Chapter 5

Plastic Biodegradation

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Abstract

Plastics that are degraded by microbial or enzymatic activity are known as biodegradable plastics. Biodegradable plastics are an alternative to conventional plastics that are chemically synthesized and are responsible for causing environmental pollution due to unwanted accumulation occurring via disposal practices. There was a serious need to introduce biodegradable plastics in the market since the level of plastic pollution in the air, water, and soil has reached its threshold values. The non-biodegradable plastics are increasingly accumulating in the environment, which can be a threat to the planet in the coming future. This chapter provides detailed insight into biodegradable polymers, mostly aliphatic polyesters that are considered as a solution against synthetic plastic. It also gives brief information on the current scenario of plastic biodegradation, recent advancements, opportunities, and future challenges. Also, it comprises precise strategies currently used at a laboratory scale to enhance biodegradation of classical synthetic plastics (e.g., polyethylene, polystyrene, etc.). Moreover, the factors affecting the biodegradation process and the characterization techniques being employed to assess degradation extent are also discussed. The overall work focuses on thrust areas to be improved concerning environmental safety and sustainable vision.

Keywords

Bioremediation, Bio-Degradation, Plastics, Bio-Degradable Plastics, Bio-Based Polymers, Enzymes

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1. Introduction

The plastic industry is one of the leading sectors in the global market. The urban and rural population is highly dependent on plastic material for their day-to-day activities. The use of plastic has made life simple and more comfortable by providing convenience in almost every need. It is difficult to imagine modern life without the use of plastics as these materials are durable, waterproof, and do not corrode. Plastic is a cheaper and lightweight alternative to metal while being easily moldable and transportable makes it widely adoptable by industries. Despite having numerous applications, they raise considerable disposal concerns. Natural degradation of plastics may take hundreds of years, and it is not suitable for landfills either. Plastics cannot be incinerated as they are known to release toxic gases that pollute the environment and affect living beings. Problems associated with environmental pollution and waste material management have become more threatening with bio-resistant synthetic plastics in the past few decades. The focus of the research community has shifted towards avoiding further adverse effects on the global environment while initiatives have been taken by respective countries to use environmental-friendly materials that can replace conventional synthetic plastics. There are various technologies to breakdown the polymers used in the manufacturing of plastic products. Still, some have drawbacks such as the involvement of time-consuming processes, high process cost, and the release of toxic gases during degradation. The

alternative solution to prevent pollution caused by the disposal of plastics is to recycle the used/waste plastic material or use biodegradable plastics or bio-based materials.

2. Environmental impact of plastics

2.1 Ocean and land pollution

The most significant sources of plastic pollution are landfills. The plastic from landfills ultimately goes to the sea, causing contamination in the aquatic life of the ocean. The degradation of conventional plastics in sea environment is a prolonged process and takes centuries to occur, which leads to their accumulation in the oceans. The sea is the ultimate receptacle for all the waste produced on land (80% of the trash found at sea comes from the land). The plastic debris found on the surface of the water has predominant particles smaller than 5mm, commonly known as microplastics. It is estimated that the concentration of microplastics in the Mediterranean Sea will increase by 8% in the next 30 years [1]. The ocean gyres are continuously producing generations of microplastic via fragmentation of plastics. These microplastics get dispersed all over the oceans. These fragments are very stable and can sometimes persist for up to 1000 years in the marine environment [2]. The plastic present on the land in the form of landfills does not have the opportunity to form small particles or microplastics. The so-called "biodegradable" plastics are emerging in the global market that promises to reduce the impact of plastic waste at sea. However, the degradation process can lead to excess formation of bacterial biomass or mineralization, which in turn is causing microbial pollution in the affected environment.

2.2 Disruption of food chains due to the toxicity of plastic

The majority of plastics found in the contaminated region of sea and soil are non-degradable plastics such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). In the environment, pollution by plastics can have several consequences. Other than the visual pollution they cause, plastics affect marine organisms directly or indirectly at different levels of the food chain [3]. Chemically, plastics consist of identical sequences (or polymers) of carbonaceous molecules, mainly hydrocarbons, toxic to many organisms, capable of accumulating along food chains. Bioaccumulation of plastic in the environment is a significant issue concerning the non-degradability of plastic. In the accumulation zones, the concentration of microplastics observed (0.5 to 5 mm in size) is comparable to that of zooplankton (between 0.005 mm and more than 50 mm). The Mediterranean Sea, for example, has microplastic/zooplankton ratios between 1/10 to 1/2 [4]. The risk for zooplankton

