

Microbial biodegradation of plastics: Challenges, opportunities, and a critical perspective

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HIGHLIGHTS

- Health hazards of plastic waste on environment are discussed.
- Microbial species involved in biodegradation of plastics are being reviewed.
- Enzymatic biodegradation mechanism of plastics is outlined.
- Analytical techniques to evaluate the plastic biodegradation are presented.

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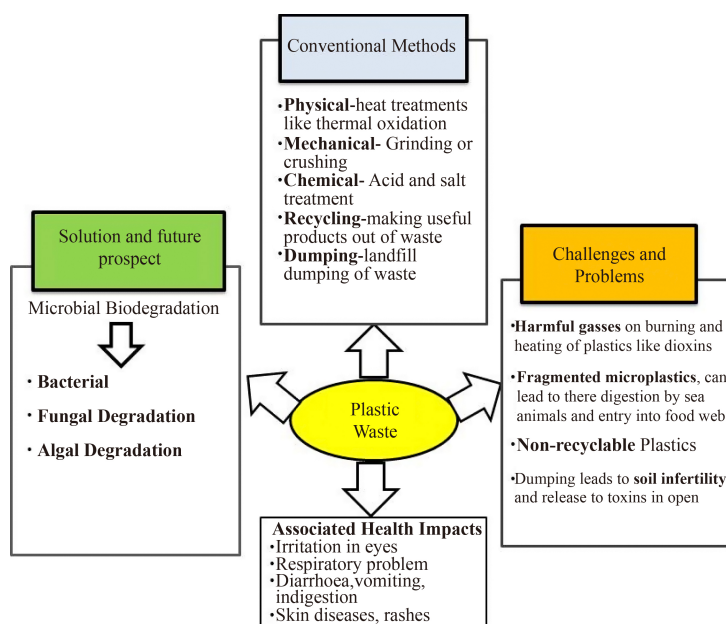
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ABSTRACT

The abundance of synthetic polymers has increased due to their uncontrolled utilization and disposal in the environment. The recalcitrant nature of plastics leads to accumulation and saturation in the environment, which is a matter of great concern. An exponential rise has been reported in plastic pollution during the corona pandemic because of PPE kits, gloves, and face masks made up of single-use plastics. The physicochemical methods have been employed to degrade synthetic polymers, but these methods have limited efficiency and cause the release of hazardous metabolites or by-products in the environment. Microbial species, isolated from landfills and dumpsites, have utilized plastics as the sole source of carbon, energy, and biomass production. The involvement of microbial strains in plastic degradation is evident as a substantial amount of mineralization has been observed. However, the complete removal of plastic could not be achieved, but it is still effective compared to the pre-existing traditional methods. Therefore, microbial species and the enzymes involved in plastic waste degradation could be utilized as eco-friendly alternatives. Thus, microbial biodegradation approaches have a profound scope to cope with the plastic waste problem in a cost-effective and environmental-friendly manner. Further, microbial degradation can be optimized and combined with physicochemical methods to achieve substantial results. This review summarizes the different microbial species, their genes, biochemical pathways, and enzymes involved in plastic biodegradation.

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1 Introduction

Plastics are high molecular weight (HMW) polymers primarily synthesized from hydrocarbons and petroleum derivatives (Zheng et al., 2005). HMW polymers such as polycaprolactone (PCL), polyethylene (PE), polyurethane (PUR), polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polybutylene succinate (PBS), and polylactic acid (PLA) are produced for varied purposes and uses (Andrady and Neal, 2009). The utilities and demand for plastic have increased exponentially in the last few decades. An estimated 367 million metric tons of plastic is produced annually compared to only 1.5 million tons in 1950 (Tiseo, 2021). However, during the COVID 19 pandemic in 2020, the plastic production decreased by 0.3% as compared to the previous years (Prata et al., 2020). Recently, it has been reported that cumulative plastic production has crossed 8 billion metric tons worldwide by 2018 and is expected to reach 34 billion metric tons by 2050 (Lebreton and Andrady, 2019).

The plastics dominance has surpassed the other materials as these polymers are highly durable, flexible, light-weight, and inert structures. There is an urgent requirement to limit the production and use of plastic, especially single-use plastic, because such materials remain in the environment for centuries after a momentarily use. Various studies have already reported that the packaging industries produce approximately 20000 water bottles per second, and merely 7% of bottles are recycled every year (Laville and Taylor, 2017).

Consequently, a considerable amount of plastic, approximately 8 million tonnes, is piling up in the oceans every year which is equivalent to a garbage truck every minute and is further expected to reach up to four by the middle of this century (Jambeck et al., 2015; van Sebille et al., 2015; MacArthur et al., 2016; Gooljar, 2018).

Plastic pollution is categorized into three categories such as macroplastics (e.g., plastic chairs, parts of vehicles, and plastic shopping bags), mesoplastics (e.g., fishing nets), and microplastics (e.g., plastic beads, pellets, and fragmented plastic particles). The most commonly found plastic waste is single-use plastic products such as bottles, plastic bags, packaging, and cutlery items (Liu et al., 2021). The major source of plastic bags in the environment has been identified as households/industrial waste, landfills, and human activities (Zhao et al., 2022). A minimal amount of single used plastic is recycled (9%), incinerated (12%), and the remaining (79%) ends up in the environment as it is considered to be unfit for further recycling (Ahmed et al., 2018).

A massive amount of plastic waste is buried under the soil, which ultimately disrupts soil fertility, destroys the marine ecosystem, and affects human health (Jambeck et al., 2015; Kumari et al., 2019). Due to their extensive

usage, industries are continuously generating different plastic products and owing to their resistant nature, plastics stay in the environment for a prolonged duration (Geyer et al., 2017). Over 99% of plastic is made from chemicals derived from fossil fuels, and therefore both industries are deeply connected. If the current consumption trend continues, the polymer industry might consume up to 20% of fossil fuels by 2050 (Godfrey, 2019; CIEL2020).

The plastic waste problem is commonly associated with marine pollution and is considered a global crisis. More than 80% of ocean plastic waste originates from land-based sources, while the remaining 20% originates from marine-based ships, oil spills, offshore oil, and gas platforms (Guern, 2019). Developing countries are always considered high waste generators. As far as plastic waste is concerned, the most significant contributors are the USA, China, India, Indonesia, Philippines, Vietnam, and Sri Lanka (Tiseo, 2021). The cumulative plastic waste is ~1–1.5 million tons annually which is contributing around 56% to global plastic waste (Ritchie and Roser, 2018).

The traditional physicochemical methods such as landfilling and incineration are not considered eco-friendly. These methods result in the leaching of hazardous waste and toxic gasses into the environment, causing significant ecological issues. Moreover, plastics are treated with harsh chemicals in the recycling process, which deteriorates the plastics and sometimes makes them more harmful to the environment (Hadad et al., 2005; Gautam et al., 2007; Okan et al., 2019).

A sustainable waste management model needs to be developed to manage the continuous accumulation of plastic waste around the globe, as landfilling and incineration are not viable options (Daftardar et al., 2017). Several initiatives, including the International Ocean Clean-up Project 2017, have been taken up to manage the plastic waste problem but such attempts are quite expensive and require active monitoring (Belhouari et al., 2017). In contrast to traditional approaches, the utilization of microorganisms found to be efficient to degrade recalcitrant, mutagenic, or xenobiotic compounds like polyaromatic hydrocarbons, phenols, pesticides, azo dyes, etc. (Christian et al., 2005; Meena et al., 2016; Narwal and Gupta, 2017). Similarly, other reports also have shown the microbiome's ability to degrade plastics in an economical and environment-friendly manner. The microbial degradation depolymerizes the plastics waste into monomers and mineralizes them into carbon dioxide, water, and new biomass (Shah et al., 2008; Krueger et al., 2015). Though multiple microorganisms have been characterized to understand the genes and the enzymes involved in biochemical pathways however the complete degradation process is still needed to explore further. Therefore, a comprehensive understanding of the

biodegradation of plastics is required, and this review summarizes the recent advancements in microbial degradation of plastic.

2 Classification of plastic, their properties, and applications

In the early 20th century, the first synthetic plastic i.e., Bakelite came into existence thereafter, plastic materials gained popularity and dominated products in the market since then (Brydson, 1999). Plastic materials can be categorized based on the source of their existence, physical properties, and synthesis. Plastics are classified as biodegradable and non-biodegradable. Biodegradable plastics are “substances that can be broken down within a fixed period by microorganisms in the environment” (Flieger et al., 2003). These biodegradable and decomposable plastics are made of biotic resources (i.e., plants, microbes, natural reservoirs) and petroleum-based plastics, which could be degraded through different biological means. To eliminate plastic waste, multi-national companies and institutions adopt eco-friendly practices under which biodegradable packing materials originate from plant waste such as sugarcane, cassava, and corn are used (Kjeldsen et al., 2019). While non-biodegradable plastics are typically based on carbon elements and oil-based raw materials which consist of small monomeric units combined through polymerization (Steinbüchel, 2005).

