [11,12]. The secondary microplastics are produced through the breakdown of large plastic items such as plastic bags, fishing nets, and bottles. The presence of microplastic particles <1 mm in large amounts in living organisms and the environment is a growing concern. Because of their imperceptible nature they can easily enter in food chains and accumulate in living tissues, as shown in Figure 1 [13,14]. The presence of microplastics in a sample and their quantification can be carried out either at the individual particle level by using spectroscopic techniques such as FTIR or RAMAN or by determining the total amount of microplastics present in a sample using Py-GCMS or thermal extraction desorption GC MS [15]. Nanoplastics are < 1 µm in diameter, are produced from the breakdown of microplastics and waste products of 3D printing, constitute a very recent area of environmental science, and are considered to be equally as harmful as microplastics, but their abundance and effects on the environment are yet to be quantified [11,16]. In this article, we reviewed the above aspects covering every biodegradable method for the degradation of plastic waste. The review of the literature for this article was based on extensive peer-reviewed research articles collected from PubMed, Google Scholar, ScienceDirect, and other trusted sources. The references in this article were organized and filtered with the help of Zotero software.

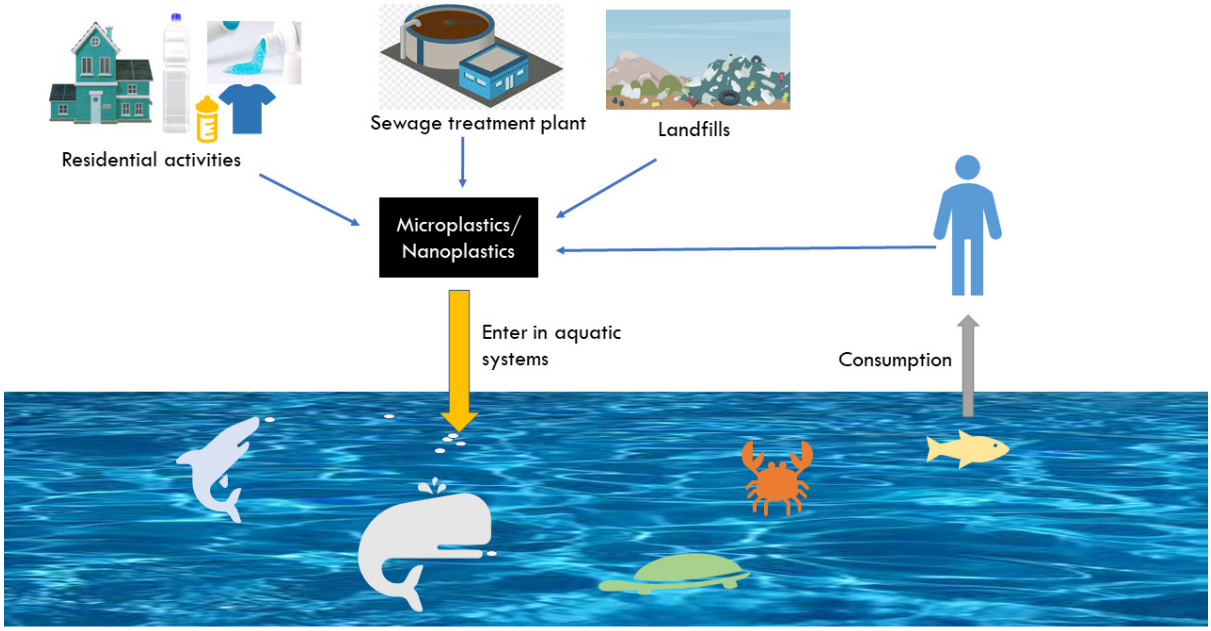

Figure 1. Life cycle of microplastics. Microplastics are produced from various sources, mainly anthropogenic in nature. Some of the examples are household activities, sewage treatments, landfalls, etc. Gradually, they enter into large water bodies and get accumulated in various habitats, which again come back to the human body via consumption of aquatic products.

2. Effect of Microplastics on Environment

Plastic waste generated on land enters the marine environment through riverine runoffs by leaching from open dumpsites or sewage effluents, washing off from beaches, spilling during transport or accident, or dumping of plastic wastes into the sea, resulting in plastic debris being found in all major ocean basins across the world [17]. Table 1 shows the countries in which the maximum amount of mismanaged plastic waste reaches the ocean [18]. Based on the mismanaged plastic waste in ocean per capita, it can be concluded that Asian countries need to adopt more efficient strategies to reduce the amount of plastic waste reaching the ocean.

Table 1. List of countries generating the highest amount of waste that reached the ocean in 2019.

Country	Mismanaged Waste Emitted to the Ocean (Metric tons Year^{-1})	Mismanaged Plastic Waste to Ocean per Capita (kg per Year)
Philippines	356,371	3.296
India	126,513	0.093
Malaysia	73,098	2.288
China	70,707	0.049
Indonesia	56,333	0.208
Brazil	37,799	0.179
Vietnam	28,221	0.293
Bangladesh	24,640	0.151
Thailand	22,806	0.328
Nigeria	18,640	0.093

The numerous effects from ingestion of macroplastics, microplastics, and nanoplastics and entanglement due to macroplastics have been well-documented in several mammal, fish, turtles, and bird species [19]. The ingestion of plastics leads to suffocation or blocking of the digestive tract and ultimately death [20]. Microplastics, due to their small size and increased surface area, can enter into tissues and cells and react with other chemicals in the environment (Figure 2). Experimental evidence demonstrates that exposure to microplastics and nanoplastics in oysters hinders feeding and has negative effects on fecundity and offspring quality [16,21].

In recent times, it has become increasingly evident that trophic transfer and bioaccumulation of plastic and other associated chemical pollutants is taking place through the food web. A well-studied example includes the filter feeders, such as mussels, wherein the microplastics and other pollutants are accumulated and are then transferred to benthic predators and humans through the consumption of wild or farmed shellfish [22,23]. Studies on mussels have established that microplastics can enter from the gut into the circulatory system, and they were found in mussel haemolymph. The presence of microplastic particles in the gastrointestinal tracts of seals and cetaceans indicates the trophic transfer taking place from the prey fish to top predators [24,25]. Moreover, studies carried out on fish, seabirds, and mussels indicate the potential of plastics to cause bioaccumulation of environmental pollutants [26–28].

Microplastic contamination is present not only in the marine environment, but there is increasing evidence that microplastics are also abundantly present in the terrestrial environment through the physical or chemical decomposition of larger plastic materials [29,30]. Plastic products abundantly used in daily life can generate microplastic particles due to fragmentation, aging, and deterioration. These microplastics accumulate in soil either by fragmentation of discarded plastic items or by the leaching of buried wastes present in the landfills [17].

Disposed plastic waste, when exposed to the natural environment, could be ingested by terrestrial biota because they mistake it as food [31]. However, very few studies have been conducted to study the occurrence of microplastics in the terrestrial biota. A survey of the presence of anthropogenic plastic waste of the size range of 0.5–5 mm in terrestrial

birds in China was conducted which found that 62.6% of total particles found in birds' digestive tract were plastics [32]. This research indicates that there is an urgent need to study the prevalence and effects of microplastics in terrestrial birds. Soil organisms such as earthworms moved 73.5% of microplastics from the soil surface down into the bulk soil by burrow formation [30]. Moreover, pollutants such as metals and organic contaminants carried by microplastics could affect the soil and groundwater quality due to the leaching of these chemicals through desorption into the soil [33].