predators (i.e., fish) from ingesting microplastics is therefore considerable. The residence time of plastic in small pelagic fish has been estimated between 1 day to 1 year [5]. Fragments of ingested microplastics are found in animal droppings and can sink with corpses or even be transferred to predators and thus reach the upper echelons of the food chain [2]. Plastics are also observed as vectors of dispersion of toxic compounds, which can also accumulate in food chains. These compounds can be directly present in the composition of plastics or adsorb on their surface. In the first case, these are additives (phthalates, biphenyls, etc.) incorporated into certain plastics to increase their resistance. Various studies have shown that these compounds can be toxic to many animals and humans [6]. Other toxic compounds (hydrocarbons, pesticides, dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyl (PCB), etc. can be adsorbed on plastics, which is likely to increase their dispersion and persistence at sea, leading to accumulation in the highest trophic levels [7]. The disastrous effects of ingestion of plastic debris confused with prey are also well documented, with severe consequences on the digestive systems of animals such as fish, birds, sea turtles, and marine mammals, sometimes leading to their death [8]. This debris is also considered to be vectors for the dispersion of toxic algae [9] and pathogenic microorganisms [10]. Moreover, another significant risk could be the use of additives in the preparation of plastics that can be observed in the form of toxins such as bisphenol-A. It is a cancer-causing agent that can also impair the immune system while leading to early puberty and sometimes may trigger the development of obesity and diabetes in humans and animals [11].

3. Types of bioplastics

Though plastic can be degraded in various ways (Fig.1), bio-degradation of plastic is the most efficient and environment-friendly approach. However, the extent of degradation of plastic in a biological environment is highly influenced by the type of plastic that is being degraded. Bioplastics can be categorized on the basis of their source of origin, the extent of degradability. There are mainly three categories in bioplastics; a) bio-based plastics, b) oxo-biodegradable plastics, and c) bio-degradable plastics.

3.1 Bio-based plastics (Hydro-biodegradable)

Bio-based polymers are plastic materials synthesized from renewable biomass sources such as sugarcane, sawdust, straw, corn starch, vegetable fats and oils, recycled food waste, etc. The raw material is mostly agricultural by-products and other recyclable materials; therefore, the cost of ingredients is comparably cheaper. These materials uptake water while the degradation process is mainly enzyme-mediated. As per the

European standards of EN 13432 plastic, the material should be susceptible to degradation by microorganisms in its natural environment. Even if this type of plastics meets the standards, its degradation in the "natural" environment remains subject to controversy [12]. Bio-based polymers are sustainable, unlike conventional fossil resources such as petroleum oil and natural gas, which leave a high carbon footprint. Interestingly, the biobased plastics are known as carbon-neutral or carbon offset materials since they do not contribute CO₂ to the environment even after incineration. The raw material for bioplastics is generally derived from sugar derivatives, including cellulose, starch, lactic acid, and is usually combined with conventional plastics for recycling. However, bioplastics are not always biodegradable, and some may be more resistant than traditional plastics. Though the share of bioplastics in the global market is only 1.1 % as of 2019, it is rapidly increasing with the demand and awareness. Examples of these plastics are polyesters, polyamides, polysaccharides, etc.

Fig. 1 Degradation of plastics using various processes.

3.2 Oxo-biodegradable plastics

Oxo-biodegradable plastics are a type of bioplastic that can be defragmented but are not depolymerized at a molecular level. They uptake oxygen due to the presence of catalytic

enzymes. It also requires UV light for the process of biodegradation. However, O₂ and UV lights are not sufficiently found in landfills, making the process of degradation a bit difficult. Typically, during the process of degradation, they form oxidized fragments by oxidative degradation catalyzed by chemicals or additives present in it. These fragments are further amiable to microbes, which are then degraded by enzymes produced by the specific microbes [13]. This type of plastic may take up to 36 months for >60% degradation to occur. Oxo-biodegradable forms of plastic have the same primary components as conventional plastics (polyethylene, polypropylene, polystyrene, even production chains). Moreover, the addition of biodegradable stabilizers or additives possibly works as pro-oxidant and predicts the lifespan of products while facilitating biodegradation using microorganisms. There is a need to generate well-documented work on the abiotic degradation of these plastics so that the demonstration of their biodegradation remains a subject of ambiguity in the field. However, developments in additive formulations seem promising. To date, only one additive named "d2w" has obtained an eco-label (environmentally friendly products) according to ISO 14020: 2002 and 14024 standards: 2004 concerning the degradation of oxo-biodegradable plastics and is ready for commercial exploration. There is still a broad scope to develop more biodegradable additives, which will direct the degradation of oxo-plastics efficiently. Several forms of this type of plastic are polyolefins, polyvinyl alcohol, etc [14].

3.3 Biodegradable plastic

Biodegradable plastics are polymers made from synthetic, semisynthetic, natural polymers or a combination that can be degraded into water and carbon dioxide by the activity of living microorganisms. The American Society for Testing and Materials (ASTM) defines biodegradable plastic as "a degradable plastic in which the degradation is the result of the action of naturally occurring microorganisms such as bacteria, fungi, and algae" [12]. By another definition given by the European standard, EN 13432:2007 plastic shows biodegradability at a threshold of at least 90% degradation, in a maximum period of six months, under composting conditions (where microbiologically active environment provided with specific conditions of humidity and temperature). A plastic (or a polymer) is called degradable when it breaks down to smaller (monomeric) subunits and loses its original properties. Extracellular depolymerase enzymes of microorganisms are majorly responsible for the depolymerization of plastics. Aromatic polyeasterase is also one of the enzymes identified in a few bacteria that play an important role in degradation. The main difference between biodegradable polymers and bioplastics is that biodegradable polymers can be completely (100 %) degraded with the help of enzyme-producing microorganisms, which are mostly present in the soil. On the other hand, bioplastics may or may not be able to degrade by the microorganisms and sometimes may

