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# Review

# Call for biotechnological approach to degrade plastic in the era of COVID-19 pandemic



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# ABSTRACT

Plastic pollution is a global issue and has become a major concern since Coronavirus disease (COVID)-19. In developing nations, landfilling and illegal waste disposal are typical ways to dispose of COVID-19-infected material. These technologies worsen plastic pollution and other human and animal health problems. Plastic degrades in light and heat, generating hazardous primary and secondary micro-plastic. Certain bacteria can degrade artificial polymers using genes, enzymes, and metabolic pathways. Microorganisms including bacteria degrade petrochemical plastics slowly. High molecular weight, strong chemical bonds, and excessive hydrophobicity reduce plastic biodegradation. There is not enough study on genes, enzymes, and bacteria-plastic interactions. Synthetic biology, metabolic engineering, and bioinformatics methods have been created to biodegrade synthetic polymers. This review will focus on how microorganisms' degrading capacity can be increased using recent biotechnological techniques.

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Abbreviations: COVID-19, Coronavirus disease-19; PPE, Personal protective equipment; PE, Polyethene; LDPE, Low-density polyethene; HDPE, High-density polyethene; PP, Polypropylene; PS, Polystyrene; PVC, Polyvinyl chloride; PET, Polyethylene terephthalate; MP, Microplastics; NP, Nanoplastics; FTIR, Fourier-transform infrared; PES, Polyethylene succinate; TCA, Tricarboxylic acid; BHET, bis(2-hydroxyethyl; MHET, Mono(2-hydroxyethyl; TPA, Terephthalic acid.

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#### 1. Introduction

Scientific and technological progress, especially in the last two decades, has led to a rise in the annual production of synthetic polymers. About 140,000,000 metric tonnes of synthetic polymers are manufactured annually. The value of synthetic plastics in modern economies and cultures is acknowledgeable (Molloy et al., 2020). Due to their low cost, stability, and resilience brought on by their polymeric characteristics, plastics are indispensable to all facets of our daily lives. Fifty years of steady expansion have resulted in 2018's record output of 359 million metric tonnes of plastic. Disposable plastic personal protective equipment (PPE) components (such as gloves, face shields, and masks) are still being used, leading to an increase in plastic waste even in the midst of the COVID-19 pandemic health crisis. Sixty-five billion gloves and 129 billion face masks are thrown away every month, contributing to the global problem of plastic pollution (Wang et al., 2022).

An increase from less than 1 % in 1960 to more than 10 % in 2005 can be seen in both high-income and developing countries' municipal solid waste. Europe had already thrown away 6.9 million tonnes of plastic by 2020. The slow rate at which plastics break down in landfills or after being released into the environment is a problem. Plastics like propylene and ethylene (derived from fossil hydrocarbons) long half-lives result from their high crystallinity, hydrophobicity, and molecular weight (Ford et al., 2022). As a result, land, freshwater, and marine environments become polluted with plastic over time. Plastic polymers often have other chemicals, and additives added to them to boost their quality. Bisphenol A, bisphenol S, octylphenol, and nonylphenol are examples of endocrine disruptors that can be harmful to living things. Some additives in plastics have been linked to endocrine disruption, diabetes, and obesity (Waring et al., 2018).

Certain petrochemical plastics have been shown to biodegrade in recent studies. These plastics are consumed by specialized marine microbes as carbon sources (such as bacteria, fungi, and algae). Some of the most well-known bacterial taxa involved in plastic biodegradation are Arthrobacter, Pseudomonas, Bacillus, Streptomyces, Micrococcus, Corynebacterium, and Nocardia. Numerous types of fungi, such as Fusarium spp., Aspergillus spp., and Penicillium spp., have been identified as contributors to plastic biodegradation (Ma et al., 2022). It is commonly believed that microbial degradation of plastic follows physicochemical degradation, which alters the structure and length of the polymer. However, little is known about how bacteria interact with plastic. Biodegradation is aided by many enzymes that aid in polymer oxidation and fragmentation. Some examples of these enzymes include oxidases, dehydrogenases, lipases, and esterases (Alim et al., 2022).

The enzymatic degradation of plastic is affected by many biotic and abiotic factors. The composition, molecular weight, crystallinity, branching structure, and critical thickness of the plastic are major factors affecting its degradation. It is observed that plastic degrading enzymes work better in elevated temperature and alkaline pH (Mistry et al., 2022).

Biotechnological developments like bioinformatics, genetic engineering, and the formation of synthetic microbial consortiums have been adopted to avoid the issues associated with traditional disposal procedures such as burning and landfilling. Genetic engineering tools have been beneficial in changing the genetic structure of microorganisms to improve their degradative capacity for plastic contaminants. The genetic potential of the natural microbial consortium was controlled using bioinformatics and genetic engineering techniques, resulting in a synthetic microbial community. This review has looked at microbial degradation strategies and other possible methods for increasing microbial potential.