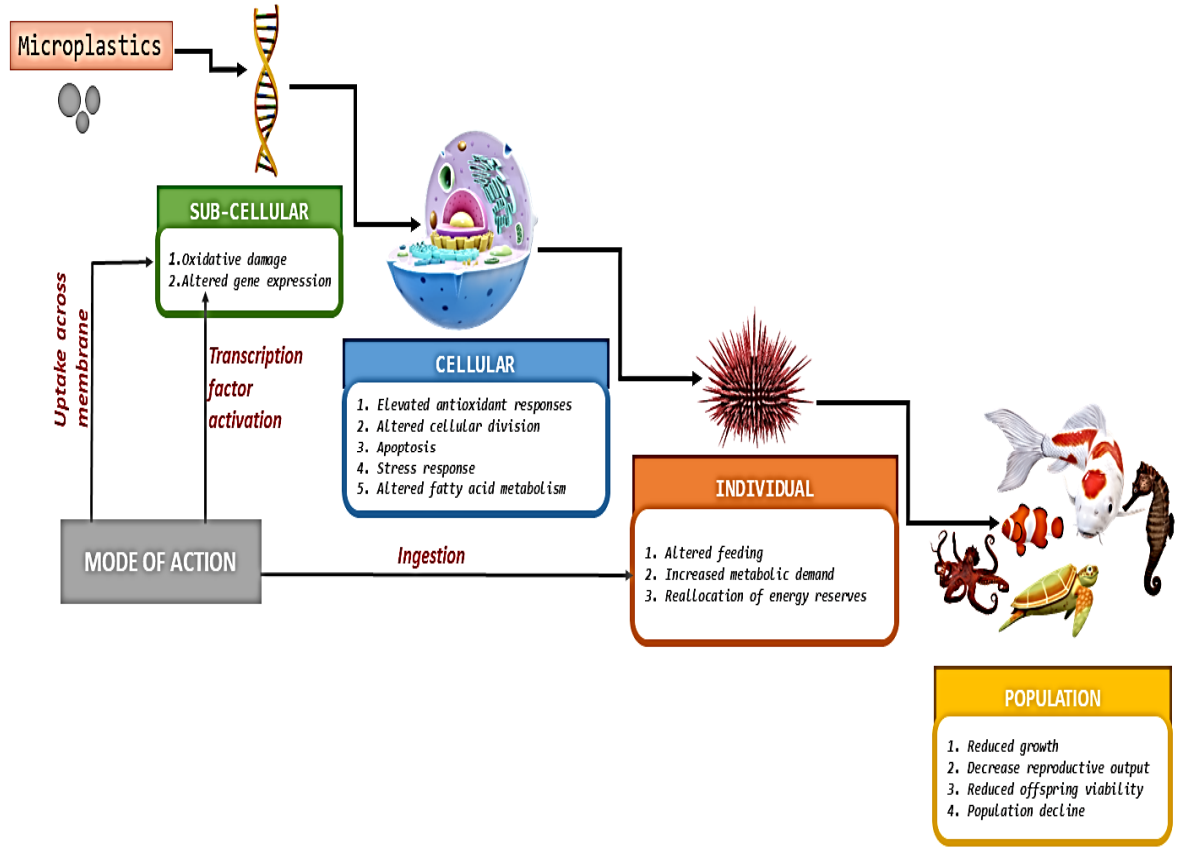

Figure 2. Effects of microplastics pollution on different levels of organizations. The biomagnification of microplastics takes place via the food chain, and they enter into the population. Gradually, they reach cell and subcellular levels in individual organisms, including human beings. It has been predicted that they can also interact with DNA of organisms to alter the gene expression and associated physiology.

The innumerable applications of plastics in day-to-day life has resulted in the presence of plastic wastes in large amounts in urban water systems [34]. Municipal wastewater treatment plants are another source for the presence of microplastics in the terrestrial environment. The wastewater collected from households, hospitals, and industries containing microplastics end up in sewage sludge [35,36]. The usage of sludge in agricultural lands as fertilizer has resulted in deposition of microplastics in the soil. A study conducted by [37] assessed that in European countries, through sewage sludge application, 125–180 tons of microplastics per million residents have been released to the terrestrial environment. The challenges faced in extraction and identification of microplastic particles from soil or sludge could be the reason for the limited availability of data on the presence of microplastics in the terrestrial environment [38] though wastewater treatment plants which used primary clarification, wherein microplastics are removed based on their densities either by sedimentation or floatation before the wastewater is subjected to biological treatment, have proven to be more efficient in the removal of microplastics from wastewater [39].

The presence of microplastics is also found in large quantities in drinking water. The effluents from wastewater treatment plants and stormwater runoff from urban and agricultural lands are the major sources of microplastics in drinking water [40]. Na et al. [41] investigated whether drinking water treatment plants, which use different processes such as coagulation, sand filtration, and disinfection using UV and UV/H_2O_2, can remove microplastics from drinking water. Through their studies, they concluded that the type of coagulant used and the pH and organic matter naturally present in water affect the efficiency of microplastics coagulation. Moreover, after sand filtration, at least 16% of microplastics which were <10 μm in size were further fragmented by UV/H_2O_2, causing the leaching of chemicals in water and resulting in increased toxicity of the water sample.

3. Health Risks Associated with the Use of Microplastics

The extensive use of plastics for several decades and their release into the environment have resulted in wide range of associated problems. All plastics are synthetic polymers which are synthesized by combining monomer chemicals, some of which are toxic and carcinogenic in nature, and these monomer residues in plastic products can be hazardous to humans [42]. Moreover, the presence of additives such as fillers and plasticizers, coloring agents, flame retardants, and other substances pose health risks for living beings and also reduce the reuse and recycling potential of plastics [27]. Plasticizers such as phthalates are widely used to make soft PVC (polyvinyl chloride), and products containing phthalates include clothing, packaging materials, toys, flooring, and other items used daily. Since the phthalates are not chemically bound to plastics, they can leach out from the plastics into the surrounding environment, causing health hazards, as they can influence the endogenous production of several hormones, hampering reproduction and development. Similarly, bisphenol A used in polycarbonate baby bottles, water bottles, and protective coating inside metal food containers has been proven to be "environmental oestrogen" [43].

The presence of primary and secondary microplastics has been proven in aquatic life, and their entry into the food chain through human consumption leads to their biological accumulation. The authors of [44,45] designed a study to check the presence of microplastics in blood using Py-GC/MS. Their experiments demonstrated the presence of PMMA (Poly (methyl methacrylate), PP (Polypropylene), PS (Polystyrene), PE (Polyethylene) and PET (Polyethylene Terephthalate)) plastic particles in 77% of donors in a quantifiable amount. PET, PS, and PE were found in a maximum amount in blood samples in about 2.4, 4.8, and 7.1 μg/mL, respectively. The plastic particles concentrations could be due to exposure of humans to personal care products (in toothpaste, face scrubs, and lip gloss), use of nanoparticles in drug delivery, inhalation through air, or consumption of food and water containing microplastics. Furthermore, [46] analyzed the presence of microplastics in the feces of patients with inflammatory bowel disease (IBD) and healthy people. They detected the presence of microplastics in the feces of patients with IBD at much higher concentrations than compared to the feces of healthy individuals. Fifteen types of microplastics were detected in feces, of which PET and polyamide were predominantly present in the form of sheets and fibers.