be found more resistant to degradation than conventional plastics. The technology used to prepare biodegradable polymers is mainly focused on maintaining their physical properties where it could replace conventional plastics while enhancing its biodegradability at the same time. A polymer is evaluated for its biodegradability based on its chemical structure along with physical properties such as solubility in water, crystallinity, glass transition temperature, melting point, storage modulus, hydrophobic, hydrophilic, etc. The lower rate of crystallization or crystallinity can also enhance its biodegradability. The complete decomposition of plastics can only be possible when a specific organism is identified and optimized for its growth at various parameters such as temperature, oxygen, pH, moisture content, nutrients, etc., to achieve the highest enzymatic activity that ultimately leads to the maximum extent of degradation. Since most aliphatic polyesters can be degraded with the help of microorganisms and their enzymes, biodegradable polymers are found to be a promising solution to tackle the plastic accumulation problem as they are environmentally friendly and known to increase the fertility of the soil.

3.4 Types of Biodegradable Plastics

Poly(ethylene adipate)

Polyethylene adipate (PEA) is an aliphatic polyester that is widely available in the market due to its low manufacturing cost as compared to other biodegradable plastics. PEA can be synthesized employing a polycondensation reaction between adipic acid and ethylene glycol. PEA is readily miscible with most other polymers except the low-density polyethylene (LDPE). Being an aliphatic polyester, PEA possesses poor mechanical properties. However, decreasing the crystallinity of the material can enhance the biodegradability and hydrophobicity of the final product [15]. Moreover, the low molecular weight of PEG as compared to other polymers further aids in enhancing its biodegradability. *Penicillium* species are found to produce lipase enzyme that breaks down the Polyethylene glycol (PEG) structure during degradation [16].

Polycaprolactoneor

Polycaprolactoneor (PCL) is a biodegradable aliphatic polyester that has broad range of applications from biomedical to the textile industry. PCL has a low melting point of around 60 °C and a glass transition temperature of about -60 °C, helping it to be readily miscible with other polymers and can be molded into various shapes. Its high biodegradability property and biocompatibility with the human body have led these biomaterials to be used in drug delivery systems, tissue engineering, prosthetics, and sutures [17]. These FDA (Food and Drug Administration) approved biomaterials are safe to use except having mild undesirable effects. PCL is often mixed with other polymers in

various concentrations to achieve desired characteristics in the final product. Moreover, the excellent physical properties of PCL enable the fabrication of a variety of structures and are used as a raw material in 3D printers.

Poly(beta-propiolactone)

Poly(beta-propiolactone), also known as PPL, is a polymer having two methylene units packed in between successive ester groups. It is a completely biocompatible and biodegradable polymer, having a similar molecular structure to those of Poly hydroxybutyrate (PHB) and PCL. Though the melting point is around 85 °C, this semi-crystalline aliphatic polyester is flexible at ambient temperature. PPL has a high molecular weight, can completely replace the conventional plastics that are used in the manufacturing of single-use cutlery, bottles, straws-cups, etc.

Polybutylene succinate

Polybutylene succinate (PBS) is a biodegradable plastic that can be easily decomposed into water and carbon dioxide using some microorganisms. The capacity to manufacture PBS is continuously being increased every year due to its high demand. PBS is a thermo-stable biodegradable aliphatic polyester polymer that contains polymerized units of butylene succinate. The synthesis of PBS is generally done by esterification of succinic acid with 1,4-butanediol. Later they are trans-esterified under vacuum using an appropriate catalyst to form a high molecular weight polymer. Since PBS possesses high heat resistance over other biodegradable polymers, these properties support the broad range of applications such as food and cosmetic packaging, fishing nets, disposable cutlery, and drug encapsulation systems used in the biomedical field. Further, they can also be used for the sustained release of pesticides and fertilizers [18].

Poly(ethylene succinate)

Polyethylene succinate (PES) is an aliphatic synthetic polyester having a melting point between 103 to 106 °C. The synthesis of PES is done either by polycondensation of succinic acid and ethylene glycol or by ring-opening polymerization of succinic anhydride with ethylene oxide using dicarboxylic acids. Similar to PBS, PES also widely used commercial polyesters with a reasonably high melting temperature and desired mechanical properties, which are now replacing the conventional non-biodegradable polymers such as polypropylene and low-density polyethylene. The lower crystallization rate and crystallinity in PES enable it to higher biodegradation rate as compared to PBS. The crystalline growth kinetics of PES was studied [19] and compared with the biodegradation process. Thermophilic *Bacillus* sp. TT96, *Paenibacillus* sp., and the fungal species of *Aspergillus clavatus* were found to be degrading PES films [20]. Among many others, *Bacillus pumilus* was found to have the highest degradation rate.