# 2. Method

In this study, we aimed to identify studies that shed light on environmental plastic contamination, plastic distribution, and plastic entry into the food web. We searched literature discussing plastic pollution in the era of the COVID-19 pandemic and its impact on human health. We focused on recent studies for plastic biodegradation. The search was done on a broad level to identify

the recent studies concerning plastic pollution and options for its degradation. We also aimed to identify the studies providing insight into the factors affecting plastic biodegradation and how we can increase plastic biodegradation through biotechnological approaches.

#### 2.1. Study selection

The literature was found using Google Scholar, PubMed, Science Direct, and ISTOR. Grey literature, such as conferences and datasets, were discovered using Google with the exact keywords: plastic pollution, plastic biodegradation, and plastic biodegrading bacteria. The search lasted from November 2021 through August

Systematic review tools were used for data screening, extraction searching, deduplication, and bias assessment. Studies older than 2015 and discussing the degradation of biodegradable plastic were excluded. The abstracts of 397 scholarly papers were read, and 200 were chosen for further investigation. Studies were selected by reading their abstracts and titles and the entire articles. They were only considered if the studies' titles and abstracts indicated their relevance. Only high-quality studies that fulfilled the inclusion criteria in their full texts were included. The compiling plan was critical in focusing the first round of searching. In addition, 63 documents from the grey literature were evaluated for their usefulness in providing a complete picture of the policymaking process. Priority was given to publications from 2019 to 2022, but other relevant publications outside that time frame were included after a thorough review of the manuscript Table 1.

# 3. Synthetic plastics biodegradation

# 3.1. Polyethene (PE)

Since its introduction in the 1940 s, polyethylene (PE), the first polyolefin material, has experienced remarkable and consistent growth in the food packaging sector. Low-density polyethene (LDPE) contains more short- and long-chain branching units than high-density polyethene (HDPE). The crystal structure of LDPE is less rigid than that of HDPE (Joshi et al., 2022).

PRISMA: Flow diagram for searches of databases and other sources.

Table 1 Identification of studies via databases Identification of studies via other methods Records removed before Berling. Dunlicate records removed (n Records identified from Records identified from\*: Databases (n= 1000) =320 )
Records marked as ineligible
by automation tools (n = 213)
Records removed for other
reasons (n =70) Reports sought for retrieval (n = 200) Reports sought for retrieval (n =3) Reports not retrieved (n = 197) Reports assessed for eligibility (n =3) Reports assessed for eligibility (n = 200) Reports excluded: Reason 1 (n = 143) Reports excluded: Reason 1 (n = 60) Reports of included other studies (n = 3)

Because of its high moisture barrier, high thermal stability, and low susceptibility to abrasion and chemicals. LDPE is the best PE to use for food packaging. Polyethene is resistant to microbial breakdown because it is composed of long chains of ethylene monomers. The study of Hou et al. (2022) identified a bacterium Pseudomonas knackmussii N1-2 which encodes genes for enzymes involved in PE breakdown. Sequencing of P. knackmussii N1-2 illustrates the encoding of enzymes that participate in metabolic pathways responsible for PE degradation (see Table 2).

# 3.2. Polypropylene (PP)

Polypropylene-based packaging, especially microwaveable food containers, is rising in popularity because of its suitability for use in food contact applications. It's a long chain of carbon atoms linked together in a linear structure and hydrophobic (Jain et al., 2022). Diapers, non-absorbable sutures, plastic tubs, stationery folders, packaging materials, and plastic mouldings are some of the uses of this versatile thermoplastic. It is easily oxidized and degraded at high temperatures when exposed to sunlight ultraviolet radiation. The degradation process resulted in a decrease in viscosity and the formation of new groups, including carbonyl and carboxyl. Bacterial strains like Pseudomonas and Vibrio have great potential to degrade polypropylene. Researchers have also found Aspergillus niger (fungus) to biodegrade polypropylene (Wang et al., 2022a).

# 3.3. Polystyrene (PS)

Polystyrene's high molecular weight and low hydrophilicity make it a unique synthetic polymer with practical applications. PS film was biodegraded to a minor extent by an Actinomycetes strain (Mor and Sivan, 2008). You can recycle it, but it won't break down in the environment. Polystyrene biodegradation was investigated by seventeen different plastic-degrading fungi. The researchers found a slow decomposition rate which could be accelerated by adding cellulose and minerals. The adhesion of Arthrobacter, Escherichia coli, Micrococcus, and Pseudomonas to polystyrene film has also been studied (Alim et al., 2022).