According to the researchers, there is a positive link between the presence of microplastics in feces and IBD. Either the exposure to microplastics causes the disease, or the patients of IBD retain microplastics in feces because of the disease. Though microplastics can have a negative impact on human health, more experimental evidence is required to prove the harmful effects of these microplastics in the human body. Moreover, Deng et al. proved the presence of fluorescent and pristine polystyrene microplastics of 5 μm and 20 μm in mice liver, kidney, and gut tissue using fluorescence spectroscopy and histological analyses [47]. Moreover, through biochemical biomarkers and metabolomic profiles studies, they concluded that exposure to microplastics affects metabolic pathways and leads to oxidative stress and neurotoxic responses. Scarcity of proper analytical tools for isolation, detection, quantification, and characterization of microplastic particles which are <10 μm has resulted in difficulty in estimating the risk of microplastics to human health. However, it has been

reported that particulate particles in air which are smaller than 2.5 μm in size and arise from diesel exhaust have the capacity to enter cells and induce the formation of reactive oxygen species (ROS) and inflammation and are linked to increased chances of death due to cardiovascular or respiratory diseases or lung cancer. By comparing these results with microplastics of smaller size, a parallel can be drawn to estimate the risk of microplastics on human health [10]. Thus, effective measures need to be taken to reduce the production of microplastics, and ecofriendly methods need to be used for the biodegradation of plastics, as the physical and chemical methods used for degradation of plastics have severely harmful effects on environment. Microorganisms have the ability to degrade plastics, but various strategies need to be adapted to increase the potential of these microorganisms to degrade plastics. In this review paper, we have discussed the various biotechnological interventions which can be used to improve the biodegradation of plastics by microorganisms and result in a reduction in the production of microplastics.

4. Role of Microorganisms in Plastic Degradation

Based on frequency of use, plastics are divided into seven broad groups. (1) Polyethylene Terephthalate (PET or PETE), (2) High-Density Polyethylene (HDPE), (3) Polyvinyl Chloride (PVC or Vinyl), (4) Low-Density Polyethylene (LDPE), (5) Polypropylene (PP), (6) Polystyrene (PS or Styrofoam), (7) Other. Out of them, many are degradable. The particular biodegradable plastics are (acrylonitrile butadiene styrene, acrylic, acetyl cellulose, cellulose triacetate, alkyd, cellophane, epoxy resin, polyamide, polyacrylonitrile, and poly(butylene adipate-co-teraphthalate)). Therefore, the above plastics are used in several sectors. Numerous studies carried out in the past few years have reported about the capability of several microorganisms in the degradation of different synthetic plastics. It is estimated that more than 50% of the plastic waste ends up in the landfill and 19% of the waste is managed by incineration. Only about 9% of plastic waste is recycled and, the remaining 22% of mismanaged plastic waste ends up in terrestrial and aquatic environments. The majority of the bacterial species which have the ability to degrade plastic are Gram-negative bacilli, and, among them, *Pseudomonas* spp. has the highest capability in the biodegradation of plastics [48–52]. For polyethylene degradation, *Pseudomonas* spp. was isolated from three different sources. Among them, the *Pseudomonas* spp. isolated from sewage sludge dump was most efficient in the biodegradation of natural and synthetic polyethylene.

Compared to the other species, *Pseduomonas* spp. formed the most viscous and flocculent biofilms on the surface within a period of three weeks [50]. Apart from *Pseudomonas* spp., several other bacterial species capable of carrying out the biodegradation of plastics include *Ideonella*, *Klebsiella*, *Streptomyces*, *Mycobacterium*, *Flavobacterium*, *Rhodococcus*, *Escherichia*, and *Azotobacter.* Along with bacteria, fungi are also involved in the biodegradation of plastics, as they hasten the biodegradation process by sharing the metabolic intermediates [53]. *Penicillum* and *Aspergillus* spp. are reported to have the capability to degrade polyethylene by the formation of biofilms which decrease the hydrophobicity of the surface [53,54]. *Aspergillus* spp. is reported to have the ability to degrade LDPE, whereas *Penicillum* spp. can degrade both LDPE and HDPE [55]. Among the *Aspergillus* spp., *Aspergillus niger* has the highest potency in degrading polyethylene (38%), followed by *Aspergillus flavus* (31%), in a period of 60 days [56].

Biodegradation of plastics takes place when microorganisms such as bacteria and fungi, through their enzymatic action, convert them into metabolic products such as methane, carbon dioxide, water, etc. [57]. Biological deterioration of plastic pollutants depends on several factors, including surface area, molecular weight, hydrophilicity or hydrophobicity, crystallinity, functional groups, chemical structure, etc. Increases in molecular weight leads to decreases in degradation rate, as the solubility also decreases, making the plastics less susceptible to microbial attack because it becomes difficult for microbes to assimilate plastics through the cell membrane [58]. Similarly, crystallinity is another important factor affecting biodegradability, with amorphous-domains-containing polymers being more

vulnerable to enzymes produced by microorganisms [59]. Moreover, the hydrophobic nature of plastics also inhibits the susceptibility of them to microbial attack by hindering water absorption, though this problem can be solved by biofilm formation on plastics [60]. The process of biodegradation of plastic is depicted in Figure 3.

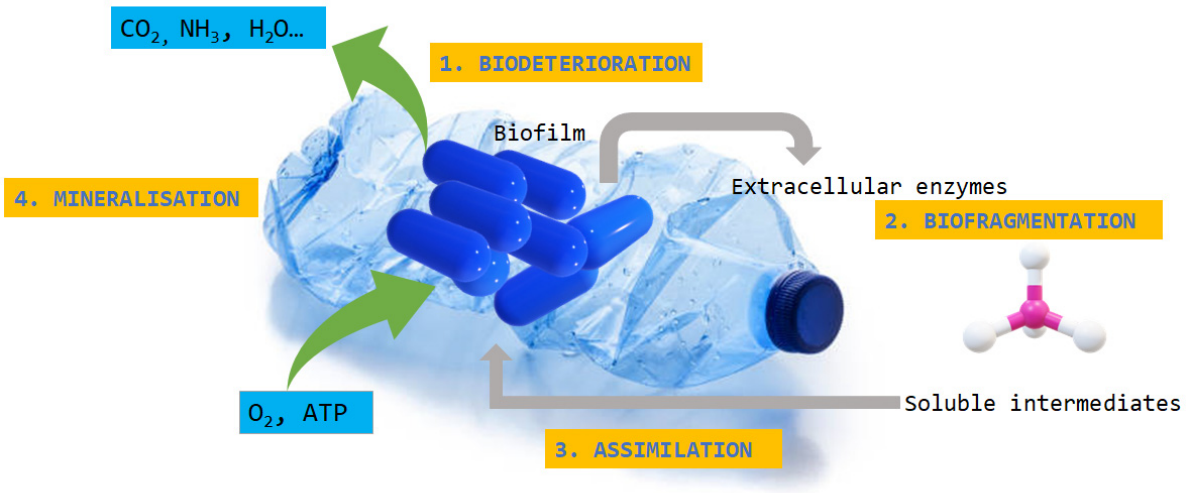

Figure 3. Different stages of plastic degradation by microorganisms. Deciphering different stages of microplastics by microorganisms reveals that different biochemical processes are involved in the event. Some of the events are action of extracellular enzymes, assimilation, mineralization, bio-deterioration, etc.