Polyhydroxybutyrate

Poly hydroxybutyrate (PHB) falls under the class of polyester that is biologically based, non-toxic, and biodegradable in nature. Biologically based means the raw materials required to produce PHB are found in nature, and *Bacillus* and *Streptomyces* microbes that are commonly found in the soil can easily breakdown these polymers into water and carbon dioxide. Typically, PHB is produced by the fermentation process, where the fully-grown microorganisms are nutritionally starved to produce bio-based polymers. This process is ecofriendly and scalable but demands high manufacturing costs when compared to conventional plastics. Therefore, scientists have come up with a transgenic plant containing genes responsible for PHB production. Now the production and the harvesting of PHB molecules are simpler and faster. The product Biopol is internal sutures (surgical stitches) manufactured for medical industries. The product Tephaflex (used in the biomedical sector) is another type of product that can be produced using a fermentation process that is being employed by recombinant microbial strains [21].

Poly lactic acid (PLA)

Poly lactic acid (PLA) is a highly thermal resistant aliphatic polyester that has derived from renewable resources like corn starch, sugar cane, cassava, etc. The raw material used for polymer preparation, such as lactic acid, can be produced by fermentative biotechnological processes using agricultural by-products and microorganisms. Its melting temperature is around 150 to 160 °C that enables its application in extended areas where other biodegradable polymers fail, making it the second-most consumed biodegradable polymer globally. PLA can replace other conventional plastics as it shows similar characteristics to polyethylene (PE), polypropylene (PP), and polystyrene (PS). The application of PLA is mostly in the manufacturing of single-use plastics, bottles and films, biomedical devices such as pins, screws, rods, etc. The melting properties of the PLA enable its application as a raw material for 3D printing as well.

4. Biodegradation of plastic

The recalcitrant materials left in the environment act as contaminants; this is called bioaccumulation. For the growth and reproduction of microorganisms, several essential elements like carbon, nitrogen, sulfur, magnesium, etc., along with energy, are needed. Depending on the type of plastic, the suitable condition and the microorganism will help in the biodegradation. Biodegradation refers to the use of microbial functions for the conversion of organic substrate or polymer to small or simple molecular fragments, which can be further converted into water and carbon dioxides [2,22,23]. The biodegradation of polymer refers to a change in polymer structure via changing the

polymer properties caused by the transformative action of a microbial enzyme, reduction in molecular weight; changes occurred on the surface, and enhanced mechanical strength [2]. Biodegradation of plastics can happen in two possible conditions; aerobic and anaerobic. In the first condition, oxygen acts as an electron receptor, while in anaerobic conditions, the other compounds such as sulfate or nitrate act as an electron receptor [24]. The oxidation reactions of organic compounds are highly exothermic (1 mole of oxygen, i.e., electron receptor has 435 kJ of energy) to give products; water and carbon dioxide. Microorganisms utilize the part of the available energy, and rest is lost as heat [25,26].

4.1 Aerobic biodegradation of plastic

Aerobic degradation refers to the deterioration of organic matter using oxygen and suitable microorganisms. CO_2 and H_2O , along with the cellular biomass of organisms, are produced during plastic degradation under aerobic conditions [2]. The aerobic degradation is more efficient in comparison to anaerobic digestion and provides more energy due to O_2 presence [2,27]. Aeration (either forced or natural) is used to provide enough oxygen and get rid of excess water present during the process. The aerobic degradation is fast and does not produce the pungent gases as in anaerobic digestion.

4.2 Anaerobic biodegradation of plastic

Anaerobic degradation of plastic refers to the breakdown of complex structures to simple forms such as CH_4 , organic acids along with CO_2 , and H_2O , without a supply of oxygen [28,29]. Under anaerobic conditions, the sulfate or nitrate ions act as a receptor and are less efficient. In the absence of oxygen, the amount of energy produced in the anaerobic condition is less as compared to aerobic digestion. Hence, anaerobic digestion is slow and requires more time for completing the degradation [2,30]. In the landfill waste, firstly, aerobic bacteria will be utilizing the oxygen present in the system to start the degradation, followed by the anaerobic digestion to get the complete biodegradation of waste. The anaerobic degradation will stop if there is an introduction of oxygen during the process, and hence the anaerobic condition has to be maintained.

5. Stages of biodegradation of plastic

The two primary sites of plastic pollutions are landfills and oceans. The stages of biodegradation of plastic are majorly affected by these sites. Biodegradation is caused predominantly by the biological action of microbes, but in nature, it is a combination of abiotic and biotic activity. The abiotic activity includes the breakdown due to UV light irradiation and ozone, followed by the mechanical breakdown of plastic due to ocean gyres. The synergistic action of both leads to the efficient biodegradation of plastics.

Figure 2 depicts the summary of different stages of plastic biodegradation by microorganisms. A plastic will first undergo abiotic degradation (not biological), which includes physical and chemical degradation. Physical (wave, temperature, and UV) and chemical (oxidation or hydrolysis) degradation will contribute to the weakening of the polymer structures and reduce the plastic into smaller pieces [31]. Followed by the second step, i.e., biological degradation, which has four successive stages [24,32].

Fig. 2 The summary of different stages of plastic biodegradation by microorganisms.