Further, plastics are commonly classified as thermoplastics and thermosetting plastics based on their physical properties. The plastic materials that can be modified and softened at respective temperatures and their chemical composition remains intact are thermoplastics such as polystyrene (PS) and polyethylene (PE). However, this

material can not regain its original shape when it cools down (Saunders, 1972; Mohammadi Nafchi et al., 2013). Thermosetting plastics include polyester resins, polyethylene terephthalate (PET), and melamine formaldehyde. These plastics can be molded into any shape by heating but can not be softened and reformed again therefore it remains permanently in that shape. The application and properties of commercially used plastics are summarized in Table 1.

Furthermore, a wide variety of additives are formulary incorporated into the plastic to improve the polymer's performance, functionality, and aging properties. Some known plastic additives are used as functional elements (e.g., stabilizers, flame retardants, plasticizers, etc.), colorants (e.g., pigments, azo dyes, etc.), filler (talc, calcium carbonate, etc.), and reinforcements (e.g., glass carbon fibres, etc.) in plastic industries (Zheng et al., 2005; Hahladakis et al., 2018). These additives not just improve the durability of the plastic but also protect the plastic from shear stress as well as microbial attack. Past studies have revealed that the plastic waste disposed of in an open environment can potentially release toxic additives, which are engineered to be highly stable and difficult to break down (Hahladakis et al., 2018).

3 Hazardous effect of plastic waste on the ecosystem

The uncontrolled exploitation of natural resources by humans has led to major disturbances in the ecosystem (Amobonye et al., 2021). The excessive usage and unmanaged disposal of plastic materials have become a significant concern (Narancic and O'Connor, 2017). With the recent amendment of the Basel Convention, plastic

Table 1 Commercially available plastics and their applications

Plastics	Chemical formula	Properties	Applications	References
PET (Polyethylene terephthalate)	(C ₁₀ H ₈ O ₄) _n	<ul style="list-style-type: none"> • Thermoplastics • Resistance to aging • Barrier properties against gas and moisture • Lightweight 	Used as an electronic component, as fibres in clothes, and in manufacturing drinking water bottles	Hui, 2006; Jankauskaite et al., 2008
PE, HDPE (High-density polyethylene), LDPE (Low-density polyethylene)	(C ₂ H ₄) _n	<ul style="list-style-type: none"> • Thermoplastics • Good weathering resistance • Water repellent 	Used as polyethylene bags, Milk carton bag lining	Hart et al., 2011
PVC (Polyvinyl chloride)	(C ₂ H ₃ Cl) _n	<ul style="list-style-type: none"> • Thermoplastic • Fire retarding properties • Resistance against acids, alkali, and inorganic chemicals • Easily bendable with other plastics 	Used in the health care sector, automobiles, building constructions, and electronics	Davis et al., 1983; Titow, 2012; Chanda, 2017
PP (Polypropylene)	(C ₃ H ₆) _n	<ul style="list-style-type: none"> • Thermoplastic • High stiffness and low density • Heat resistance and transparency 	Used in making syringes, Petri plates, and disposable cups and plates	Barbeş et al., 2014; Rocha-Santos and Duarte, 2015
PS (Polystyrene)	(C ₈ H ₈) _n	<ul style="list-style-type: none"> • Thermoplastic • Impact resistance and toughness • Poor barrier against water and oxygen • Crystal-like appearance if unfilled 	Used in thermal insulations, plastic cutlery, license plate frames, and plastic model assembly kits.	Lithner et al., 2011; Rochman et al., 2013

wastes are now listed as toxic waste and restricted the transboundary movement of any such materials (Peiry, 2019). Critical research and analysis are required to evaluate plastic under the toxic waste agenda (Newman, 2021).

Undeniably, plastic-based waste accumulation in the environment has become a pressing issue of the 21st century. Most plastic waste ends up in the natural environment, which affects the land through its excessive accumulation in landfill sites (Payne et al., 2019). Plastic waste can exist in the environment for hundreds of years as its degradation rate is prolonged, for example, an ordinary PET bottle and plastic straws take approximately 200–400 years for complete degradation in the environment (Scott et al., 2020). In recent years, microplastics (particles less than 5 mm in size) have gained much attention as they are causing damage to the ecosystem (Huerta Lwanga et al., 2017). The microplastic fragments accumulate in water bodies and enter the terrestrial food webs (Miloloža et al., 2021). The excessive contamination of plastic waste in the soil impacts the soil-water interaction as it increases the water requirements of the soil (Leslie et al., 2022). The presence of plastic contaminants in the soil alters the microbiome composition and their functioning which causes the enrichment of certain taxonomic members in the soil. Furthermore, the fragmented plastic monomers leach out into the ground, resulting in elevated concentrations of toxins and causing significant shifts in microbial community and activity of enzymes in soil (Lear et al., 2021). The fragmented plastic particles in the environment can also cause serious health hazards like respiratory sickness, cancer, and skin allergies (Derraik, 2002). Forte et al. (2016) have reported the effects of PS nanoparticles on the cell viability, inflammatory gene expression, and cell morphology of human gastric adenocarcinoma epithelial cells. A study was conducted to evaluate the transfer of LDPE plastic pollution through terrestrial food webs by investigating the microplastic pollution in home gardens. Further examined the egestion of plastic by earthworms and chickens (*Gallus gallus domesticus*), along with the concentration of plastic in the chicken corps, gizzards, and the gizzards prepared for human consumption. The study revealed that the concentration of microplastic increased from soil (0.8%) to earthworm cast (14.8%) to chicken feces (129.8%) (Huerta Lwanga et al., 2017).

The other major issue that makes plastic waste more toxic is additives in the plastic synthesis process. Plastic additives, such as plasticizers and polybrominated biphenyls, are highly carcinogenic and cause organ disruption. Research-based evidence shows that phthalates, bisphenol A (BPA), and other additives in plastic are toxic and carcinogenic. Traces of these toxic elements were found in human clinical samples (Rudel et al., 2008; Talsness et al., 2009). A recent study discovered the quantification method for the analysis of plastic particles

in human blood samples. The study was conducted with a small set of donors, the quantifiable concentration of plastic particles in the human bloodstream was found to be 1.6 µg/mL (Leslie et al., 2022). The toxic monomers of polystyrene polymer may lead to cancer, and reproductive defects in humans, rats, and invertebrates (Wang et al., 2016). The study reported that the long-term exposure of plastics to UV radiation due to weathering conditions could increase the leaching of hazardous substances such as BPA (bisphenol A), phthalates, etc. (Koelmans et al., 2014). The leaching of BPA was experimentally proven through laboratory-scale studies by placing the untreated and treated granulated polycarbonate plastics (0.8–2.0 mm) in seawater for 60 d. The results showed that the treated plastics leached 72 mg/100 mL of BPA, having a 600 ng/L concentration in the seawater compared to untreated plastic showing less than 20 ng/L leached BPA concentrations (Koelmans et al., 2014; Hahladakis et al., 2018).

During the corona pandemic, unprecedented demand for PPE kits and single-use medical equipment has resulted in the massive generation of plastic biomedical waste. Most medical equipment comprises polyvinyl chloride (PVC) which is a significant source of hazardous chlorine emissions from incineration units (Silva et al., 2018; Thind et al., 2021). Additionally, the uncontrolled disposal of polypropylene and polyethylene masks had led to microplastic accumulation in the water bodies (Peng et al., 2021). As per the reports of the European Commission (EU) regulation, PVC materials contain vinyl-chloride monomers, which possess acute toxicity to the human body, and are considered carcinogenic. Hence, it was stated that plastic materials contacting the food items must not contain vinyl-chloride monomers exceeding 1 mg/kg (Lithner et al., 2011; Hahladakis et al., 2018).

Plastic waste has adversely affected the aquatic environments, as the major portion of plastic pollution ends up in rivers and, ultimately, oceans (Rajmohan et al., 2019). As per records, 170 marine species have been reported to ingest polyethylene waste, causing severe ailments like gut rupture and impactions, exchange of lethal mixes, and lessened nourishments (Taylor et al., 2016). Certain incidents have been reported to embed plastic materials in the gastrointestinal tract of annelid and lugworms (Prinz and Korez, 2020). Sea turtles are also being reported to consume plastic bags and consequentially suffer from an obstruction in the oesophagus, leading to these creatures' death (Nelms et al., 2016). As seafood is one of the popular food sources in coastal areas, these microplastic fabrics get ingested by the fish and indirectly incorporated into the food chain. Moreover, the seabirds are also getting affected. They end up eating floating plastic debris in the sea. This ingested plastic discharges plasticizers in the tissues of birds and leads to impairment disorder, immune disorder, and reproductive inability (Alimba and Faggio, 2019).