**Table 2**Plastic degradation potential of various bacterial strains reported in literature.

Sr. no.	Organism	Plastic-type	% Loss	Reference
1	Moraxella sp.	Polyethylene terephthalate polyurethane	64 %	Nikolaivits et al. (2022)
2	Klebsiella pneumoniae CH001	High-density polyethylene (HDPE)	18.40 %	Awasthi et al. (2017)
3	Streptomyces sp.	Polyethylene terephthalate (PET)	68.80 %	Farzi et al. (2019)
4	P. knackmussii N1-2	Polyethylene	5.95 %	Bakht et al. (2020)
5	P. aeruginosa RD1-3 strains	Polyethylene	3.62 %	Bakht et al. (2020)
6	B. wudalianchiensis UMT	Polyethylene	6.60 %	Bakht et al. (2020)
7	Xanthomonas sp. HY-71	Polyester-polyurethane	23.95 %	Kim et al. (2022)
8	Acinetobacter pitti	Low-density polyethylene (LDPE)	26.80 %	Montazer et al. (2018)
9	Serratia sp.	LDPE	40 %	Nadeem et al. (2021)
10	Bacillus siamensis	LDPE	8.46 %	Maroof et al. (2021)
11	Bacillus cereus	LDPE	6.33 %	Maroof et al. (2021)
12	Bacillus wiedmannii	LDPE	5.39 %	Maroof et al. (2021)
13	Proteus mirabilis	LDPE	89.72 %	Akhiqbe et al. (2022)
14	Enterobacter sp.	Polypropylene	64 %	Skariyachan et al. (2021)
15	Staphylococcus sp.	Polythene	20 %	Sing et al. (2016)
16	Sporosarcina globispora	Polypropylene	11 %	Helen et al. (2017)
17	Lysinibacillus fusiformis	Polythene	21.87 %	Shahnawaz et al. (2016)
18	Bacillus paramycoides	Polyethylene	2.25 %	Widyananto et al. (2021)
19	Stenotrophomonas pavanii	Polyethylene terephthalate (PET)	91.40 %	Huang et al. (2022)

# 3.4. Polyvinyl chloride (PVC)

While reports on PVC's photo- and thermal-degradation are common, biodegradation is rarely seen. Insect larvae from the Coleopteran and e3Lepidopteran orders have degraded PVC. For instance *Tenebrio molitor* has potential to biomineralize PVC into HCl. It has been established that the microbiota in *T. molitor's* gut is necessary for the organism to biodegrade plastic (Pivato et al., 2022). Fungal enzymes' potential for PVC breakdown has also been explored. The ability to decompose lignin has been connected to PVC biodegradation, as evidenced by the change in PVC structure caused by fungal lignin peroxidase from *Phanerochaete chrysosporium* (Temporiti et al., 2022).

# 3.5. Polyethylene terephthalate (PET)

With an annual global output of 70 million tonnes, polyethylene terephthalate (PET) is commonly used in disposable containers for soft beverages, juices, and drinking water. The accumulation of PET waste in the environment is recognized as a global concern with far-reaching effects that require rapid and significant interventions due to the material's extraordinary resilience to degradation. Research has identified a bacterial species that can metabolise amorphous PET without the aid of any other consortium participants. *Ideonella sakaiensis* (rod-shaped, gram-negative, aerobic bacterium) is a new PET-degrading bacterium with PETase and METHase enzymes (Tamargo et al., 2022).

# 4. Increase of plastic pollution in the COVID-19 pandemic

According to reports, plastic waste will more than double by 2030, posing a substantial transboundary threat to animal and human health. Personal protective equipment like gloves, face masks, and sanitiser bottles are made of synthetic plastic. The COVID-19 coronavirus's increased demand for single-use plastic has exacerbated the world's plastic waste problem. Face masks and hand sanitiser bottles are both composed of plastic. Face masks constructed of polypropylene, a famously difficult-to-recycle plastic, are widespread.

Personal protective equipment is required to follow administrative procedures for COVID-19 pandemic management, and people are turning to internet shopping and food delivery. The appropriate recycling of worn PPE, packing materials and food containers may not be guaranteed (Fig. 1). Secondary microplastics and nanoplas-

tics are assumed to result from poorly disposed plastics that break down into smaller particles and are then discharged into the environment (Wang et al., 2022).

# 4.1. Plastic effects on human health

Marine macro-plastic pollution is a major problem for aquatic animals because they often use plastic bags and sheeting for their preferred food. Animals in the water may not be able to untangle themselves from plastic debris as humans can. Large pieces of plastic are safe for human consumption, except for plastic sauce packets (Waring et al., 2018).