5.1 Abiotic deterioration

Deterioration refers to the artificial degradation of various properties (chemical, physical, and mechanical) of plastic. These include thermal, chemical, mechanical, and photodegradation. Photodegradation occurs due to the sun-sensitive components that trigger the physical disintegration of plastic when exposed to sunlight [33]. The abiotic deterioration sometimes has a synergistic effect or helps to initiate the biodegradation process.

5.2 Bio-deterioration or biotic deterioration

Bio-deterioration refers to the deterioration of the chemical and physical properties of plastics due to the action of microorganisms, which will result in the superficial degradation of plastics [34,35]. The microorganisms forming biofilms have a major role in plastic biodegradation. They are highly effective in the first stage of biodegradation.

5.3 Biofilm formation

The first step of biodegradation of plastics is colonization or biofilm formation of bacteria or fungi cells onto the surface of the plastic. These biofilms of cells are attached to the surface of plastic due to the exopolysaccharide secreted by them. The degradation of the polymer takes place with the help of exo-enzymes secreted by the microbes [36]. The cells take up the polymer fragments for growth, in turn, secrete exopolysaccharides. Chemical degradation can also be orchestrated by the great diversity of species present in the biofilm, such as the production of acidic compounds by chemolithotrophic and chemoorganotrophic bacteria. The microbes exert mechanical, chemical, and enzymatic action on the plastics, leading to the changes in properties of the polymer. The overall process is dependent on various environmental factors such as humidity, atmospheric pollutants, and weather, especially in the case of degradation of plastic in the sea. The microbial biofilms give rise to different chemical and physical deterioration processes. The microbial biofilm formation secretes extracellular polymeric substances (EPS), which reinforce the adhesion to the surface of the plastic. This EPS helps the growth of microorganisms inside the pores, which results in cracking of pores, and hence physical properties deteriorate [37]. In the development of biofilm, the microorganism secretes the acid compounds. Chemo-lithotrophic bacteria release nitric acid, nitrous acid, or sulfuric acid. Similarly, they release other types of organic acids, such as glutaric, oxalic, gluconic, citric, fumaric, and glyoxylic acid. The release of acids results in a change in pH inside the pores and helps to degrade the structure of plastic, called chemical deterioration resulted from biofilm formation [10].

5.4 Depolymerization

The hydrophobic nature of the polymer, along with lengthy chain structure, makes microorganisms incapable of directly transporting them inside the cells [30]. The changes in the chemical and physical properties due to the formation of biofilms results in the breaking of plastic material in small molecule, i.e., dimers, oligomers, and monomers of low molecular weight. This will help to accommodate the small molecules inside the cells, which can be further converted to H₂O and CO₂ [26].

5.5 Bio-fragmentation

Fragmentation refers to the degradation of plastic in terms of modifying the mechanical, physical, and chemical properties of a material. Bio-fragmentation refers to the action of bacterial enzymes released outside cells to cleave plastic polymers into shorter sequences; oligomers and monomers [38]. Oxygenases, for example, make plastic polymers more water-soluble, making them easily degradable by bacteria. Lipases and

esterases can individually attack carboxylic groups and endopeptidases amine groups. Different bacterial species can be employed in the process of bio-fragmentation [31].

5.6 Assimilation

It involves the transfer of plastic molecules of size <600Daltons (Da) into bacterial cells, followed by their transformation into cellular compounds and biomass [39]. The bio-deterioration and fragmentation of plastic materials result in the formation of small substrates, monomers. Its incorporation inside the microbial cells is called assimilation. During the process, microorganisms utilize the small molecules to obtain energy in the form of electrons along with essential elements for cell growth (carbon, nitrogen, oxygen, phosphorus, sulfur, and so forth) [2].

5.7 Mineralization

The process of biodegradation in which the plastic polymer is completely converted to carbon dioxide is called mineralization [26]. It corresponds to the complete degradation of plastic into oxidized molecules (CO_2 , N_2 , CH_4 , and H_2O). However, the process in the natural environment is much more complex and involves many bacterial species.

6. Molecular mechanism of plastic biodegradation

Microorganisms or microbes consist of a broad range of living organisms that can live alone or in groups and are trillions in number, including bacteria, protozoa, fungi, and some algae. They can be heterotrophic or autotrophic, depending on their food production capacity. These microorganisms can actively participate in the biochemical transformation of several substrates. The conversion of organic chemicals into simple chemicals is termed biochemical transformation. Various bacteria, fungi, including yeast and molds, and few insects have been reported for plastic biodegradation, as depicted in Table 1 [40-51].