4 Abiotic factors affecting plastic degradation

The degradation of plastic materials is commonly categorized based on any deleterious change in its physical, mechanical, chemical, or structural properties. Plastic degradation is a complex process, as it can not be measured and controlled easily, especially under open environmental conditions (Law and Narayan, 2021). Regardless of environmental conditions, the polymer degradation rate depends on the composition of polymeric substances (Kyrikou and Briassoulis, 2007). Extreme environmental conditions such as weathering, sunlight, aging, water, and soil burial can accelerate plastic degradation. Plastics subsequently undergo thermal, mechanical, chemical, and photo-degradation, altogether acting synergistically to enhance plastic degradation rate (Fig. 1). Temperature also plays a significant role in the plastic degradation process (Al-Salem et al., 2019). The thermal properties of different plastic materials such as glass transition temperature, crystalline/amorphous ratio, and melting temperature affect the rate of plastic degradation. Likewise, plastic waste in landfills and industrial composters experiences prolonged sunlight exposure resulting in reaching a higher temperature than the surrounding air and causing “heat build-up” (Chamas et al., 2020). The temperature in some landfills and composters has been reported to reach 80–100 °C, which accelerates the degradation rates, provided sufficient moisture and oxygen is present for thermal oxidation degradation and hydrolysis (Briassoulis, 2004, 2006). Ding et al. (2020) performed a study to evaluate the effect of high temperature (75 °C) on the aging property of PS

microplastic particles. The study reported that the temperature affects the degradation of microplastics in three different environmental conditions (seawater, pure water, and air) after exposure for a certain number of days. The combinatorial effect of UV light and elevated temperature causes microstructure and surface morphology changes in the microplastics, resulting in efficient aging and degradation (Brandon et al., 2018).

Similarly, chemical degradation causes covalent bond breakage and produces free radicals depending upon the structure of the polymer. The degradation pattern of the polymer is greatly influenced by the presence of branched chains or unsaturated carbon bonds. Additionally, the amorphous phase in the polymer promotes the oxidative and hydrolytic degradation of the polymer (Khorasani-zadeh, 2013). The pH of the environment plays a crucial role in the plastic degradation process. Polymers such as PLA and PCL show a low rate of degradation in the neutral environment while basic and acidic conditions offer a high degradation activity. Hydrolysis of ester bond results in the release of low molecular weight particles from polymer structure, consequently leading to degradation of the polymer (da Luz et al., 2014).

The mechanical factors such as compression, shear stress, and tension accelerate the degradation process at the molecular level. The synergistic effect of the mechanical degradation factor works best in combination with chemical, thermal and other degradation methods (Siracusa, 2019).

Photo-degradation is also one of the well-known abiotic factors responsible for the degradation of plastics. It is performed by inducing light exposure to the polymer surface. It takes place through Norrish reactions, by

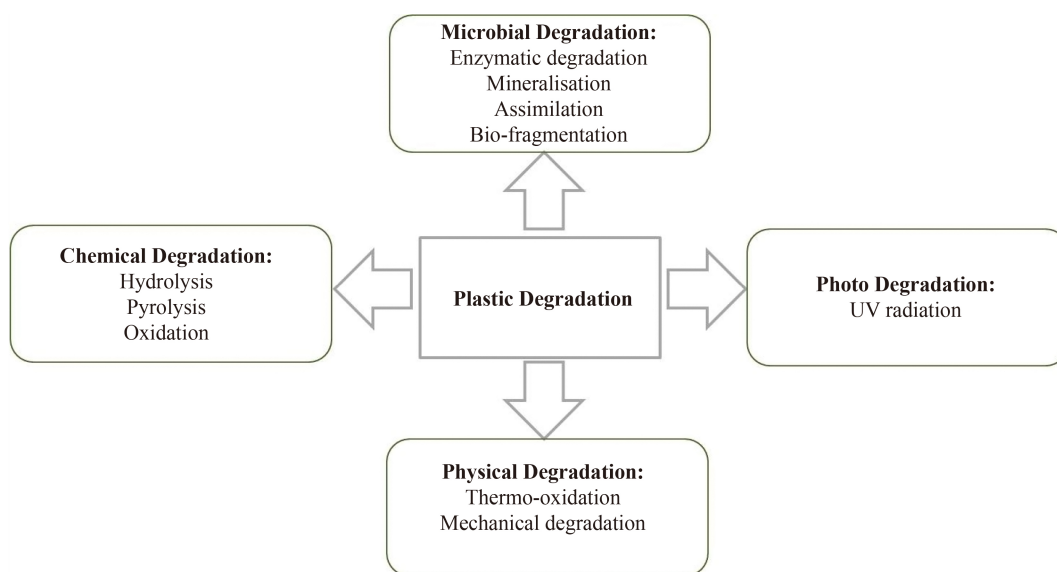


Fig. 1 Different modes of plastic waste degradation: 1) Physical degradation i.e. thermal oxidation and mechanical methods; 2) Chemical degradation i.e., hydrolysis, pyrolysis, and oxidation; 3) Photo-degradation i.e., weathering and UV radiation by the sunlight; 4) Microbial degradation i.e., enzymatic degradation, biochemical transformations, and assimilation.

photoionization (Norrish I) and chain scission (Norrish II) (Chamas et al., 2020).

5 Biodegradation

Biodegradation refers to the degradation of plastic with the help of enzymes or microbes in a specified period. It is a catabolic process that results in the degradation of plastic (Tschan et al., 2012). Biodegradation of plastic can be studied *in situ* or under laboratory conditions by screening and isolating plastic degrading microbes from the environmental reservoirs which are further quantified using analytical methods (Grover et al., 2015). Several studies have been conducted on plastic waste degradation, including municipal or landfill dumping soil samples for isolation and screening of plastic degrading microbes (Table 2). Plastic contains a carbon-carbon backbone structure, and microbes utilize plastic as a source of food, energy, and reproduction, resulting in biodegradation. Microorganisms catalyze energy-producing chemical reactions in which breakage of chemical bonds occurs, and electrons get transferred from plastics, which are further taken up by the microbes. Aerobic microbes use carbon from plastics to produce carbon dioxide, water, and new cells. In contrast, an anaerobic microorganism uses carbon to produce methane, hydrogen sulphite, nitrogen gas, and biomass (Mohan et al., 2020). Different studies have reported the use of non-carbonaceous media such as Bushnell Hass broth, minimum salt medium, and other synthetically designed media for the isolation and characterization of specific microbes (Zahra et al., 2010; Meena et al., 2016; Park and Kim, 2019).

Biodegradation seems to be a promising solution to tackle the plastic waste issue but requires a thorough understanding of the efficient microorganisms, gene clusters, pathways, and enzymes involved in the process (Abraham et al., 2017; Wilkes and Aristilde, 2017; Urbanek et al., 2020). The following sections discuss the studies carried out on the biodegradability of plastic waste in controlled environments.

6 Relevance of microbes in plastic degradation

The microorganisms are known for their diverse environmental adaptability as they have distinct catabolic pathways which may help to degrade polymers based on their types and properties (Moharir and Kumar, 2019).

Numerous efficient plastic degrading microbial strains such as *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Streptomyces* sp., *Pseudomonas* sp., *Comamonas* sp., and *Ideonella sakaiensis* sp. have been reported earlier (Kyaw et al., 2012; Sangeetha Devi et al., 2015; Skariyachan et al., 2016; Amobonye et al., 2021; Priya et al., 2022).

These microbial strains were mostly isolated from the plastic contaminated landfill sites and were found as prominent plastic degraders (Fig. 2). Notably, these studies are primarily focused on the biodegradation of low density and high-density polyethylene materials (Bhatia et al., 2014; Sangeetha Devi et al., 2019; Bardaji et al., 2019; Kumari et al., 2019).

Kumari et al. (2019) have reported degradation of PVC (polyvinylchloride), LDPE, and HDPE through *Bacillus* sp. isolated from the marine ecosystem. Similarly, Park and Kim (2019) demonstrated the degradation of micro-sized polyethylene particles with the help of bacterial isolates from the municipal landfill site. Further investigation revealed the dominance of *Bacillus* and *Paenibacillus* sp. among other plastic degrading isolates. Besides that, other bacterial species, i.e., *Actinomadura*, *Streptomyces*, *Laceyella*, *Brevibacillus* sp., and *Aneurinibacillus* sp. are also well-established plastic degraders, reportedly (Hadad et al., 2005; Skariyachan et al., 2018; Sriyapai et al., 2018). Similarly, *Bacillus cereus* isolated from a dumping site soil sample was documented as polyethylene degrader (Sowmya, 2014). Tribedi et al. (2012) isolated *Pseudomonas* sp. which could efficiently degrade LDPE plastics. Further, the addition of mineral oils could lead to higher microbial activities due to biofilm formation on the surface of LDPE.

Microbulbifer hydrolyticus, a bacterial strain isolated from marine pulp mill wastes, could degrade LDPE after 30 d of incubation. The degradation was shown by observing morphological changes on the PE surface using scanning electron microscopy and was further confirmed by the appearance of additional carbonyl groups through Fourier transform infrared spectroscopy (Li et al., 2020).

Biological treatment of polyethylene sheets with *Anabaena spiroides* (blue-green algae), *Navicula pupula* (diatom), and *Scenedesmus dimorphus* (Green microalga) has been studied and *Anabaena spiroides* found to be the most efficient plastic degrader (Kumar et al., 2017).