Macroplastic breaks down into microplastics (MP) and nanoplastics (NP) as it travels through the environment. Particularly shellfish and crustaceans can introduce these through ingestion or inhalation into the human food chain. Notably, the intestinal uptake of non-toxic MPs is low. NPs are easily absorbed and can build up in the brain, liver, and other tissues of animals and aquatic organisms. A negative effect on the central nervous system or the reproductive system from exposure to nano-plastic is possible, but only at very high exposure levels and with the aid of physiological factors in absorption (Mai et al., 2021).

Discarded plastics pose significant risks to human health through inhalation and ingestion. Micro-plastics have been linked to a wide range of adverse biological effects, including tissue damage, fibrosis, and carcinogenesis when exposed over long periods; inflammation, genotoxicity, apoptosis, oxidative stress, and necrosis have all been linked to micro-plastic persistence. Humans and animals that ingest micro- and nano-plastics may facilitate the entry of adhered or endogenous pollutants into cells (Ali et al., 2021).

# 5. Environmental plastic degradation

Both PE and PP can be broken down by photo- and thermal-degradation in aerobic and anaerobic conditions. Plastic photo-degrades into subsequent polymer fragments when exposed to sunlight due to autooxidation. Auto-oxidation occurs during thermo-oxidative degradation, and it can be triggered by heat or mechanical stress (Mai et al., 2021). PE and PP undergo an initiation, propagation, and termination stage during photo- and thermal-induced oxidation. The presence of chromophoric or thermolabile groups in the polymer chain triggers the formation of free radicals, which marks the beginning of the initiation step. During



Fig. 1. Increase of plastic use in SARS- CoV-2 leading to micro/nano plastic pollution (Haque and Fan, 2022).

the propagation stage, oxygen reacts with products of the initiation step (free radicals) to produce hydroperoxides. Hydroperoxide breaks down into alkoxy and hydroxyl radicals. Different products can be obtained after the alkoxy and hydroxyl radicals react, either through  $\beta$ -scission, hydrogen abstraction, or the cage reaction. The reaction terminates when the radicals recombine into a stable form. While the visual signs of degradation like discoloration and brittleness are easy to spot, the chemical effects like bond scission and the formation of new functional groups require further investigation (Canopoli et al., 2020).

# 5.1. Complete degradation of plastic

The photo-degradation and thermal degradation of plastic lead to the production of primary and secondary micro-plastic hazardous to aquatic and terrestrial organisms (Fig. S1). Microplastics, or small plastic particles, cannot degrade in nature (Akarsu et al., 2022). As a result, primary and secondary microplastics form and persist in the environment. Micro-plastics have been discovered in both marine and freshwater habitats. Microplastics had previously been found in over 114 aquatic species in freshwater and marine ecosystems until that year (2018). It is also critical to remove existing micro-plastics from the environment. One option considered was the employment of microbes capable of degrading synthetic micro-plastic polymers. The presence of several bacterial and fungal species allows for the biodegradation of polystyrene, polyester polyurethane, and polyethylene (Fig. 2). These microbes could be helpful to polluted environments such as sewage overflow (Jaiswal et al., 2022).

# 5.2. Plastic degradation by fungi

Aside from environmental factors and the activities of microbial species, the biodegradation of manufactured plastics is a slow process. Fungal enzymes like proteases, laccases, cutinases Lipases as well as the presence of pro-oxidant ions and lignocellulolytic enzymes, all play a key role in plastic biodegradation (Gao et al., 2022).

The oxidation or hydrolysis of the enzyme produces functional groups that increase the hydrophilicity of the polymer, causing the molecular weight to decrease. Clasdoporium cladosporiodes, Bjerkandera adusta, Penicillium griseofulvum, Phanerochaete chrysosporium, and other saprotrophic Aspergillus species have been studied for plastic degradation. According to specific research, the simultaneous engagement of photo-degradation and thermo-oxidative processes in biodegradation facilitates rapid and effective plastic degradation (Srikanth et al., 2022).

# 5.3. Plastic degradation by algae

Biodegradation of LDPE has been discovered in several bacteria, fungi, and actinomycetes (*Streptomycetaceae*). There have been few investigations on LDPE biodegradation by photosynthetic algae. The use of microalgae for the biological degradation of polyethene, as compared to traditional degradation methods, has the added benefit of being environmentally friendly and cost-effective. *Oscillatoria, Phormidium, Lyngbya, Nostoc, Spirulina, Hydrocoleum, Chlorella, Pithophora, Stigeoclonium tenue, Anomoeoneis,* and *Nitzschia* have all been found to colonize polyethene bags in water. The water jet was ineffectual in eliminating the cyanobacteria because they were firmly bonded to the surface of the plastic (Bhuyar et al., 2019).