5. Enzymes Involved in Biodegradation of Plastic Polymers

Enzymes are primarily responsible for the degradation of plastics due to their ability to carry out hydrolysis and oxidation [61]. The enzymatic degradation of plastic can be measured by weight loss and addition of functional groups [54]. Hydrolases including esterases, cutinases, and lipases play an instrumental role in plastic degradation [62–65]. Enzymes from different microorganisms with the ability to degrade different synthetic plastic polymers are shown in Figure 4. Cutinase-like hydrolase isolated from *Thermobifida fusca* had the ability to degrade low-crystallinity PET bottles and pellets up to 50% of its initial weight at 50 °C in 21 days [66]. LC-cutinase, having the ability to hydrolyze low-crystallinity PET films, resulting in 50% of weight loss at 50 °C in 7 days, were identified from a leaf-branch compost metagenomic library [67]. Yoshida et al. [2] found *Ideonella sakaiensis* 201-F6, which, on adherence to PET substrate, produces PETase (hydrolase) and MHETase. These enzymes have the ability to degrade PET to bis (2-hydroxyethyl) terephthalate (BHET), mono (2-hydroxyethyl) terephthalate (MHET), terephthalic acid, and ethylene glycol (Figure 5). Terephthalic acid is converted to protocatechuic acid (PCA), which, upon cleavage by PCA 3,4 dioxygenase, is converted to 4-carboxy-2-hydroxymuconic acid. On further dehydrogenation, 4-carboxy-2-hydroxymuconic acid forms 2-pyrone-4, 6-dicarboxylic acid, which enters the TCA cycle. On the other hand, the glycoaldehyde reductase enzyme oxidized ethylene glycol to glycoaldehyde, which is further oxidized to glycolate. Enzyme glycolate oxidase oxidizes glycolate to glyoxylate, which condenses with acetyl CoA to form malate, which enters the TCA cycle [68].

Santo et al. [69] isolated *Rhodococcus ruber* C208, which degraded polyethylene by secretion of extracellular laccase. The laccase enzyme required the presence of copper ions for its induction and activity. The biodegradation of polyethylene took place due to the oxidation of amorphous regions of PE films by laccase, which resulted in the formation of easily accessible carbonyl groups, which was confirmed by FTIR analysis and a decrease in molecular weight of polyethylene film.

Jeon et al. [70] compared the low molecular weight polyethylene-degrading ability of two alkane monooxygenases (AlkB1 and AlkB2), which are expressed in *Pseudomonas*

aeruginosa E7, by measuring the transcription levels of *alkB1* and *alkB2* using quantitative real-time PCR (qRT-PCR). The transcription levels of *alkB2* were higher than compared to *alkB1* when *P. aeruginosa* E7 was grown in a medium containing low molecular weight polyethylene as the sole source of carbon. Polyurethane esterase isolated from *Comamonas acidovorans* TB-35 comprised a catalytic domain and surface-binding domain, which was essential for polyurethane (PU) degradation [71]. Polyurethane was hydrolyzed by PU esterase into polyisocyanate and ethylene glycol. Ethylene glycol is further converted to glycoaldehyde and glycolic acid and finally enters the TCA cycle (Figure 6).

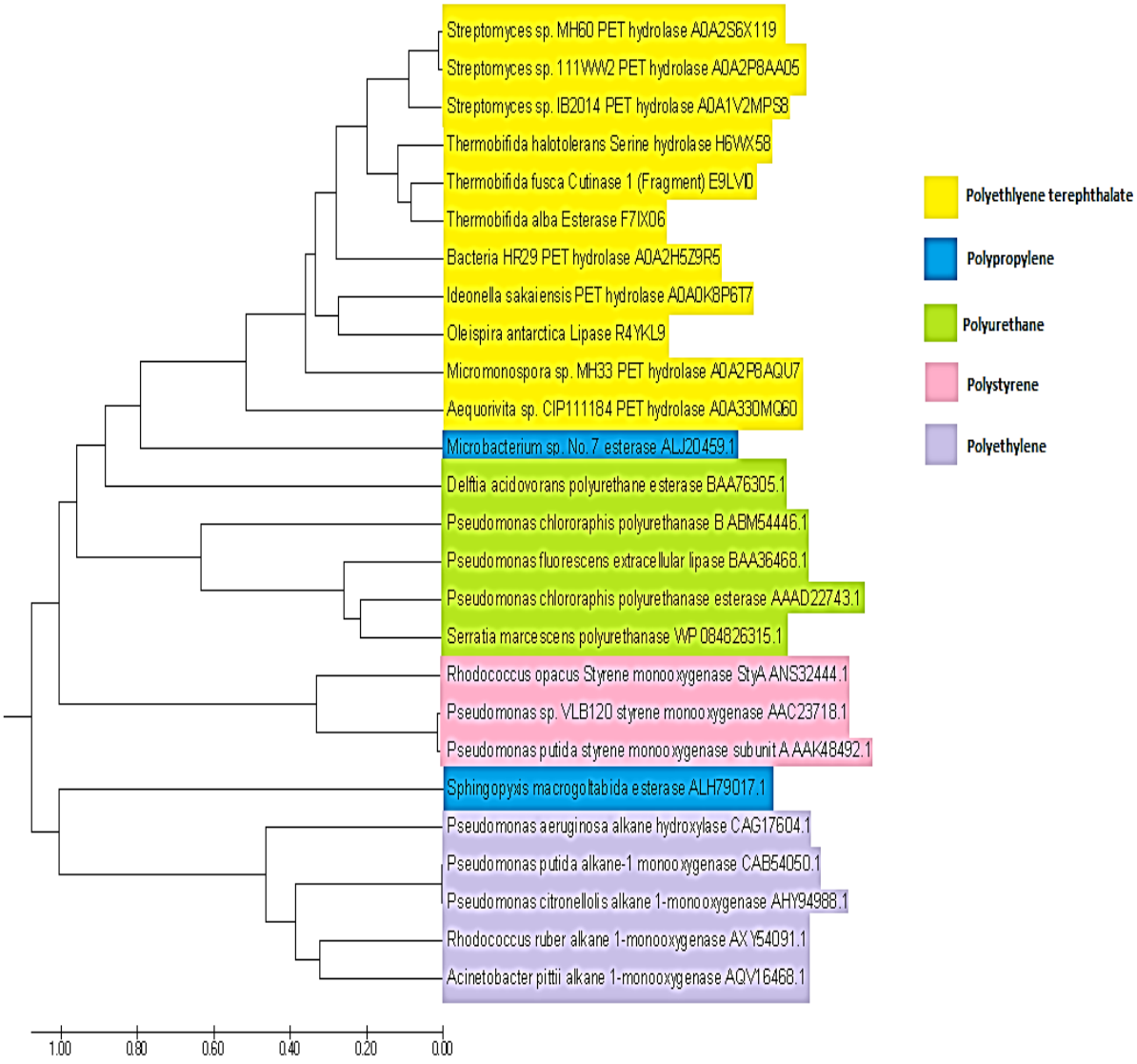

Figure 4. Phylogenetic relationships among bacteria for degradation of microplastics. A phylogenetic tree constructed based on amino acid sequence homologies of synthetic plastic polymer-degrading enzymes in bacteria is drawn. The tree was constructed with Molecular Evolutionary Genetics Analysis v7 (MEGA7, https://www.megasoftware.net/ (accessed on 20 November 2022)).