Ascomycetes (*Xylaria* sp.) have also shown low-density polyethylene degrading properties (Thilagavathi et al., 2018). Esmaili et al. (2013) reported that the higher degradation rate for the UV-irradiated LDPE film as compared to non-UV-irradiated in the soil environment by *Aspergillus* sp. and *Lysinibacillus* sp. Other researchers have also identified the plastic degrading capabilities of *Aspergillus terreus* and *Aspergillus fumigatus* species (Zahra et al., 2010).

Actinomycetes and *Streptomyces* species have reportedly shown degrading abilities for polycaprolactone (PCL), polylactic acid (PLA), and poly (butylene succinate) (PBS) (Penkhrue et al., 2015). PLA-degrading bacterial isolates, i.e., *Stenotrophomonas pavanii* CH1 and *Pseudomonas geniculata* WS3, were isolated from the sanitary landfill site and wastewater sludge, respectively (Bubpach et al., 2018). Likewise, *Trichoderma virens*, *Paecilomyces variotii*, *Chaetomium globosum*,

Table 2 Plastic degrading microbial strains with their optimization conditions

Polymer targeted	Media	Microbe isolated	Sample source	Analytical technique(s)	% degradation and incubation time	References
LDPE	Minimal salt medium	<i>Enterobacter</i> and <i>Pseudomonas</i>	Plastic dumping landfill, Karnataka, India	Hydrophobicity analysis, SEM, FTIR, and AFM	Observed 12.5%, 15%, 15%, 10%, 10% and 15% weight loss of LDPE post 150 d incubation by <i>Enterobacter</i> and <i>Pseudomonas</i> isolates	Skariyachan et al., 2021
LDPE beads and LDPE films	Minimal salt medium	<i>Stenotrophomonas</i> sp. and <i>Achromobacter</i> sp.	Plastic waste dumpsite near IIT Kharagpur campus and drilling fluid site in Maharashtra, India	Atomic Force Microscope (AFM), Scanning Electron Microscope (SEM), and Fourier Transform Infrared Spectroscopy	Observed 8% weight reduction of LDPE beads in 100 d	Dey et al., 2020
LDPE, HDPE, and PVC	Bushnell–Haas minimal medium	<i>Bacillus</i> spp.	Water samples from a plastic polluted coastal area	SEM, AFM, and FTIR analysis	Observed 0.2%, 0.9%, and 1% weight loss after 90 d for PVC, LDPE, and HDPE films respectively	Kumari et al., 2019
HDPE and LDPE	Synthetic media (SM)	248 bacterial isolates dominantly from <i>Bacillus</i> spp. and <i>Pseudomonas</i> spp.	Plastic waste dumped sites	FTIR analysis	Observed a high percentage of weight loss among 25 isolates from different districts after 30 d of incubation	Sangeetha Devi et al., 2019
LDPE	Artificial modified media	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Brevibacillus</i> , <i>Cellulosimicrobium</i> , <i>Lysinibacillus</i> and fungi <i>Aspergillus</i>	Dumpsite samples	FTIR and GC-MS	Observed the mean weight reduction of 36.4% in <i>Aspergillus oryzae</i> strain A5 and 20.2% reduction in case of <i>Bacillus cereus</i> strain A5 culture in the incubation period of 8, 12, and 16 weeks	Ndahebwa Muhonja et al., 2018
LDPE, HDPE and PP	Minimal media	Consortia of thermophilic <i>Aneurinibacillus</i> spp. and <i>Brevibacillus</i> spp.	Highest plastic polluted eight spots across different districts of Karnataka, India	FTIR, EDS, AFM, NMR, and GC-MS	Observed the highest percentage weight reduction of 58.2%, 46.6%, and 56.3% for LDPE, HDPE, and PP, respectively, after 140 d	Skariyachan et al., 2018
LDPE	Minimal media broth	Consortia of bacteria having gram-negative bacilli <i>Proteus</i> sp., <i>Enterobacter</i> sp., <i>Pantoea</i> sp., and <i>Pseudomonas</i> sp.	Plastic processing garbage area soil samples	Weight loss determination LDPE pellets and strips, FTIR, SEM, and GC-FID analysis	81% and 38% of weight reduction in LDPE strips and pellets for 120 d	Skariyachan et al., 2016
LDPE	Minimal Salts medium	<i>Pseudomonas</i> sp., <i>Sphingobacterium</i> sp., <i>Stenotrophomonas</i> sp., <i>Ochrobactrum</i> sp., <i>Citrobacter amalonaticus</i> , <i>Micrococcus luteus</i> , and <i>Acinetobacter pittii</i>	Landfill soil	FTIR, SEM, and gravimetric weight loss analysis	26.8% gravimetric weight loss of polyethylene films over 4 weeks	Montazer et al., 2018
LDPE and corn starch	Czapek–Dox agar Nutrient and potato agar	<i>Bacillus</i> , <i>Clostridium</i> , <i>Micrococcus</i> , <i>Aspergillus</i> , <i>Penicillium</i> , and <i>Mucor</i>	Soil burial at 5 cm or 15–30 cm depth	Electret-thermal analysis (ETA) and Thermally stimulated currents (TSC) spectra	10%–15% of degradation after 1.5 months	Pinchuk et al., 2004
Food packaging grade virgin LDPE film	Malt Extract Agar	<i>Chaetomium globosum</i> , <i>Corynascussepdonium</i> , <i>Trichoderma longibrachiatum</i> , <i>Fusarium</i> sp., <i>Paecilomyces variotii</i> , and <i>Aspergillus niger</i>	Horse manure, fresh grass waste, partially rotted plants material, and straw	SEM, FTIR, and tensiometry analysis	Microbial colonization at 30 °C for 2 weeks and 28 d in corona discharge treatment	Matsunaga and Whitney, 2000
LDPE	Basal mineral solution	<i>Phanerochaete chrysosporium</i>	Soil sample	FTIR analysis	56% reduction in elongation in the inoculated sample while 12% in uninoculated soil for 6 months	Orhan and Büyükgüngör, 2000
LDPE	Mineral salt medium	<i>Xylaria</i> sp.	Fungal garden of termite ecosystem	Zone of clearance analysis	Heat treatment for 20 d, UV treatment for 1 to 2 h, and chemical treatment for 10 d. Agar plates were incubated for 2–4 weeks	Thilagavathi et al., 2018

(Continued)

Polymer targeted	Media	Microbe isolated	Sample source	Analytical technique(s)	% degradation and incubation time	References
1) Nano additives containing plastic bags, 2) Oxo-biodegradable plastic bags, 3) LLDPE and HDPE, 4) Plastic bags with additives	Medium containing Peptone, Glucose, Sodium chloride, and Meat extract	<i>Bacillus</i> sp.	Compost agricultural residue	Weight loss, structure, surface morphology, FTIR, and tensile strength analysis	Observed 60.7%, 11%, and 4.4% of weight loss in three different types of plastics within 30 d	Dang et al., 2018
PE microplastics	Basal medium	<i>Paenibacillus</i> sp. and <i>Bacillus</i> sp.	Municipal landfill sediment	GC-MS, dry weight, TGA, and SEM analysis	Observed 14.7% dry weight reduction in PE and 22.4% mean diameter reduction after 60 d	Park and Kim, 2019
PE mulch film	Czapek Dox medium and liquid carbon-free basal medium	<i>Arthrobacter</i> sp. and <i>Streptomyces</i> sp.	Soil plastic from Gansu province, China	CO ₂ evolution, FTIR	Observed decreased hydrophobicity, and increased carbonyl index in 90 d incubation time	Han et al., 2020
PE	Marine broth, Nutrient Broth, Czapek-Dox Broth, and R2A Broth	<i>Comamonas</i> , <i>Delftia</i> , and <i>Stenotrophomonas</i>	Soil sample from plastic debris site	ATR/FTIR, AFM, SEM, and Raman spectroscopy	Crystalline content loss was confirmed by Raman spectroscopy, while a 46.7% decrease in viscose area revealed by phase imaging	Peixoto et al., 2017
PE	Nutrient medium, Mineral Salt Medium	<i>Bacillus subtilis</i>	—	Gravimetric, weight loss, and FTIR analysis	Observed 9.2% weight loss in 30 d	Vimala and Mathew, 2016
PE	Nutrient broth containing Cow dung (500 g) + paper cup waste (500 g) + microbial consortium	Microbial consortia such as different <i>Bacillus</i> spp. and <i>Acinetobacter baumannii</i>	Waste like plastic paper cups	FTIR, X-ray diffraction, and SEM analysis	Observed 52.9%–33.1% reduction in total organic matter (TOM) after 90 d of incubation	Arumugam et al., 2018
PE	Minimal media containing potassium and ammonium salts	<i>Pseudomonas</i> sp., <i>Staphylococcus</i> sp., and <i>Bacillus</i> sp.	Soil samples	Determination of weight Loss	42.5%, 20%, and 5% of weight loss by <i>Staphylococcus</i> sp. (P1A), <i>Pseudomonas</i> sp. (P1B), and by a consortium (PID) in 40 d.	Singh et al., 2016
PE	Mineral Salt medium	<i>Bacillus cereus</i>	Local dumpsite	SEM and FTIR spectroscopy analysis	Observed 7.2% weight loss of autoclaved polyethylene in 3 months	Sowmya, 2014
PE bag and plastic cup	Mineral salt agar plates	<i>Streptomyces</i> sp., <i>Bacillus</i> sp.	Garbage soil	Determination of weight Loss	28.4% of plastics and 37% of polythene degraded	Usha et al., 2011
PE	Nutrient broth medium	<i>Pseudomonas</i> sp.	1) Domestic waste disposal site 2) Soil from textile effluents drainage site; and 3) Soil dumped with sewage sludge	Dry weight estimation	46.2% and 29.1% weight reduction in natural and synthetic polyethylene, respectively, in 8 weeks	Nanda et al., 2010
PE	Minimal media and soil mulching	<i>Rhodococcus ruber</i>	Soil samples from 15 plastic dumping sites	SEM and FTIR analysis	Observed 8% degradation (gravimetrically) in polyolefin in 30 d	Orr et al., 2004
PE	Nitrogen-free mineral salts, malt extract, and yeast extract	<i>Streptomyces</i> strain, <i>Mucorrouxii</i> , and <i>Aspergillus flavus</i>	—	Tensile strength, percent elongation, and FTIR Spectrometry	Observed 28.5% and 46.5% reduction elongation by <i>Streptomyces</i> and fungal culture	El-Shafei et al., 1998
PE	Bold's Basal Medium and Diatom medium	<i>Diatom</i> medium, <i>Anabaena spiroides</i> , and <i>Navicula pupula</i>	Photosynthetic microalgae samples from freshwater bodies like pools, ponds, and ditches	Scanning electron microscopic (SEM) analysis	An average of 3.7%, 8.1%, and 4.4% degradation was reported by <i>Scenedesmus dimorphus</i> , <i>Anabaena spiroides</i> , and <i>Diatom</i> , respectively	Kumar et al., 2017