A similar discovery was made in a study of a heavily urbanized freshwater lake where the garbage bags were placed, involving the microalga *Uronema africanum* Borge. LDPE sheets were biodegraded using this microalga. Based on the results of light microscopy, dark field microscopy, GC–MS, FT-IR, SEM, and AFM after 30 days of incubation, the microalga was identified as low-density polyethene degrading organism (Sanniyasi et al., 2021).

# 5.4. Plastic degradation by insects

Insects, which are among the most diverse creatures on the planet, are being studied for a variety of potential applications. Biotechnological research extensively employs agriculturally important insects such as the silkworm, the black soldier fly, *Galleria mellonella* (Greater wax moth), and others (Yamamoto et al., 2022).

A new method utilizing insect degradation of plastic is fascinating in the context of plastic pollution challenges. Microbes discovered in the digestive tract of the Indian mealworm (*Plodia interpunctella*) are capable of degrading polyethene (Fig. S2). It was found that *Tenebrio molitor* (Yellow mealworms) could chew and consume polystyrene, also known as Styrofoam. Polystyrene degradation in yellow mealworms was studied using isotopic analyses. *Galleria mellonella* caterpillars, commonly known as Greater wax moth larvae, have been found to degrade PE more quickly than *Plodia interpunctella* and *Tenebrio molitor* (Kesti and Shivasharana, 2018).

# 5.5. Plastic degradation by bacteria

Bacteria are the most common type of microbe and the most common type of organism. They can be found almost anywhere, including in soil, water, and the air, and many species are known

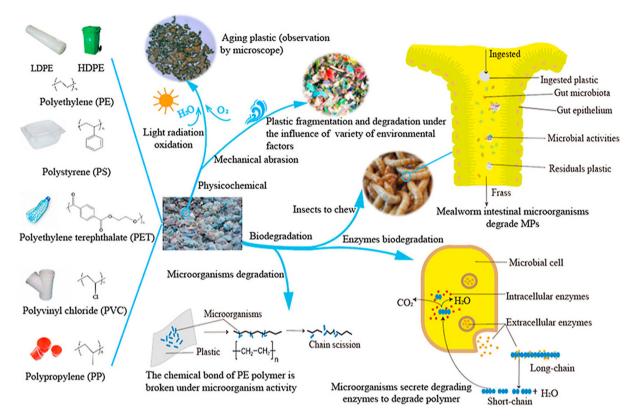


Fig. 2. Degradation of plastic (PE, PS, PET, PVC, and PP) by the environment, insects, and microorganisms (Lin et al., 2022).

for being able to break down pollutants. In the past few years, there has been much more research on how bacteria might help break down plastics and micro-plastics (Li et al., 2022).

Researchers have used pure bacterial cultures in a controlled laboratory to study how microorganisms break down plastic. Most of the time, enrichment culturing was used to separate these cultures from sediment, sludge, and wastewater. However, some of the cultures were obtained through culture collections. Using pure strains in studies of how plastic breaks down is helpful because it makes it easier for researchers to look into metabolic pathways or figure out how different environmental factors affect and how plastic breaks down. Also, the mechanism of functional bacteria breaking down plastic and the changes that happen to plastic can be carefully observed (Urbanek et al., 2018).

In the past few years, many bacterial isolates that can break down plastic have been found. Skariyachan et al. (2021) found that novel combinations of Enterobacter and Pseudomonas from cow dung helped polyethene and polypropylene break down faster. After 160 days in the lab, the Enterobacter strain and the Pseudomonas strain caused LDPE to lose 64 % of its weight. Because of the microorganisms' activity, the treated LDPE's surfaces also had different pores and irregularities. Based on these results, pure cultures of bacteria taken from the environment could adhere to, take over, and break down plastic. Maroof et al. (2021) found and described the microorganisms that break down several types of plastic. These microorganisms were Bacillus siamensis, B. cereus, and B. wiedmannii, and they were all found in the soils of waste disposal sites. These three strains each caused LDPE to lose weight by 8.46, 6.33, and 5.39 %, respectively.

Studies have found that bacteria can change not only plastic appearance but also functional groups and other features. For example, when PE was incubated with *Alcaligens faecalis*, the carbonyl group in PE's FTIR spectrum disappeared. Also, when PE was mixed with *A. faecalis*, new absorption bands appeared at

1740 cm1 and 1460 cm1, which showed that C=O and CH3 bonds were present (Nag et al., 2021).

After being incubated with *Streptomyces* sp., the molecular weight and tensile properties of polyethylene terephthalate decreased quickly (Farzi et al., 2019). This shows that bacteria can change plastic's molecular weight and tensile properties. In a separate study, *Lysinibacillus fusiformis* was used to break down polythene. The highest degradation determined was 21.87 % (Shahnawaz et al., 2016).