Table 1 Biodegradation of plastics using microorganisms [40-51]

S. No.	Organism	Plastic	Method	Results	References
	Bacteria				
1.	Heterotrophs (<i>Burkholderia</i> sp.), Nitrifying bacteria (<i>Nitrosomonas</i> sp. AL212, <i>Nitrobacter winogradskyi</i>), Type I methanotrophs (<i>Methylobactor</i> sp. and <i>Methylococcus capsulatus</i>), Type II methanotrophs (<i>Methylocystis</i> sp., <i>Methylocella</i> sp.)	PE, PP, HDPE, LDPE, PS	Semi-aerobic landfill Bacterial degradation	Epoxides, alcohol detected after 3 months, weight loss of 15-20% HDPE at 1 mL/min, long degradation time, high aeration to increase the reaction rate.	[40]
2.	<i>Pseudomonas</i> sp. AKS2	Polyethylene succinate (PES)	Bioaugmentation in soil,	A higher number of PES-degrading organisms were found in the bio augmented microcosm.	[41]
3.	<i>Bacillus subtilis</i>	PE	UV irradiation and surfactant addition	9.26% degradation in 30 days	[42]
4.	<i>Ideonella sakaiensis</i> 201-F6	PET Poly(ethylene terephthalate)	No pretreatment	Novel strain was obtained	[42]
5.	<i>Bacillus subtilis</i> V8, <i>Acinetobacter calcoaceticus</i> V4, <i>Pseudomonas putida</i> C 25, <i>Pseudomonas aeruginosa</i> V1	LDPE Low density polyethylene	UV light of 365nm wavelength, Nitric acid, 80°C Surfactant tween (0.05%v/v)	Degradation by each bacterium was at different rates. But enhancement in degradation has been obtained	[43]

6.	<i>Bacillus amyloliquefaciens</i>	Linear low-density polyethylene (LLDPE)	Gamma irradiation, at 90°C for 7 days	3.2% weight loss	[44]
7.	<i>Pseudozymaantarctica</i>	Polybutylene succinate-co-adipate (PBSA), polybutylene succinate (PBS)	5 weeks	Pre-treatment enhanced the biodegradation in soil	[45]
	Fungi				
8.	<i>Engyodontium album</i> MTP091 (F2), <i>Phanerochaetechrysosporium</i> NCIM 1170 (F1) and	ST-PP: Starch blended, MI-PP: metal ions blended polypropylene	UV irradiation for 10 days	79-57% weight loss	[46]
9.	<i>Trametes versicolor</i>	PVC	Liquid inoculation by mycelial fragments and subsequent fermentation	Degradation occurred in 21 days	[47]
10.	<i>Pleurotusostreatus</i>	Biodegradable PE	120 days sunlight exposure, 30 days incubation by fungi	UV exposure before composting was found highly efficient.	[48]
	Insects				
11.	<i>Zophobasatratus</i>	Polystyrene	Containers stored in incubators maintained at 25 °C	In 16 days, 36.7% of ingested Styrofoam was mineralized into CO ₂ .	[49]
13.	<i>Tenebrio molitor</i>	Polystyrene and polyethylene	Containers were stored in incubators maintained at 25 °C and 70% humidity	Conversion up to 49.0 ± 1.4% of the ingested PE into a putative gas fraction (CO ₂) was observed.	[50]

	Microalgae/Diatoms				
14.	<i>Phaeodactylum tricornutum</i>	PET, PET glycol	Active degradation at 21oC & 30oC, PETase secreting gene was inserted in microalgae and expressed	degradation products terephthalic acid were detected, 80-fold high activity on PET glycol than PET	[51]

Several studies have attempted to describe the physical, chemical, and biological stages involved in the decomposition of plastic [8]. Biological degradation is carried out by microorganisms, mainly bacteria [28]. The degradation capacity of different types of plastics by bacteria has been widely discussed in the literature, showing a wide variety of bacteria capable of degrading them [30,34,35]. Even though bacterial degradation of plastics is occurring in nature, the current environmental challenges have to be aimed at better understanding and characterizing the biodegradation of plastics by bacterial communities. Bacteria form the primary source of biodegradation, which occurs naturally at the sea coast and in the sea. The most abundant organisms in the oceans (~100 million bacteria and >500 species per liter of seawater) are invisible to the naked eye, have extremely varied metabolic capacities. In their natural environment, bacteria play the role of garbage collectors of the oceans (saprophytic organisms) since they re-mineralize half of the organic carbon that comes from waste in the food chain. Many bacteria are also specialized in the degradation of hydrocarbons, a major component of plastics, are known as hydrocarbonoclastic bacteria. Chemi-lithotrophic and -organotrophic bacteria are the major classes of bacteria responsible for plastic biodegradation in nature. There are few reports on the usage of fungal species for plastic biodegradation as well, including *Rhodococcus ruber*, *Phanerochaete chrysosporium*, *Pleurotus Ostreatus*, etc. The mechanism of degradation of insoluble synthetic hydrophobic polymers by microorganisms is yet not clearly explained in reports. Microorganisms follow a very complex mechanism in synthetic polymer degradation. Lipolytic enzymes such as lipase and esterase can hydrolyze not only fatty acid esters and triglycerides but also aliphatic polyesters. Laccases oxidize the polyethylene hydrocarbon chain, whereas alkane monooxygenases have been reported in the breakdown of polyethylene.