(Continued)

Polymer targeted	Media	Microbe isolated	Sample source	Analytical technique(s)	% degradation and incubation time	References
PET	Mineral medium	<i>Streptomyces</i> sp.	–	GC-MS analysis	49.2%, 57.4%, 62.4%, and 68.8% reduction in weight of 500, 420, 300 and 212 μm size PET particles, respectively, after 18 d	Farzi et al., 2019
PET	Minimal salt medium	<i>Acinetobacter baumannii</i>	Soil and plastic waste sample	Percentage weight loss, FTIR, and SEM analysis	Observed 27.3% weight reduction in 90 d of incubation time	Hussein et al., 2018
PET	Yeast extract, sodium carbonate, and Vitamins medium	<i>Ideonella sakaiensis</i>	PET contaminated dumpsites	SEM analysis	75% degradation of PET films	Yoshida et al., 2016
PET	Yeast extract sodium carbonate vitamins (YSV)	<i>Ideonella sakaiensis</i>	PET-bottle recycling site in	–	–	Tanasupawat et al., 2016
Poly (butylene adipate- co - terephthalate) (PBAT)	Murashige and Skoog medium	<i>Stenotrophomonas</i> sp.	Soil sample from apple farms in Yanxia Town, Shaanxi Province, China	LC-MS, NMR, XRD, ATR-FTIR, and SEM	Observed stretching vibrations at 2964 cm^{-1} and 2871 cm^{-1} , respectively, and the C = O stretching vibration was found at 1715 cm^{-1}	Jia et al., 2021
Polybutylene succinate-co-adipate (PBSA), Poly-(butylene succinate) (PBS), Polylactic acid (PLA), and PCL	Basal medium	<i>Actinomadura</i> , <i>Streptomyces</i> , and <i>Laceyella</i>	Compost soil	Morphological, physiological, and chemotaxonomic analysis	Observed 1% (w/v) PBS (19.4 U/mL), 0.5% (w/v) PLA (22.3 U/mL), 1% (w/v) PCL (18 U/mL), and 0.5% (w/v) PBSA (6.3 U/mL) polyester degrading activity	Sriyapai et al., 2018
Poly (L-Lactide)	Basal liquid medium	<i>Actinomycetes</i> strain	Soil samples	Clear zone method	PLA-degrading activity at 22 U/mL, while 15 and 10 U/mL of activity by other strains	Sukhumaporn et al., 2011
Poly (L-Lactide)	PLA agar plates and Basal medium	<i>Actinomadura</i> sp. and <i>Bacilli</i> sp.	Soil surface layer samples	Clear zone method	22 U/mL highest PLA-degrading activity by <i>Actinomycetes</i> strain	Sukkhum et al., 2009
PCL and PVC films	Agar into nutrient-salt solution	<i>Chaetomium globosum</i> , <i>Penicillium funiculosum</i> , <i>Aspergillus brasiliensis</i> , <i>Paecilomyces variotii</i> , and <i>Trichoderma virens</i>	–	Colour and morphological changes, mass loss, SEM and optical microscopy (OM)	Observed the mass loss of up to 75% after 28 d of incubation	Vivi et al., 2019
PVC	Anaerobic specific medium containing sodium chloride	Anaerobic consortia, i.e., acidogenic/methanogenic, nitrate-reducing, and sulfate-reducing microorganisms.	Marine samples from Elefsis Bay, Greece	Thermogravimetric analysis (TGA), Gel permeation chromatography (GPC)	Observed gravimetric weight losses up to 11.7%	Giacomucci et al., 2020
PUR	(a)SDA agar plate (b)Liquid Minimal salt medium (c) Soil burial	<i>Aspergillus tubingensis</i>	Waste disposal site, Islamabad, Pakistan	SEM, Attenuated total reflectance Fourier transform Infrared spectroscopic (ATR-FTIR) analysis	Observed higher PU degradation in plate culture than in liquid culture and soil burial technique	Khan et al., 2017
PUR	Lysogeny broth-Miller (LB) or minimal medium	<i>Pseudomonas protegens</i>	–	NMR spectroscopy	–	Hung et al., 2016

Penicillium funiculosum, and *Aspergillus brasiliensis* have also been reported as the potential PVC and PCL degraders (Vivi et al., 2019). The unique potential of *Pestalotiopsis microspora* was investigated, as the fungal strain has shown the ability to utilize PUR as the sole carbon source under anaerobic and aerobic conditions (Russell et al., 2011). Similarly, the fungal species

belonging to genera *Gliocladium*, *Aspergillus*, *Trichoderma*, *Fusarium*, *Penicillium*, and *Emericella* were also found to be potential polyurethane degraders (Bhardwaj et al., 2013).

The degradation of PET is comparatively difficult because of its complex nature. Multiple microorganisms have been used to degrade PET in laboratory conditions.