Schimdt et al. [72] analyzed the ability of four polyester hydrolases, LC Cutinase (LCC), TfCut2, Tcurl278, and Tcur0390 to degrade polyester PU Elastollan B85A-10 and C85A-10. After 200 h of incubation at 70 °C, LCC caused weight loss of 4.9% and 4.1% of Elastollan B85A-10 and C85A-10, respectively. LCC was most efficient because of its higher thermostability at 70 °C, and FTIR analysis confirmed that the weight losses were due to cleavage of the ester bonds. Styrene monooxygenase, styrene oxide isomerase,

phenylacetaldehyde dehydrogenase, and phenylacetyl coenzyme A ligase are the enzymes which reported in styrene degradation and are believed to be also responsible for depolymerization of polystyrene to styrene monomers and conversion of styrene monomers to phenylacetate and its incorporation in the TCA cycle [73].

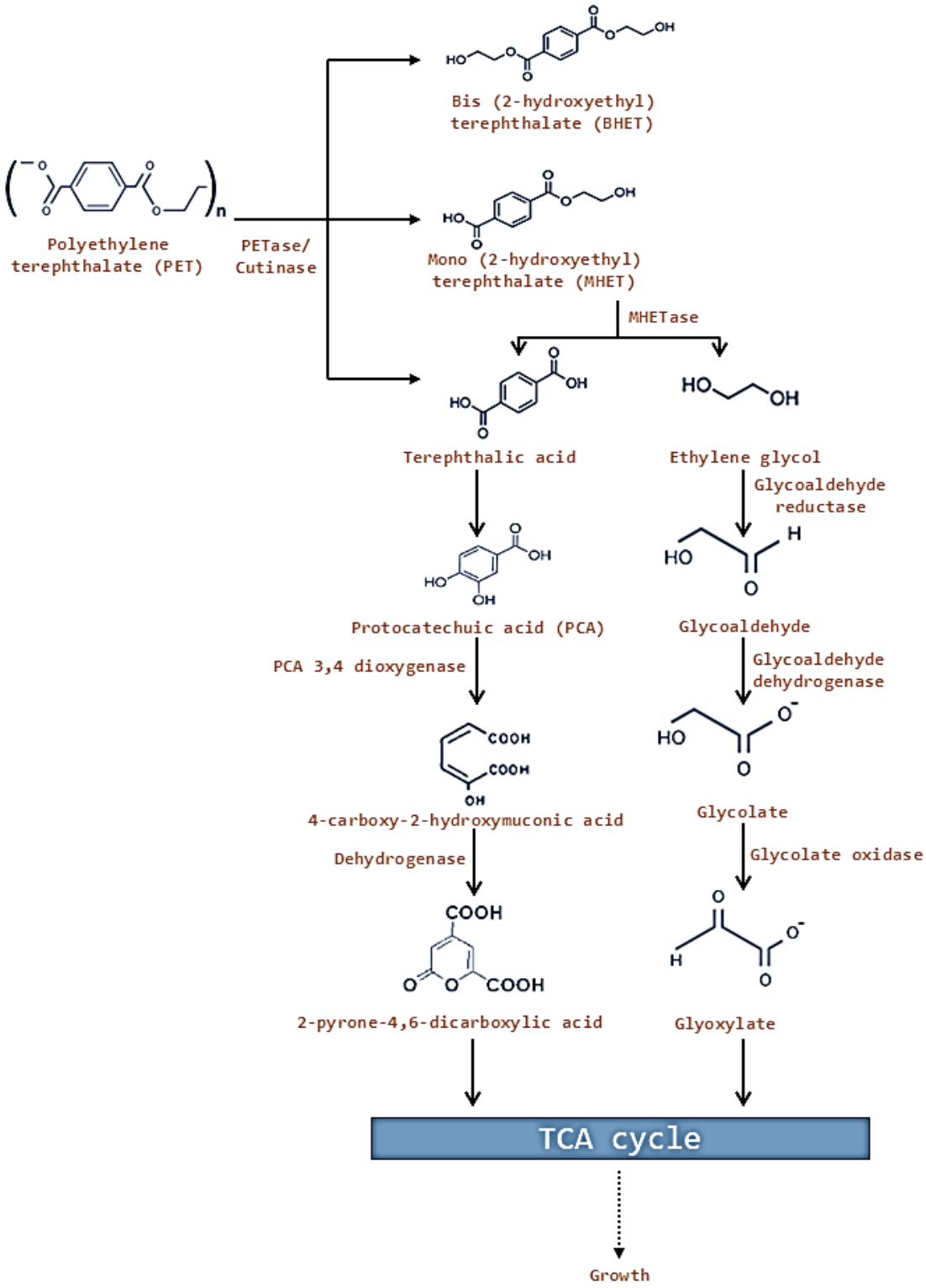

Figure 5. Enzymatic reactions involved in biodegradation of PET. The biodegradation of PET is stated with the enzyme PETase, and, subsequently, the by-product enters into TCA cycle to culminate energy.

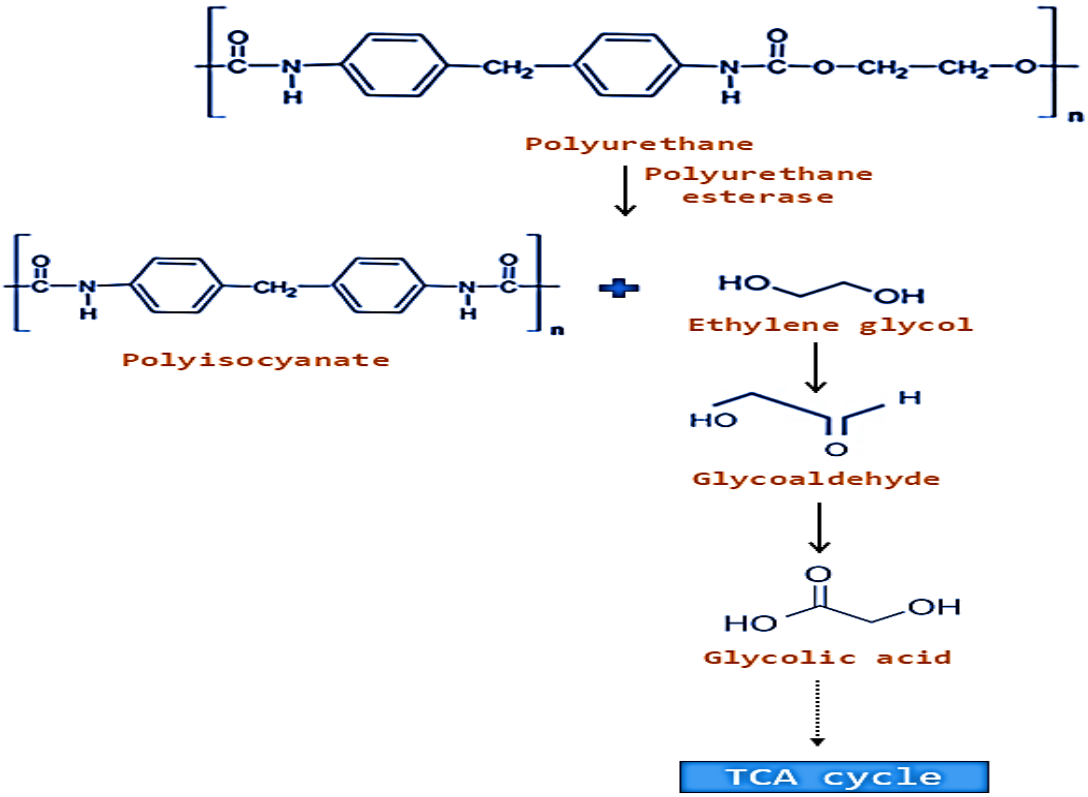

Figure 6. Enzymatic reactions involved in biodegradation of polyurethane (PU). The enzyme PU esterase is the enzyme that helps in biodegradation of PU to polyisocyanate and ethylene glycol. Finally, the produced glycolic acid enters into TCA cycle.