In addition, bacteria from the gut of insects have been isolated, which helps the insects in plastic degradation. The larvae of the *Galleria mellonella* insect were used to collect bacteria from the adult insects. These bacteria are known as *Bacillus aryabhatai*, *Microbacterium oxydans.*, and *Lysinibacillus fusiformis*. Cell mass production and LDPE weight loss in PE-containing media were significantly influenced by bacteria isolated from greater wax moth larvae. The degradation of LDPE by a group of bacteria was more effective than by any single species (Montazer et al., 2021).

# 6. Factors affecting plastic biodegradation

# 6.1. Temperature

The effects of temperature on chemical reactions are universal. Biodegradation of plastic by microbes or their enzymes is temperature dependent due to the sensitivity of biochemical reactions to heat. Biodegradation rates of plastics tend to be slower at low temperature due to a decrease in enzymatic activity (Lin et al., 2022).

# 6.2. Branching structure and molecular weight

Although high molecular weight polyethene is not biodegradable, pure linear paraffin molecules with a molecular weight of less

than 500 are used by microorganisms. High molecular weight plastics can be broken down by enzymes produced by the bacteria Nocardioides zeae EA12, Stenotrophomonas pavanii EA33, Gordonia desulfuricans EA63, and Chitinophaga jiangningensis EA02. The copolymerization of metabolically active groups along the chain or at the chain ends did not affect the biodegradability of the polymer. Biodegradation of straight-chain alkanes begins at molecular weights below 500 Da, indicating that enzymes responsible for  $\beta$ -oxidation degradation of regular paraffin are unable to complex with the chain ends in higher molecular weight materials. These terminal groups are likely to be dispersed and inaccessible due to the folded chain configuration of the polymer molecule (Mistry et al., 2022).

# 6.3. Crystallinity

The more crystallinity of the plastic material decreases its biodegradation Because there are no exposed sites for enzymes and other catalysts to work with, and due to their well-defined repeated structure, oxygen and other gases cannot diffuse, retarding the process of biodegradation. Highly crystalline polymers, such as high-density polyethene, are impermeable to oxygen and show slow biodegradation (Yang et al., 2022).

# 6.4. Critical thickness

A water-insoluble polymer's degradability is determined by its critical thickness (Critical). A polymer thicker than  $L_{\rm critical}$  degrades only on its surface (through surface erosion). In contrast, a polymer thinner than  $L_{\rm critical}$  degrades on its surface and core (i.e., bulk erosion) (Van Roijen and Miller, 2022).

# 6.5. Alkaline hydrolysis

Recent studies have shown that elevating the pH can speed up the biodegradation of plastics. When wet oxidation is combined with alkaline hydrolysis, water-soluble and biodegradable products are produced. A more basic environment promoted plastic biodegradation by increasing the activities of protease, esterase, and lipase. Researchers have discovered that in alkaline environments, hydrolysis of plastic ester bonds is increased due to the catalytic effect of hydroxyl ions. According to studies, plastic degradation is easy when combined with soil containing high nitrogen volumes from dairy wastewater sludge (Mistry et al., 2022).

# 6.6. Microbial consortia

Mechanical recycling, chemical recycling, landfill incineration, and pyrolysis are imperfect methods for disposing of plastic waste. Incorporating microbial consortiums or cocultures into existing microbiological waste management practices is an efficient, economical, and ecologically sound option. By-products from plastic recycling can be used to generate cheap energy in bio-digesters. The study of Edwards et al. (2022) proved that bacteria could work synergistically better for plastic degradation. They used a consortium of five bacteria for PET degradation and observed high degradation. Thus, the establishment of microbial consortia, the application of microbial enzymes, and the screening of novel plastic-degrading microorganisms will all play a role in managing plastic waste.

# 7. Mechanism of plastic enzymatic degradation

Depolymerases and hydrolases, acting as extracellular enzymes, can lower the size of polymers used in plastics. Hydrolytic cleavage can occur at the end of the polymer chain (*exo*-attack) or in the center of the chain (called *endo*-attack). The outcomes of the two approaches differ. Bacteria that attack plastic with extracellular enzymes can consume the resulting small oligomers or monomers. When a polymer is attacked from the inside, its molecular weight (MW) is significantly diminished, and the resulting products are unlikely to be assimilable without further degradation. *Pseudomonas* sp. extracellular depolymerase decomposes brominated high-impact plastic effectively, and *P. stutzeri* employed an intracellular polyethylene glycol dehydrogenase to convert PEG to glyoxylic acid. *Pseudomonas* sp. E4 decomposed PE using AlkB family alkane hydroxylases.