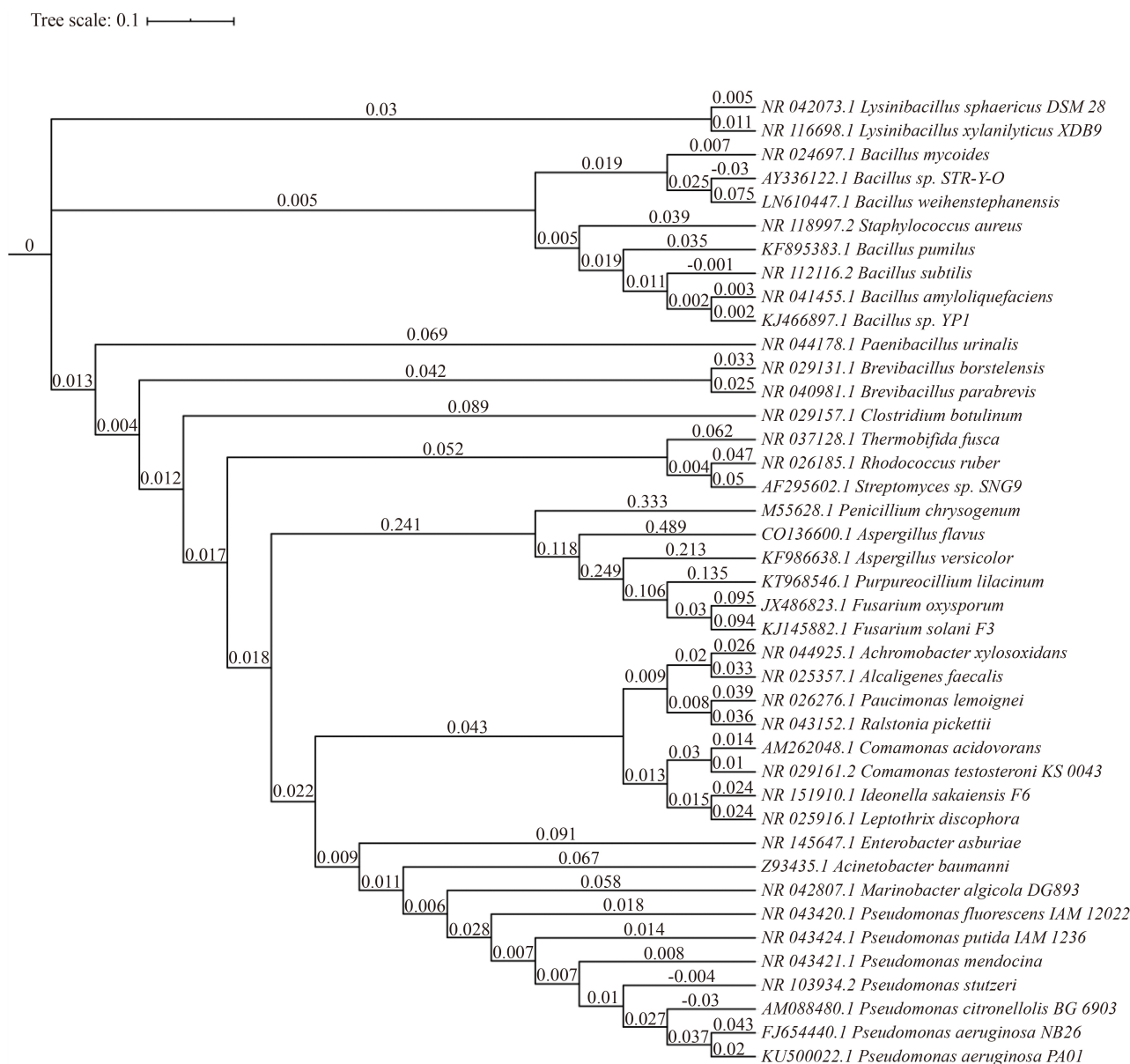


Fig. 2 The diversity analysis of plastic degrading microbial strain was studied through phylogenetic tree construction. The tree was generated using the neighbour-joining method. The nodes are supported with their appropriate bootstrap values.

Yoshida et al. (2016) have isolated a novel bacteria *Ideonella sakaiensis* from PET debris which has shown effective results as a plastic degrader. While Austin et al. (2018) have isolated the PETase enzyme from the *Ideonella sakaiensis* and studied its role in PET degradation. Further, the structural analysis of PETase revealed the similarities to other plastic degrading enzymes such as cutinases and lipases.

Streptomyces species were also evaluated for PET degradation and crushed PET bottles with different particle sizes were used (Farzi et al., 2019). It has been reported that the surface area and reaction time plays a significant role in PET biodegradability. The small particle size is advantageous for reducing the biodegradation

assay time and eliminating the surface limiting effects to assess a material's intrinsic biodegradability (García-Depraect et al., 2022). Similarly, the study reported that the plastic pellets have a slower biodegradation rate than other samples i.e., plastic powder (Chinaglia et al., 2018).

The multi-Omic techniques i.e., metagenomics can be applied to understand the PET degradation by marine bacterial isolates. The study investigated the degradation potential of two novel marine isolates i.e., *Thioclava* sp. BHET2 and *Bacillus* sp. BHET2 and the presence of PET hydrolytic intermediates confirmed their degradation ability (Wright et al., 2021). The exploration of microbial communities through metagenomics could serve as a great tool for mining novel enzymes and genes involved

in plastics degradation processes (Shilpa et al., 2022).

Recent studies have reported the role of the gut microbiome in polymer degradation. Gut microbiota consists of trillions of microbial species that harbour living organisms' intestinal tracts. The gut microbiota has the capabilities to adapt according to different foods (Srivastava et al., 2021; Lindell et al., 2022). Mealworms (*Tenebrio molitor* Linnaeus) can ingest and biodegrade the polyester (PS) to CO₂ and low molecular weight compounds within the gut. *Exuglobacterium* sp. has been isolated from the gut microbiota of mealworms and was found to be an efficient PS degrader as it could degrade 7.4% PS after 60 d of incubation (Yang et al., 2018). However, the study concluded that the isolated strain works poorer outside the living host than inside the mealworm's gut. The mealworm gut act as an efficient bioreactor for PS degradation (Banerjee et al., 2022). Similarly, the yellow mealworms were also being tested for their PS degradation ability and have shown degrade half of the supplemented PS within 12–15 h inside the gut. Further, FTIR results revealed the chemical modifications in the structure of plastics egested by the mealworms (Brandon et al., 2018). A recent report has shown the potential of waxworms in utilizing polyethylene as the sole diet source (Cassone et al., 2020). Different worms have great plastic degrading efficiency because the gut microbes of waxworms work synergistically to break down PE and excrete glycol. Cassone et al. (2020) have identified 30 waxworms which could eat up to 30 cm² of the plastic sheet within a week. Similarly, the Indian-meal moth, *Plodia interpunctella*, was identified as the potential candidate for the degradation of PE. The study reported *Bacillus* sp. and *Enterobacter asburiae* sp. as the two prominent species in the larvae gut, which might be responsible for the PE breakdown (Bombelli et al., 2017). The waxworms and mealworms are the potential candidates which can be beneficial in PE degradation because of their adaption to different ecological conditions. These insects feed on the beeswax, which is similar to the PE, as they consist of identical hydrocarbon bonding. The complete mechanism and pathways of PE degradation inside the gut of waxworms are not depicted in the literature, which further needs to be explored (Yang et al., 2014; Bombelli et al., 2017). In a recent study, the rumen content from the cattle (*Bos taurus*) was being explored to identify synthetic polyester degrading enzymes based on the fact that these ruminants feed on natural plant polyesters. The hydrolysis activity of rumen samples demonstrated the degradation activity of polyesters. Further, it was found that *Pseudomonas* sp. was the most dominant species in the rumen microbiome (Quartinello et al., 2021). But the further reinvestigation is required to find out other enzymes present in the rumen, which could work synergistically to degrade plastic materials on a large scale.

7 Molecular mechanism of plastic biodegradation

Biodegradation of plastic is described as any alteration and breakdown in their structure by microbial enzymes or microbial digestion which ultimately results in weight reduction, loss of mechanical strength, and change in surface properties. Microorganisms like bacteria and fungus can deteriorate the plastic by enzymatic and non-enzymatic hydrolysis (Amobonye et al., 2021). Such microbial degradation processes are tangible and healthy alternatives in maintaining the balance of the ecosystems. Microbes accomplish this process by different enzymatic actions and bond cleavage mechanisms. The most common way for plastics to biodegrade is by oxidation (Ghatge et al., 2020).

Microbial biodegradation is a multi-step process; firstly, in the depolymerization phase, microorganisms bind to the surface of plastic material and secrete degrading enzymes that convert complex polymers into their simpler forms (Bahl et al., 2021). Further, microbes utilize these fragmented polymer products from plastic degradation as a food and energy source. In the mineralization process, short fragments of plastic get degraded and form water, methane, and carbon dioxide as the end products (Vignesh et al., 2016; Chaurasia, 2020). Finally, the assimilation process begins to form secondary metabolites/byproducts by integrating the atoms inside the microbial cell (Tokiwa et al., 2009). The excreted secondary metabolites get further utilized by other microbes or stay in the pool as non-assimilable compounds. Fragmented molecules which are transferred across the cell membrane get oxidized through catabolic pathways for structural cell elements and energy storage purposes. Alternatively, fragmented plastic monomer undergoes the sequential degradation into a common metabolite of the TCA cycle and enters into central carbon metabolism; however, no clear evidence of microbial cell metabolism is available (Chinaglia et al., 2018).

Genetic and molecular-level analysis of genes involved in plastic degradation pathways can be a breakthrough in this field. Very few reports discuss the genes responsible for plastic degradation and the pathways followed. In the early '90s, esterase enzyme from *Comamonas acidovorans* was purified which could effectively degrade polyester polyurethane (PUR) under controlled conditions. Further, the structural gene, *pudA*, for the PUR esterase was cloned in *Escherichia coli* (*E. coli*), which enhanced the degradation activity of *Comamonas acidovorans* (Nomura et al., 1998). Similarly, a gene encoded for polyester hydrolase (*Pseudomonas aestusnigri*) was cloned in *E. coli* to enhance the PET degrading ability of microbe. This study gives insights into the structural characteristics required for polyester degradation (Bollinger et al., 2020). Likewise, the PET encoding

hydrolyses genes, cloned from *Thermobifida cellulolytica*, and *Thermobifida fusca* were expressed in *E. coli*, responsible for the efficiency of the cutinase enzyme (Acero et al., 2011). Sasoh et al. (2006) also identified two identical clusters of TPA degradation genes isolated from *Comamonas* sp.. As predicted, these genes were coded for TPA binding receptors and a large subunit of the oxygenase. This report could be a reference for degradation as TPA is a primary compound to produce polyethylene terephthalate. Chen et al. (2020) have developed whole-cell biocatalysts by displaying PETase on the surface of *Pichia pastoris* resulting in improved degradation efficiency with enhanced pH and thermal stability. The newly developed form of catalyst showed a high turnover rate under optimal conditions.

The role of different microorganisms such as bacteria, and fungi are evident in the plastic degradation processes. Besides this, microalgae have also been used to isolate plastic degrading enzymes. The photosynthetic *Phaeodactylum tricornutum* microalgae were used as a chassis to secrete an engineered PETase enzyme. This hybrid enzyme was capable to degrade PET and copolymer i.e., polyethylene terephthalate glycol (PETG) in the supernatant culture at mesophilic temperatures (21 °C). Further, two compounds i.e., (2-hydroxyethyl) terephthalic acid (MHET) and terephthalic acid (TPA) were detected from PET degradation (Moog et al., 2019).