6. Genetic Engineering Approaches to Enhance Plastic Biodegradation

Genetic engineering approaches have been used by the identification of genes to study the potential of microbes to metabolize xenobiotic compounds by cloning and transforming them under appropriate host microorganisms under the control of promoters [74]. The chief objective of the genetic engineering approach is to isolate the genes involved in degradation pathways, modify them, and clone them in appropriate hosts, such as *E. coli*, using techniques such as PCR, site-directed mutagenesis, antisense RNA technology, etc. [75,76]. In order to be used for recycling processes, the enzymes should be stable and have the ability to function at high temperatures. A plant disease causing bacteria produced Cutinase, which was present in the outer coat of the plants. This enzyme, capable of breaking down polyester linkages, was used in the PET degradation process, but the major limitation was that the enzyme was unfolding and clumping with itself at higher temperatures.

A yeast strain was genetically engineered to produce bacterial Cutinase by incorporating sugar residues at strategic positions that keep the enzyme folded at elevated temperatures and do not allow the clumping of the enzyme to take place by creating physical barriers. The genetically engineered enzyme was able to degrade PET at an optimal temperature and concentration [77]. Oda et al. [78] genetically modified Cutinase (Cut190) isolated from *Saccharomonospora viridis* AHK190, which is active when bound to calcium and inactive in a calcium-free state. Of the three calcium binding sites, introduction of a disulfide bond at site 2 between Asp250 and Glu296 residues resulted in increased melting temperature of the mutant enzyme, signifying that disulphide linkage imitates the presence of a bound calcium effect. Moreover, replacement of surface Asn and Gln residues with Asp, Glu, and His residues resulted in increased melting temperature of the enzymes. Engineered mutant enzymes were more efficient in degradation of PET compared to the native enzyme. Moreover, genetic modifications were also carried out to enhance the plastic degrading capacity of enzymes. PETase (PET digesting enzyme) has potency to carry out

biodegradation of polyethylene terephthalate (PET), and the X-ray crystal structure of the enzyme revealed that PETase has features which are frequently observed in cutinases and lipases.

Moreover, it was observed that though the enzyme retained the ancestral α/β hydrolase fold, the active site of the enzyme had a more open cleft compared to homologous cutinases. To increase the PET-degrading capability of PETase, two mutations were introduced in the cleft, which resulted in a narrowed opening of the cleft which, in turn, increased the ability of PETase to degrade PET [79]. Yan et al. [80] isolated a thermophilic Cutinase which has the ability to degrade PET at high temperatures of around 70 °C from the plant compost metagenome. The Cutinase was cloned in *Clostridium thermocellum*, wherein a strong promoter of a constitutively expressed gene was used to obtain a high level of gene expression, and signal peptide of exoglucanase Cel48S was used for extracellular secretion of the enzyme. Though the thermostable PET hydrolases have been expressed in *E. coli*, *B. subtilis*, *B. megatarium*, and *Pichia pastoris*, only the recombinant *C. thermocellum* has been able to express cutinases and carry out biodegradation of PET at 60 °C. *Pseudomonas* sp.

E4 isolated from beach soil contaminated with crude oil had the ability to degrade polyethylene in the molecular weight range of 1700–23,000, of which 4.9–28.6% of carbon was mineralized into CO_2 after incubation for 80 days at 37 °C. The alkane hydroxylase gene (*alkB*) of *Pseudomonas* sp. E4 was cloned in *E. coli* BL21. During the biodegradation process in the compost for 80 days at 37 °C, the recombinant strain mineralized 19.3% of carbon into CO_2, indicating that the *alkB* gene plays a significant role in polyethylene degradation [81]. By incorporation of a novel biosynthethic pathway using plasmids, recombinant *E. coli* acquired the ability to convert terephthalic acid derived from plastic into vanillin, which is widely used in the food and cosmetic industries. The advantage of this genetic engineering approach is that no hazardous waste is generated, and the reaction takes place at room temperature in an aqueous medium with a pH of 5.5–7 and without the need for the addition of any cofactors or reagents [82]. Thus, using the genetic engineering approaches, the thermostability and ability of enzymes to degrade plastics can be greatly enhanced, and plastic waste can be converted into value-added compounds.

The survivability and stability of GMOs is quintessential in their application in bioremediation. In order to use GMOs for plastic waste management, the organism should be able to survive and grow under such environmental conditions. Moreover, factors such as growth rate, inoculum size, and interactions of GMOs with the indigenous bacterial community and other components of the ecosystem play a crucial role. Another limitation of GMOs is the possibility of release of GMOs into natural ecosystems because of horizontal gene transfer. Thus, the introduction of GMOs into field sites and their effect on the structure and function of the natural ecosystems needs to be taken into consideration [83]. Although several different microorganisms with the ability to degrade plastic polymers have been identified, most of these studies were carried out in laboratory conditions; hence, it is too difficult to predict the effectiveness of these microorganisms, in natural conditions, to biodegrade plastics. So, new methodologies need to be designed which improve the survival of GMOs in foreign environments, and we must increase our understanding of the mechanism of biodegradation.

7. Gene Editing Tools for Plastic Waste Degradation

Gene editing uses engineered nucleases, known as molecular scissors, to modify DNA sequences. CRISPR-Cas, ZFN, and TALENs are the main gene editing tools, and they work by introducing double strand breaks in the target gene sequence, which is then repaired by either a homology-directed repair (HRD) or a nonhomologous end joining (NHEJ) pathway [84,85]. Zinc finger nucleases (ZFN) are artificial restriction enzymes which have Zinc finger proteins (ZFPs) comprised of alpha-helices, which are 30 amino acids long and act as DNA binding domains, and Folk1 cleavage domain, which introduces double strand breaks (DSB) at the target site. ZFPs recognize 18 bp specific sequences on

DNA, and around four to six ZFPs interact with the cleavage domain, depending upon the target site, thus allowing target-specific gene editing. Transcription activator-like effector nucleases (TALENs) use TAL proteins, which consist of two domains: one domain for specific sequence recognition and one domain for sequence cleavage which results in DSB in the target DNA [86].

Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated proteins (Cas) act as an adaptive immune system of microbes by incorporating short sequences of invading genomes (spacers) into the CRISPR locus. There are three types of CRISPR systems and several subtypes which have been identified, of which the type II system is best characterized. It comprises Cas9 nuclease, the guide crRNA, and transactivating crRNA, which forms a CRISPR complex and associates with the target DNA using the mature guide crRNA. Cas9 endonuclease introduces double strand breaks which are then repaired by the host cell machinery, resulting in either insertion or deletion of genes, thus resulting in disruption of open reading frames of the genes. Thus, by using these gene-editing tools, knock-in or knock-out mutations can be introduced, wherein the expression of enzymes which play a crucial role in plastic degradation can be increased, or such genes coding for the enzymes could be introduced in the host microorganism [87]. CRISPR-Cas systems have been used by researchers in carrying out gene editing in *Pseudomonas* sp. [88,89] and *Escherichia coli* [90,91]. However, carrying out gene editing of indigenous microorganisms which are already present at the contaminated site would be more beneficial, as they have the ability to survive and harbor themselves in stress conditions.

8. Immobilized Enzymes for Bioremediation of Plastics

Though microorganisms can survive in extreme conditions, it is difficult to maintain these conditions in the outside environment [92]. Many factors, such as temperature, pH, moisture content, oxygen, the bioavailability of nutrients, contaminants, and the presence of other toxic compounds, can influence the enzyme expression in microorganisms [93]. Moreover, the expression of enzymes in microbial cells is regulated by specific inducers, repressors, and cofactors [94]. This problem can be overcome by using immobilized enzymes. The advantage of using immobilized enzymes is that it will not lead to inhibition by inhibitors, can work effectively at low and high concentrations of pollutants, and will be more mobile compared to microbes [95].

Since the biodegradation of plastics would be more efficient by microbial consortia rather than single species, an immobilized multienzyme system needs to be designed to carry out complex biological processes which involve several chemical conversions. Physical adsorption onto octyl-agarose and octadecyl sepabeads of microbial lipase from *Yarrowia lipolytica* resulted in ten-fold-higher stability and better yield than free microbial lipase [96]. Barth et al. [97] reported a two-fold increase in yield of biodegradation products by using a dual-enzyme reaction system consisting of polyester hydrolase and immobilized carboxy esterase from *Thermobifida fusca*.

9. Designing Synthetic Microbial Consortium for Plastic Degradation

Numerous studies have reported the presence of different types of bacteria, fungi, and actinomycetes in polluted environments, with the surroundings becoming acclimatized to plastics and utilizing these waste products as sources for carbon and nitrogen. The limiting factor affecting the rate and range of hydrocarbon degradation by microorganisms appears to be the lack of ability of most microbial strains to utilize different components of plastic. Different strains can degrade different components, but a single strain can usually attack a limited number of hydrocarbons. Hence, a microbial consortium is more nutritionally versatile than a single strain and exhibits considerable competence in utilizing a large number of hydrocarbon components from plastics [98,99].

There are innumerable microorganisms with the potential to degrade plastics in a consortium, but it is practically difficult to experimentally test all combinations to develop

the most suitable bacterial consortium. These microbial species can be filtered by simulation to test their compatibility in silico. FLYCOP, which stands for FLexible sYnthetic Consortium Optimization, is an in silico tool which can be used for designing and optimizing microbial communities. Instead of using a trial-and-error approach for multiple random consortium configurations, FLYCOP uses a stochastic local search. It takes multiple consortium configurations as the input and gives back the best configuration as the output. FLYCOP can be used to carry out simulations and detailed evaluations of diverse situations before doing actual in vivo experiments. It is a flexible in silico tool applicable to different microbial communities with diverse objectives to study numerous community configurations computationally.

Two other tools, which use a hybrid approach, Microbial Community Modeller (MCM) and COMETs, can also be used for designing the microbial consortium [100]. The use of in silico tools for designing microbial consortia will circumvent the trial-and-error efforts and the need for chemical optimization and will save resources and time. A microbial consortium comprising *Mycobacterium* spp. PO1 and PO2, *Novosphin-gobium pentaromativorans* PY1, *Ochrobactrum* sp. PW1, and *Bacillus* sp. FW1 exhibited a three-fold-higher degradation rate for pyrene compared to individual bacterial strains. The degradation process was initiated by *Mycobacterium* spp, and *Novosphin-gobium pentaromativorans* PY1, *Bacillus* sp. FW1, and *Ochrobactrum* sp. PW1 degraded the intermediates aided by the release of biosurfactant by *Bacillus* sp. FW1, which helped in increasing the solubility of pyrene [101]. A consortium comprising *Streptomyces* sp. A5, A11, M7, and MC1 removed 86% of Cr(VI), which had an initial concentration of 50 mg/kg in soil and 46% of lindane, which was present at an initial concentration of 25 mg/kg of soil [102]. *Arthrobacter* sp. DNS10, Variovorax sp. DNS12, *Arthrobacter* sp. DNS9, and *Bacillus subtilis* DNS4 removed atrazine present at an initial concentration of 100 mg/L, with 100% efficiency compared to a single microbial strain when used alone [103].

Similarly, synthetic microbial consortia can be designed for the degradation of different types of plastic polymers. Furthermore, hybrid consortia comprising indigenous and engineered microorganisms can be designed to have better plastic bioremediation capabilities. While designing synthetic microbial consortia, the spatial arrangement of the mixed cultures is important to invigorate the interactions locally and to improve their ability to survive in environmental stress conditions. Moreover, over a long period, the microbial strains in the consortia can undergo mutations, which can lead to nonproductive phenotypes and can decrease population-level performance. Hence, while designing microbial consortia, care must be taken to decrease mutations along with the competitive and antagonistic interactions taking place between the native species. However, to ensure that the engineered microbial communities do not disrupt the natural ecosystem, the biocontainment of synthetic microbial consortia is important, along with population control [104]. In this regard, many specific pathways have been targeted for microbial degradation of plastics (Table 2).

Table 2. List of biodegradation databases and pathway prediction systems.

Sr. No.	Database	Link	Importance in Plastic Degradation	Reference
1	Biodegradation Network-Molecular Biology Database (Bionemo)	https://bionemo.bioinfo.cnio.es	Manually curated database providing information about genes and proteins involved in biodegradation metabolism	[105]

Table 2. *Cont.*

Sr. No.	Database	Link	Importance in Plastic Degradation	Reference
2	University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD)	http://eawag-bbd.ethz.ch/	Provides information pertaining to metabolic pathways along with microbial biocatalytic reactions, enzymes, and genes in the process of biodegradation	[106]
3	Metacyc	https://metacyc.org/	Highly curated database providing information about experimentally elucidated metabolic pathways and enzymes for all domains of life	[107]
4	Biocyc	https://biocyc.org/	It gives information pertaining to entire genomes and has predicted a metabolic network of an organism	[108]
5	PathPred	http://www.genome.jp/tools/pathpred/	Retrieves information from KEGG reaction and KEGG RPAIR databases of query compound and predicts possible enzyme-catalyzed reaction pathways	[109,110]
6	Biochemical Network Integrated Computational Explorer (BNICE)	http://minedatabase.mcs.anl.gov	Using the reaction rules based on the Enzyme Commission classification system, it designs novel chemical structures and pathways	[111]
7	From Metabolite to Metabolite	http://FMM.mbc.nctu.edu.tw/	Proposes metabolic pathways including the information about enzymes involved and genes and organisms which can be compared between different species and is based on KEGG databases and other integrated databases	[112]
8	Metabolic Tinker	http://osslab.ex.ac.uk/tinker.aspx	Used to design metabolic pathways between source compounds and end-products synthetically	[113]
9	MetaRouter	http://pdg.cnb.uam.es/biodeg_net/MetaRouter	Maintains diverse information related to bioremediation and biodegradation pathways stored in an integrated framework	[114]

10. System Biology Approaches for Plastic Degradation

The Systems biology approach gives insight into the difference in the response of microbial systems under different environmental conditions. Moreover, it helps in understanding the microbial interactions within the communities and the survival of microorganisms under conditions of extreme temperature and pressure [115,116]. This is possible through the interdisciplinary field of study combining computational biology and multiomics approach.