# 7.1. Hydroxylases

Polyethylene, polystyrene, polypropylene, and polyvinyl chloride all include carbon atoms in their molecular structures, and alkane hydroxylases play a role in their degradation. The formation of carboxylic acids begins with the oxidation of C—C bonds to aldehydes or ketones and ends with reducing these compounds to primary or secondary alcohols. During microbial oxidation, carboxylic acids are formed, reducing the number of carbonyl groups (Pinto et al., 2022).

Microorganisms can degrade carboxylated n-alkanes through the  $\beta$ -oxidation route in a manner similar to how bacteria can degrade fatty acids. However, the best-known enzymes involved in PE breakdown in the  $\beta$ -oxidation pathway have been discovered as alkane hydroxylases (AlkBs), which are a component of the alkane hydroxylase system route and are known to degrade linear alkanes. The monooxygenases involved in the alkane hydroxylase system are the main enzymes of interest. There are many distinct alkane hydroxylase numbers and types because different bacteria have varied induction conditions and amounts of target carbon in the alkane chain (Tan et al., 2022).

# 7.2. Hydrolases

Several hydrolases, such as esterases, lipases, and cutinases, are essential in the breakdown of the plastic. Hydrolases are necessary for enzymatic polymer cleavage, in which ester bonds are ruptured by a nucleophilic attack on carbonyl carbon atoms left behind from previous oxidation processes. *Pseudomonas* sp. AKS2 hydrolase and dehydrogenase activity in a bio-augmented soil assisted in polyethylene succinate (PES) degradation, according to enzyme studies. Esters, whether naturally existing in the polymer or produced through oxidation processes, can be hydrolyzed into alcohols, phenols, and acids by esterases. *Pseudomonas* sp. AKS2 generated an esterase that degraded PES to yield the tricarboxylic acid (TCA) cycle metabolite succinic acid (Abdelghafour et al., 2022).

# 7.3. Petases and MHETases system

Two enzymes have been discovered in newly isolated, *Ideonella sakaiensis*, bacterium (PETase and MHETase). PETase has been proposed to catalyse the depolymerization of PET by hydrolyzing the ester linkages contained in the polymer. As a result, terephthalic acid (TPA), bis(2-hydroxyethyl) (BHET), and mono(2-hydroxyethyl) (MHET) are produced, which are hydrolyzed by MHETase, releasing ethylene glycol (EG) and TPA. The intracellular mechanism of plastic degradation by microbes is still unknown, and further investigations are needed (Knott et al., 2020).

# 8. Plastic degrading enzymes databases

As plastic pollution is a pressing issue of this age, microbial enzymes for plastic degradation have been sequenced. The record of these enzymes, relevant genes and microorganisms is collected in databases. This development is beneficial for analyzing, manipulating, and cloning plastic degrading genes in future.

# 8.1. Plastic microbial biodegradation database (PMBD)

Eco-conscious communities widely adopt biodegradation by microorganisms because it is a naturally occurring process. To aid in the investigation of plastic biodegradation, the Plastics Microbial Biodegradation Database (PMBD) is established. Information on this topic is compiled and shared through the Plastic Microbial Biodegradation Database (PMBD). The 79 genes and 949 relationships between microorganisms and plastics in this database are assembled by hand and verified through literature reviews (PMDB).

#### 8.1.1. PlasticDB

The microorganisms and proteins involved in plastic biodegradation are catalogued in the PlasticDB database. There are currently 573 known microbial species. Additionally, over 180 proteins with the ability to break down plastics are identified and recorded in PlasticDB.

# 8.1.2. Plastics-active enzymes database (PAZy)

Several publications and PMBD erroneously reported the presence of plastic-degrading enzymes due to widespread annotation errors in genome and metagenome datasets. Humans have carefully selected only those enzymes shown to degrade synthetic polymers in biochemical or genetic complementation studies for inclusion in the PAZy. This results in 100 % enzyme and gene activity throughout the PAZy organism. Short oligomers and additives are particularly interesting to the putative enzymes, microbes, and microbial consortiums in PMDB (PAZY).

# 9. Biotechnology approach for plastic biodegradation

Microorganisms' genetic material can be manipulated via genetic engineering to improve their efficiency in dissolving plastic waste in the environment. It includes gene cloning, recombinant DNA technology, and other forms of genetic engineering. Using this technology, researchers could efficiently investigate the bioremediation of a wide range of pollutants, including hydrocarbon compounds, heavy metals, and others.