A thermophilic bacterial strain i.e., *Thermobifida alba* has also been reported to biodegrade aliphatic-aromatic copolyester film and minimize the polymer particle sizes to a certain extent at 50 °C. The esterase coding gene (*est119*) was cloned to enhance the enzyme activity between 20 °C to 75 °C (Hu et al., 2010). Similarly, Ndahebwa Muhonja et al. (2018) reported the presence of alkane hydroxylase encoding genes in multiple bacterial and fungal isolates the hydrolases known to have a crucial role in LDPE degradation.

8 Biofilms forming properties of microbes

Microorganisms display diverse characteristics including biofilm formation representing the complex microbial life-form. Biofilms form with a high degree of interaction between cells which develops an extracellular polymeric matrix by self-immobilization. Biofilms mimic the hydrogels, a 3D hydrophilic polymers complex, and consist of a vast quantity of water. The formation of biofilm may result in the development of stable and functionally coordinated microbial groups (Morohoshi et al., 2018). The phylogenetic analysis reveals that the biofilms, also known as microbial gatherings or periphytons or biofouling networks, may belong to different algae, fungi, and bacteria groups. It has been observed that microbial biofilm formation on polymer surfaces is considered a prerequisite for biodegradation

(Ghosh et al., 2019). The extensive biofilm formed after the initial attachment of microbes and colonization on the surface of the polymer. Consequently, the biofilm formation alters the polymer properties such as changes in functional groups, hydrophobicity/hydrophilicity, surface morphology, molecular weight, and crystallinity. Biofilm initiation enhances the carbon source utilization from immiscible substrates (Han et al., 2020). A recent study provided insights into the basis of biofilm formation on plastics (i.e., PLA, PET) surfaces and the role of conditioning films. This study concluded that the biofilms formed as plastic surfaces come in contact with the aquatic medium and the adsorption of biomolecules (i.e., carbohydrates, lipids) took place on its surface. Hence, microbial interaction and morphological changes on the surface of plastics may occur (Bhagwat et al., 2021).

Poly (3-hydroxybutyrate-co-3- hydroxyhexanoate) (PHBH) biodegradation by bacterial genus *Undibacterium* and *Chitinimonas* has reportedly been known to produce biofilm on the surface of PHBH under freshwater conditions (Morohoshi et al., 2018). Similarly, *Exiguobacterium* species were observed to form biofilm on the surface of synthetic polymer polystyrene. The study claimed that biofilms possess more potential for biodegradation. Further, the atomic force spectroscopy analysis revealed that the cell shape was altered during the biofilm formation as observed during their planktonic stage (Chauhan et al., 2018). *Rhodococcus ruber* strain is also known to form biofilm on the surface of polyethylene and efficiently degrade other synthetic polymers. Additionally, the growth kinetics study revealed the early appearance of biofilm that proves the cells were found to be active for quite a long period which is usually required for polymer degradation (Sivan et al., 2006).

9 Enzymes involved in biodegradation of plastic

The microorganisms are known to produce several enzymes based on their requirements in different biochemical processes. Enzymes are typically specific in their functionality as they catalyze key reactions in various pathways. Microorganisms secrete extracellular and intracellular enzymes for the biodegradation of plastic (Amobonye et al., 2021). These enzymes attach to the plastic surface and then hydrolyze into the plastic monomeric units, as shown in Fig. 3. Scientific communities have reported certain enzymes produced by microbes that can effectively degrade plastic materials, as mentioned in Table 3. Enzymatic degradation has played a vital role in the degradation of PCL polymer and reported by multiple researchers. The hydrolysis of polycaprolactone by lipase enzyme secreted from *Pseudomonas aeruginosa* has also been reported which shows the formation of a dimeric ester of hydroxyhe-

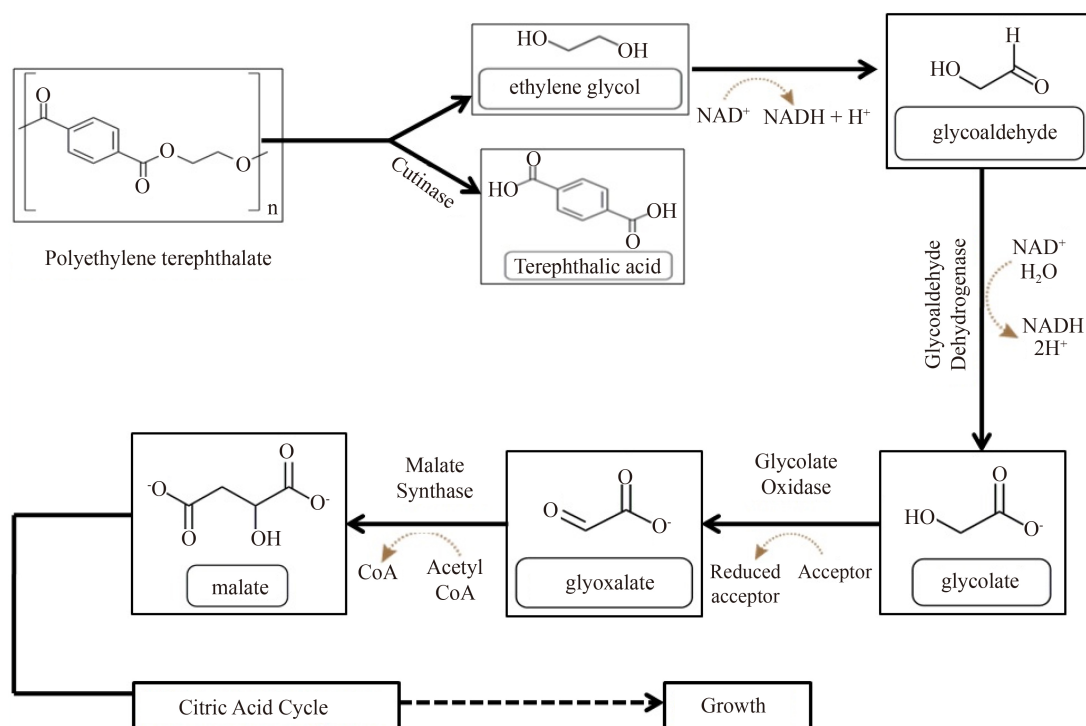


Fig. 3 Enzymes associated with PET degradation are cutinase, glycolaldehyde reductase, glycolaldehyde dehydrogenase, glycolate oxidase, and malate synthase. Breakdown of PET by cutinase enzyme results in two monomeric units: ethylene glycol and terephthalic acid, further ethylene glycol monomer is reduced to glycolaldehyde, which gets dehydrogenated by glycolaldehyde dehydrogenase enzyme to glycolate, further glycolate is oxidized to glyoxalate, finally malate synthase act on glyoxalate and convert it to malate, which is further utilized by microbes for their growth.

xanoate (Jaeger et al., 1995). Researchers have investigated the role of cutinase secreting phytopathogens primarily fungus and bacteria, in PCL degradation. The reports further suggested the possibility of PCL degradation by esterases and proteases (Jaeger et al., 1995; Urbanek et al., 2020). Murphy et al. (1996) purified PCL depolymerase enzymes (i.e., cutinase and lipase) from fungal phytopathogen *Fusarium*. The enzymatic degradation of the PCL takes place by hydrolytic cleavage of high molecular PCL into water-soluble low molecular weight oligomers, monomers, and finally it mineralized into CO₂ and H₂O (Antipova et al., 2018).

As per a report, yeast belonging to *Cryptococcus* sp. could produce lipases enzyme which is homologous to the cutinase family. It has been found that this lipase enzyme can degrade high molecular weight PLA and other biodegradable plastics like polybutylene succinate and poly (3-hydroxybutyrate) (Masaki et al., 2005). Nakamura et al. (2001) isolated *Amycolatopsis* sp. able to utilize and degrade Poly (L-lactic acid) as the only carbon source by secreting novel PLA depolymerase enzyme. This novel enzyme also exhibited the properties of degrading casein and fibrin. Recently researchers have identified the potential PLA degrading species like *Stenotrophomonas pavanii* CH1 and *Pseudomonas geniculata* WS3 which can produce PLA degrading enzymes. Further, the study proved the correlation of

increasing lactic acid content with PLA weight reduction (Bubpachat et al., 2018).

The degrading ability of two hydrolytic enzymes i.e., ureases and papain were investigated for polyurethane polymer and both were found to be polymer degraders. Papain can hydrolyze the polymer by breaking urea and urethane linkages while ureases degrade the urea linkages. Comparatively, papain was found to be an efficient degrader due to its border specificities and small molecular size (Phua et al., 1987).