Microbial consortia play an important role in the efficient biodegradation of xenobiotic compounds by cooperative metabolic activities, and hence, the final fate of the compound by partial or complete degradation to a nontoxic compound can be predicted by in silico analysis [93,117]. Moreover, several biodegradation databases and pathway prediction

systems have been designed which assist in implementing microbial consortia to an environmental site for remediation and in hazardous waste management [118]. Since an enormous number of microbes are present in the natural environment, phylogenetic analysis and pathway prediction systems can be used to filter, by simulation, the number of possible combinations which are essential for formulating novel microbial consortia [93]. The different databases which provide data related to biodegradation of chemicals, the genes and enzymes which are involved in the biodegradation, and the metabolic degradation pathway are mentioned in Table 2.

Autodock, Schrödinger, Vina, YASARA, Glide, GEMDOCK, PatchDOCK web server, GOLD, and DARWIN are the different softwares which can be used to carry out molecular docking analysis, wherein the ability of an enzyme to adhere to a pollutant can potentially be displayed and its functionality in the biodegradation process can be understood [119–121]. Skariyachan et al. [122] studied the molecular interactions between lipases from *Pseudomonas* spp., polyethylene, and polystyrene, which showed that both polyethylene and polystyrene had good interaction with lipases, and hence, bioinformatic analysis can be used for hypothesizing degradation mechanisms. In molecular docking analysis, the protein is held static; this limitation can be overcome by using molecular dynamic simulations. The stability of a ligand-receptor complex proposed by molecular docking analysis can be estimated by using molecular dynamic simulations, as it allows conformational change in both ligand and enzyme in complexes during biological premises. The programs which can be used to study molecular simulations are Abalone, Amsterdam Density Functional (ADF), and Desmond [123].

The multiomics approach includes genomics, transcriptomics, proteomics, metagenomics, and metabolomics and can be used for identifying uncharacterized biosynthetic gene clusters within the genome of sequenced organisms (Figure 7). The metagenomics approach is garnering lot of interest because of its possibility to degrade oil, petroleum, plastics, and other hydrocarbons by carrying out direct genome analysis of nonculturable microorganisms. Fang et al. [124] investigated, through metagenomics, the biodegradation genes involved in degradation of DDT (dichlorodiphenyltrichloroethane), HCH (hexachlorocyclohexane), and atrazine, where they were able to identify the genes involved in biodegradation after carrying out genome annotation.

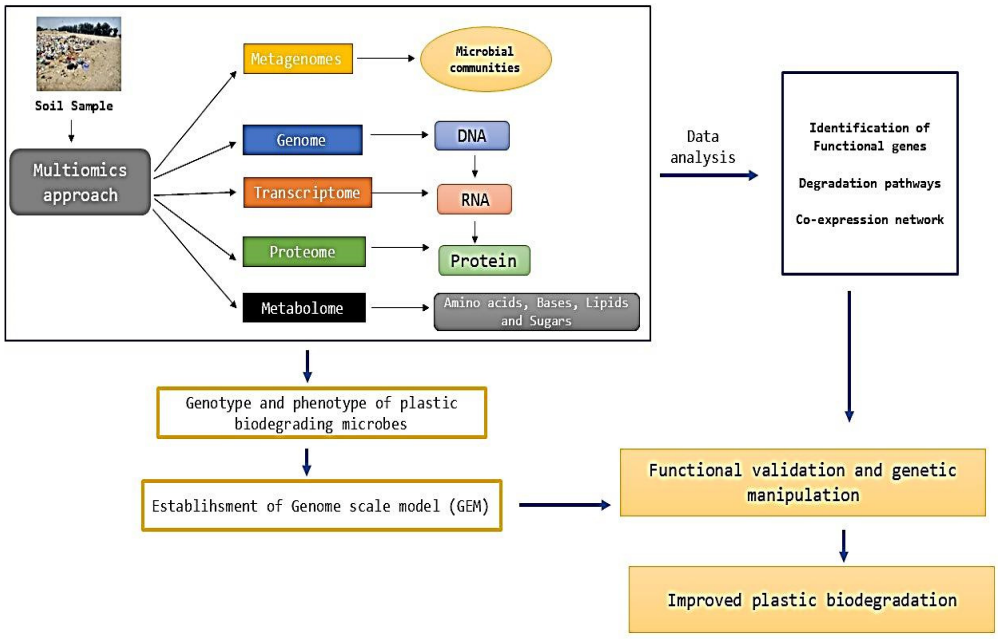

Figure 7. The multiomics approach for plastic degradation. Various approaches such as metagenomic, genomic, transcriptomic, proteomic, and metabolomic approaches are followed for the degradation of plastics. High-throughput techniques can be followed to do so.

Transcriptome-based studies were conducted on a marine bacterium *Bacillus* species, AIIW2, which provided insights into the hydrolytic enzymes involved in the PET degradation process. The process of PET degradation was confirmed by carrying out weight loss assay, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) analysis, and by measuring the hydrolytic products of PET degradation. Aldehyde dehydrogenase and carboxylesterase were found to be involved in the PET degradation process. By observing the changes in the functional groups and HPLC analysis of degraded products, the degradation pathway was elucidated. Moreover, engineered microbial systems with higher PET degradation capabilities could be developed using the gene annotations [125]. A similar approach could be used for studying the genes involved in biodegradation of plastics. The genome scale model (GEM) can be used to predict the microorganisms with the highest potential for bioremediation using the genotype and phenotype data about the particular microorganism available from proteomics, transcriptomics, and metabolomics [126].

11. Conclusions

This article focuses on the ubiquitous use of plastics in all aspects of life, which has caused a tremendous increase in the amount of plastic waste generated. The degradation of this plastic waste into smaller particles gives rise to microplastics, which have proven to have negative impact on the aquatic and terrestrial environment. Lack of proper analytical tools to study microplastics < 10 µm has made it difficult to study the detailed effects of microplastics on human health. Researchers have reported accumulation of microplastics in animal tissues, and their harmful effects have been found in cell lines. The presence of microplastics has been reported in human blood, and a positive correlation has been found between the presence of microplastics in human feces and IBD, but more experimental evidence is required to prove the deleterious effects of microplastics on human health.

The current methods employed for the degradation of plastic wastes, including in landfills and incineration, result in the release of more toxic chemicals in the environment. Microorganisms have the ability to degrade plastics in an ecofriendly manner. In order to increase the potential of these microorganisms to degrade plastic waste, modifying these microorganisms using genetic engineering techniques and genome editing tools can be used. *Pseudomonas aeruginosa* strains were engineered which were capable of removing microplastics from seawater samples by secretion of exopolysaccharides using a 'trap and release' mechanism [127]. Modification of bacterial proteins to enhance their ability to remove microplastics from wastewater can be employed [128]. The major challenge in using these engineered microorganisms at the industrial scale is the safety concerns associated with the use of GMOs. By using omics technology and pathway prediction tools, microbial consortia can be designed, which have more potential to degrade plastics and reduce the amount of microplastics released in environment.

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