Table 2 Factors affecting the biodegradability of various types of plastics [53-58]

Sr . N o.	Name of the Plastics	Melting Point (°C)	Glass Transition Point (°C)	Crystallinity (%)	Enzyme	Incubation Temperature (°C)	Microorganism	Reference
1	Poly-ethylene adipate (PEA)	55	-50	28.1	lipase	45	<i>Penicillium</i> sp.	[53]
2	Polycaprolactone (PCL)	60	-60	53.6	lipase, cutinase	45	<i>Streptomyces thermoviolaceus subsp.thermoviolaceus</i> 76T-2	[54]
3	Polybutylene succinate (PBS)	114.1	-28.5	49.1	lipase B	37	<i>Candida antarctica</i>	[55]
4	Poly-ethylene succinate (PES)	103–106	-6.15	64	hydrolytic enzyme	30	<i>Bacillus</i> and <i>Paenibacillus</i>	[56]
5	Polyhydroxybutyrate (PHB)	175	2	60	PHA depolymerases	30	<i>Acidovorax</i> Sp.	[57]
6	Polylactic acid (PLA) or polylactide	170	60	37	lipase, esterase, and alcalase	40	<i>Bacillus licheniformis</i>	[58]

7. Factors affecting the biodegradation of plastic

The extent of biodegradation of bioplastic can be influenced by several factors such as the type and amount of microbial community present in the surrounding, temperature, moisture content, available gases to exchange, along the O₂ availability. Moreover, several factors such as the pH of the system, distribution of polymers at the molecular level, and the presence of chemical linkage influence the biodegradable properties of the material. The biodegradation of plastic in the soil is majorly altered by temperature, as detailed in a prior study [52]. The biofilm-forming microbial community can lead to a fast-paced degradation than others. Furthermore, the type of enzymes produced by the organisms also affects the biodegradation rates. The degradation rates differ according to the location where the biodegradation is taking place. In soil, degradation depends on whether the environment is supplied with dry or humid air and the type of site of disposal

(soil or a landfill). Moreover, a composting environment is drastically influenced by the location of degradation (sewage, freshwater, or marine environment). The moist air or aquatic environment can commence the first step of the hydrolysis of polymer since radical formations accelerate these reactions. Table 2 [53-58] depicts the factors affecting the biodegradability of various types of plastics. These factors affecting the degradation process can further be classified as follows: a) environmental parameters (abiotic/biotic) and b) type of plastic (polymer properties).

7.1 Environmental Parameters

All polymer types are susceptible to biodegradation to some extent considering their multi-component system. The organic constituents (hardeners, resins, etc.) of their composition support the degradation. Polymer structure can comprise random copolymers while varying its structure. A branched network structure of the polymer can influence the cleavage of the polymer chains. An ample amount of qualitative/semi-quantitative information has been documented concerning the several factors that influence the overall degradation process of polymer in biological environments. Environmental parameters affecting the biodegradation process can further be classified as abiotic and biotic factors.

Abiotic factors

Abiotic (environmental) factors such as humidity (moisture), temperature, pH, sunlight, available gases to exchange along with O₂, and other stress conditions direct the abiotic degradation of the polymer. These factors further affect the growth of microbes and the expression of enzymes that are actively involved in the process [59]. Elevated moisture and temperature can enhance the hydrolysis process while increasing microbial activities that ultimately improve the depolymerization of polymers, making it easier for microbes to attack targeted sites [60].

Biotic factors

Several biotic factors that largely influence the biodegradation process are as follows; a) expression of enzyme, b) enzyme-substrate (E-S) complex formation, and c) hydrophobicity.

Expression of enzyme (extracellular)

The type of extracellular enzyme produced in the process shows its efficiency concerning its ability to attack available sites on that specific polymer and varies as the type of polymer changes. For instance, fungal species (*Aspergillus flavus* and *Aspergillus niger*)

can express enzymes that efficiently attach 6-12 carbon di-acid-derived (aliphatic) polyesters when compared to other types of monomers [61].

Enzyme-substrate (E-S) complex formation

E-S complex formation can be described in two ways; lock-key or induced-fit model [62]. The activity of an enzyme is directly influenced by the geometry of both units involved in complex formation. Plastic, as a substrate, must possess a geometry that aligns with the enzyme in order to attain better enzymatic action that ultimately yields a better rate of degradation.

Hydrophobicity

Though the water solubility of the polymer does not assure its degradation, the strong hydrophobic nature of the polymer can make the degradation process difficult for microbial communities. Several enzyme-producing microbes may be able to degrade the plastic more efficiently when it has low hydrophobicity compared to others [63].

7.2 Type of plastic (Polymer properties)

Properties of polymer used in the synthesis of bioplastic also influence the overall degradation process. Several properties of polymers that affect the process are described below.

Conformational flexibility

The flexibility of polymers directs the rate of degradation by affecting the enzyme-substrate complex formation. Since most of the polymers are cross-linked and branched, they hinder the reach of microbes for possible attack sites. For instance, aliphatic polyester (highly flexible) is degraded when compared to PET (highly rigid) [64].

Molecular weight (MW)

Polymers with low molecular weights are easy to degrade for microbial communities during microbial transformation. Synthetic polymers (mol. wt. below 1000) are observed to be more favorable to the biodegradation process when compared to the polymers with higher molecular weight [53].

Crystallinity

Polymer with amorphous physical properties is easily susceptible to microbial degradation when compared to highly crystalline structures. An amorphous structure can accommodate more microbial interactions with polymer since they are loosely packed [65]. Moreover, the amorphous structure allows more permeability to O₂ that plays a

crucial role in the formation of peroxy radicals that are actively involved in the dissociation of C–H bonds of the substrate (polymer) [66].