In the early '90s, a study reported the *Streptomyces* species producing extracellular enzymes capable of degrading polyethylene in 3 weeks at 37 °C in shake flasks (Pometto et al., 1992). Likewise, *Bacillus cereus* has been shown to produce both laccase and manganese peroxidase enzymes during submerged fermentation for degrading polyethylene (Sowmya, 2014). A copper-binding enzyme, laccase secreted by *Rhodococcus ruber*, was reported for its polyethylene degradation and oxidation properties. Researchers have observed a 13-fold increase in mRNA levels of laccase in the cultures treated with copper compared to untreated control culture. Furthermore, FTIR analysis of polyethylene films incubated with the extracellular laccase exhibited an increase in the carbonyl peak, which validates that enzymatic oxidation by laccase plays a vital role in polyethylene biodegradation (Santo et al., 2013). A novel

Table 3 Enzymes involved in plastic degradation

Plastics	Enzyme	Microorganism	Polymer target	References
Polyethylene (LDPE, HDPE, PE)	Laccase-like multicopper oxidases	<i>Aspergillus flavus</i>	PE	Zhang et al., 2020
	Laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP)	<i>Pleurotus ostreatus</i>	LDPE	Gómez-Méndez et al., 2018
	Laccase and manganese peroxidase enzyme	<i>Penicillium simplicissimum</i>	PE	Sowmya et al., 2015
	Alkane hydrolase, rubredoxin, and rubredoxin reductase	<i>Pseudomonas aeruginosa</i>	LDPE	Jeon and Kim, 2015
	Alkane hydrolase	<i>Pseudomonas</i> sp.	PE	Yoon et al., 2012
	Laccase and manganese peroxidase enzyme	<i>Bacillus cereus</i>	PE	Sowmya, 2014
	Laccases	<i>Rhodococcus ruber</i>	PE	Santo et al., 2013
PET	PETase	Microalga <i>Phaeodactylum tricorutum</i>	PET	Moog et al., 2019
	PETase (IsPETase)	<i>Ideonella sakaiensis</i>	PET	Joo et al., 2018
	Hydrolase	<i>Thermobifida fusca</i>	PET	Müller et al., 2005
Polyurethanes	Cutinases, lipases, proteases, and ureases	<i>Aspergillus niger</i> , <i>Chaetomium globosum</i>	Polyurethanes	Magnin et al., 2020
	Polymerases	<i>Bacillus</i> and <i>Pseudomonas</i> sp.	Polystyrene	Mohan et al., 2016
	Cysteine hydrolase	<i>Pestalotiopsis microspore</i>	Polyurethane	Russell et al., 2011
Other Polymers	Carboxylic ester hydrolase	<i>Pseudomonas aestusnigri</i>	Polyester	Bollinger et al., 2020
	PLA depolymerase	<i>Amycolatopsis</i> spp.	PLA	Nakamura et al., 2001
	PETase-like gene (SM14est)	<i>Streptomyces</i> sp.	Polycaprolactone	Almeida et al., 2019

gram-negative microorganism *Ideonella sakaiensis* sp. nov has been identified as the PET degrader which is also known to produce the PETase enzyme. The study revealed that PETase exhibits higher activity at mild temperature (30 °C) than other enzymes, such as cutinases and lipases (Tanasupawat et al., 2016; Joo et al., 2018).

10 Analytical techniques for plastic biodegradation assessment

Different analytical techniques are being utilized to evaluate the rate of plastic degradation as described in Table 4. The analytical methods may differ among distinct plastic groups and laboratory conditions under which degradation has been carried out (Shah et al., 2008; Eubeler et al., 2009). The most convenient way to determine the extent of plastic degradation involves measuring the changes in their mass. This quantification method has been widely used to assess the plastic degradation in soil, compost, and microbial-treated batches at laboratory conditions (Singh and Sharma, 2008; Chamas et al., 2020).

Another study reported the biodegradability of polyethylene treated with bacterial and fungal species and was determined by the percentage weight loss method. The average mean weight reduction of polyethylene was recorded as 36.4%, 35.7%, and 20.2% by *Aspergillus oryzae*, *Bacillus cereus*, and *Brevibacillus borstelensis*,

respectively (Ndahebwa Muhonja et al., 2018). Bacterial species *Bacillus cereus* isolated from local dumpsites was identified to degrade autoclaved, surface sterilized, and UV pre-treated polyethylene. Analysis based on loss in weight of polyethylene after treatment revealed that *Bacillus cereus* could degrade UV-treated polyethylene (14%) more efficiently, followed by autoclaved (7.2%) and surface sterilized (2.4%) (Sowmya, 2014).

The biodegradation ability of this *Exiguobacterium* was evaluated based on the reduced surface hydrophobicity of the PS samples (Chauhan et al., 2018). Multiple structural changes in the carbon and hydrogen contents of LDPE and PP were detected through XRD spectrum analysis after treatment with microbial consortia (Jeon et al., 2021). Furthermore, researchers observed the presence of cracks, cavities, and grooves on the surface of plastic film with the help of SEM analysis (Skariyachan et al., 2021).

11 Conclusions and future perspective

Chaotic disposal of plastic waste in landfill sites and oceans has contributed to a level of significant global threat. The release of microplastics and fragmented plastic particles from dumping sites into the open atmosphere is causing multiple serious issues. As plastics cause severe ailments in humans and adversely impact the environment therefore one should be vigilant in their usage and disposal. Self-sustainable policies need to be adopted regarding the institutional and industrial use and

Table 4 Existing analytical techniques for the assessment of plastic biodegradation

Variation in the properties of plastics	Techniques used	Function	References
Morphological changes and surface changes	SEM, AFM	SEM reveals the presence of cracks, cavities, and erosion. AFM estimates the roughness of material at low magnifications	Harrison et al., 2018; de Santana et al., 2019
Molecular weight	HT-GPC	Detection of changes in molecular weight of plastics	Yabannavar and Bartha, 1994; Suresh et al., 2011; Jeon and Kim, 2016
Contact angle, density, and viscosity	Software-controlled hanging drop method.	Detect changes that occur in the surface density of the functional group and surface energy	Suresh et al., 2011
Crystallinity changes	X-ray diffraction (XRD) and differential scanning calorimetry (DSC)	Detect crystallinity changes in the plastic material	Capitain et al., 2020
Tensile Strength & Modulus of polymer	Dynamic Mechanical analysis	Detect changes in the tensile strength and percentage elongation of polymer	Huang et al., 2005
Chemical properties	FTIR	Detection of certain polar functional groups, like ester carbonyls and ketones, to quantify oxidative degradation pathway	Celina et al., 1997; Ioakeimidis et al., 2016
CO ₂ evolution test	Traditional trapping, titration methods, and Sturm test	Used as an indication to prove that biological degradation is happening	Alshehrei, 2017; Castro-Aguirre et al., 2017
Electrical properties	pH changes	Used to detect the degradation based on biomass growth on plastics	Krueger et al., 2015
Colour alteration	Visualization test and colorimetric test	Detect the biochemical alteration and changes in the colour of plastics	Ali et al., 2014; Pastorelli et al., 2014
Metabolites formation	Gas Chromatography-Mass Spectrometry (GC-MS)	Detection of bio-fragments and the presence of saturated linear alkanes in the culture media	Kyaw et al., 2012
Weight of polymer	Gravimetric weight loss	Detection of percentage weight loss of polymer	Skariyachan et al., 2016

disposal of such recalcitrant synthetic polymers. Furthermore, bio-monitoring needs to be incorporated into human and animal systems to clarify the toxic effects of plastic waste materials. The way plastic waste is piling up in the environment is an utmost requirement to develop eco-friendly solutions rather than relying on traditional methods to cope with plastic waste. Multiple reports have suggested that the microorganisms belonging to *Ideonella* sp., *Bacillus* sp., *Streptomyces* sp., *Pseudomonas* sp., etc. have great potential against plastic waste at laboratory-scale investigations. Although research performed in the particular area are not very descriptive therefore extensive studies are still required to identify the degradation pathways involved in the biodegradation of plastic materials. As per observations, the previously published biodegradation studies seem little biased towards the results achieved under optimized conditions, thus painting an excessively configured picture of minimal transferability to natural environments.

Therefore, future plastic removal or degradation technologies/research needs to focus on improving the pre-existing or developing new approaches. The identification of highly efficient microbial consortia needs to be studied and optimized. The availability of plastic degrading enzymes is very low; hence, further studies on identifying species and enzymes with multi-functionality on dominant polymers need to be done. The cultivation techniques have not yet led to the discovery of highly active enzymes for most plastics therefore exploration of the diversity of non-cultivated microbes could be a promising source of novel biocatalyst identification.

Specific differential genes are expressed in microbial species that code for enzymes/proteins involved in plastic degradation. Therefore, the high-throughput transcriptome-based approaches can help to find out the differential up-regulation and down-regulation of genes expressed under distinct growth conditions during the plastic biodegradation process. Moreover, the studies based on identifying the interaction between the genes and proteins and elucidation of the functions of the gene of interest can be beneficial to get in-depth insights into the process of plastics degradation. Lastly, plastic waste treatment technologies must be sufficiently durable and feasible for large-scale use where microbial adaptability in the environment is an utmost requirement. Plastic waste treatment technology through microbes on a large scale could be the most rewarding trouble-shooters for the global plastic waste problem.

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Conflict of Interest

The authors of this manuscript declare that they have no conflict of interest.

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