# 9.1. Gene manipulation

The development of plastic-degrading enzymes has focused on this area since the interaction between the enzyme active site and the substrate is a crucial feature influencing the effectiveness of plastic depolymerization. Active site substrate binding groove hydrophobicity is another potential engineering goal. According to studies, increased hydrophobicity may be advantageous for binding to plastic substrates because of increased affinity, leading to improved breakdown efficiency. By simultaneously changing an enzyme's hydrophobicity and opening size, catalytic performance can be increased. Enzyme-substrate interactions have been improved by taking advantage of the surface properties of enzymes that break down plastic. Substance binding is regulated by electrostatic and hydrophobic interactions between substrate molecules and amino acid residues on the enzyme's surface. Therefore, it is usual practice to alter the surface's electrostatic and hydrophobic

qualities. More specifically, lessening electrostatic repulsion between the enzyme and the plastic substrate, making the enzyme surface electrically neutral, could increase binding and degradation efficiency (Jaiswal et al., 2020).

Similarly, it was reported that the interaction between the PET substrate was essential in single-mutation site variations. Modifying the 119th position to an aromatic residue (Q119F), like the 119th position change to Q119Y, may improve hydrophobic packing in the binding pocket of the PETase structure through interaction with the aromatic motif of the PET substrate. Depending on the substrate's length, increasing the hydrophobicity of one end of the catalytic site may improve substrate binding while decreasing it at the other (Charupanit et al., 2022).

In position 238, the interaction between the PETase and the PET complex is improved by replacing the polar-uncharged Ser with the polar-charged Cys (S atom). While the S238C substitution reduced the number of hydrogen bonds in the catalytic residue loop, it was compensated for by increased hydrophobic contact between the substrate and the more hydrophobic S atom. The alterations at position 238 have been the subject of extensive research because of their proximity to one of the catalytic triads (H237). By exchanging the conserved Phe at position 238 for a short side chain residue, the active site of the PETase version is made more flexible, and the enzyme's interaction with PET is improved (such as Ser or Arg). However, the combined mutation W159H/S238F improved PETase activity by shortening the distance between S160 and the substrate in the binding cleft. The S238C variation, depending on the characteristics of the side chain and the distance between S160 and the substrate, may increase flexibility while decreasing the binding pocket that brings the substrate close to S160. A variety of side chain features were uncovered by mutations at positions 87, 214, and 280 in our protein's sequence space, all of which potentially improve the interaction between substrate and PETase (Charupanit et al., 2022).

#### 9.2. Genetic modification

Possible genetic engineering targets include halophilic bacteria and fungi. The accumulation of waste in the ocean is a major problem. However, most bacteria and other tiny organisms are doomed to die due to the high salt content of the ocean. There is good news, though; halophile bacteria do well in these conditions. Furthermore, this bacterial strain has been demonstrated to be an exceptionally amenable host for genetic engineering (GE). As a result of their distinct reproductive processes, fungi have become attractive targets for GE research. Most fungi disperse their spores into the air, so they can quickly aid in the fight against plastic pollution (Xia, 2022).

To degrade plastic, scientists have genetically engineered *E. coli* to produce the appropriate enzymes. *E. coli* is a popular host for gene cloning due to the relative simplicity with which DNA molecules can be introduced into cells. Protein production in *E. coli* is expected because of the strain's rapid growth and high protein expression levels. One of the enzymes necessary for plastic degradation is manganese-dependent peroxidase, engineered from *S. cerevisiae* BY 4741 and *E. coli*. In another study, *E. coli* BL21 and *P. chrysosporium* strains were genetically modified and found to produce laccase (Jaiswal et al., 2020).

For the biocatalytic degradation of PET plastics, the curli of an *E. coli* cell was genetically modified to produce BIND-PETase. Under a wide range of reaction conditions, BIND-PETase can depolymerize 9.1 % of highly crystalline post-consumer PET waste in 7 days at room temperature and humidity. This study's results provide the crucial groundwork for designing eco-friendly biocatalytic strategies for plastic degradation and recycling (Zhu et al., 2022).

#### 10. Conclusion

There is a need to focus on plastic biodegradation due to the increased use of plastic material in the COVID-19 era. Many investigations have identified microorganisms like bacteria, fungi, and algae with good potential to degrade synthetic plastic. The genes and enzymes responsible for plastic degradation have been studied as well. A completely new approach to recycling plastic and managing garbage resulted from identifying bacteria and enzymes that break down plastic. The catalytic efficiency of these enzymes can be improved by genetic modifications. The genes responsible for plastic degradation can also be engineered in non-degrading bacteria like *E. coli*. To reduce the amount of plastic, we need to design and use natural or artificial biocatalysts that can break down plastic on an industrial scale. Biotechnological approaches will help reduce plastic pollution, which is the biggest global issue of this era.

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# Appendix A. Supplementary data

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