Melting temperature (T_m) and the size of the polymer

Polymers with lower T_m support the degradation process, whereas polymers with higher T_m are harder to depolymerize [53]. Similarly, the size of the polymer also affects the process by generating hindrance in mass transfer due to the lower surface area available in large-sized polymers. On the contrary, polymers with small sizes have a larger surface area, leading to higher rates of mass transfer that allow better interaction between microbes and polymers.

Plasticizer or additive

The type of plasticizer or additive used for the synthesis of plastic also partially influences the rate and extent of degradation. Chemically synthesized polymers are modified by blending additives during the synthesis. Co-monomers blended plastics are easy to degrade since it reduces the crystallinity of the material that favors microbial degradation by providing easy access to H_2O . Moreover, residues from catalytic processes, along with pigments/fillers, can create resistance to degradation. In contrast, the addition of metals in polyolefins can promote the pro-oxidants in the system leading to a better extent of degradation [67].

Mode of synthesis

The stability of the polymer is also influenced by the mode of synthesis employed. For instance, polystyrene (having peroxide residues) produced may favor a better photodegradation when synthesized in its free radical form compared to the photo-stable form. Similarly, polypropylene (PP) in copolymerized form is less susceptible to degradation than the PP synthesized employing a catalytic-polymerization (bulk) process [68].

8. Characterization/analytical estimation of plastic biodegradation efficiency

Biodegradability of a particular plastic can be assessed primarily by two parameters. The first standard is an assessment practice that precisely mimics the proposed environment that also defines a method to measure the extent of degradation. The second standard comprises a designed standard that designates a minimum value to set the biodegradation. Both standards are essential to establish the biodegradation performance of material adequately. For biodegradation, performance is checked by simply measuring the weight loss of plastic before and after biodegradation. There are some gravimetric methods that are used to measure the extent of biodegradation. The qualitative analysis of

biodegradation can be done by sophisticated instruments such as scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FT-IR) that help analyze the changes in the functional group on the surface of the plastic material. Plastic waste has been disposed of in landfills leading to the formation of solid waste environments. Similar environments for plastic deposition include home/industrial compost, litter pits/landfills, anaerobic digestion systems, and ocean sites. The test methods and biodegradation performance standards established to date are only being explored for two types of disposal environments; industrial compost and marine environment. However, only test method standards are available for anaerobic-digestion and landfill environments. The extent of biodegradation means checking the performance; the standards for landfill, anaerobic-digestion systems, and home composting environments are not feasible at this moment. Therefore, the majority of plastics and bioplastics can claim coinciding performance standards of biodegradation with respect to industrial composting and marine environments, among others. To characterize and analyze the extent of degradation, several techniques can be employed in the process.

8.1 Dynamic Mechanical Analysis using universal testing systems

Dynamic Mechanical Analysis (DMR) includes the study of several mechanical properties of plastics such as tensile strength, polymer modulus, and elongation. The various physical properties relating to change in density, viscosity, glass transition temperature, amorphous and crystalline nature, and morphology are also considered to assess the information of degradation.

8.2 Scanning Electron Microscopy

The changes in the surface morphology of polymer can be studied with the help of Scanning Electron Microscopy (SEM). The method involves the sputter coating of the sample with gold or some metal ion (platinum) before analyzing. Researchers have reported the use of variable pressure SEM at suitable magnification as an alternative for direct analysis of samples [42].

8.3 Thermogravimetric analysis

Thermogravimetric analysis (TGA) records the change in the mass of samples with respect to heating. Dynamic thermogravimetry helps to study the temperature corresponding to the highest rate of decomposition along with the activation energy and decomposition reaction rate [69].

8.4 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) helps to find the changes in the chemical structure of polymer due to the biodegradation [33,70,71]. It can provide information about reactions, hydrogen bonding, copolymer composition in solid and liquid form, end group detection, and chemical-physical structure changes that occurred during/after degradation.

8.5 Nuclear Magnetic Resonance Spectroscopy

The degradation reaction pathway is very useful information to understand the role of microbes and conditions in the degradation. Nuclear Magnetic Resonance Spectroscopy (NMR) is very useful to analyze this information along with the formed product composition using ^1H and ^{13}C [28,50].

8.6 Gel permeation chromatography

Gel permeation chromatography (GPC), also referred to as size exclusion chromatography (SEC), is very useful to analyze the distribution of molecular mass of polymer and alterations in the molecular weight of degraded polymer [72,73].

8.7 High-pressure liquid chromatography

High-pressure liquid chromatography (HPLC) is used for the separation of formed molecules after degradation and depends on the size of molecules [74]. The product formed after the metabolic degradation of xenobiotics can be analyzed using reverse-phase HPLC.

8.8 Gas chromatography-mass spectroscopy

Gas chromatography is also used to separate the formed products based on boiling point and chemical polarity. Coupling GC with MS (GC/MS) helps to identify and quantify the volatile components, residual monomers, trace contaminants, and for on-line or at-line monitoring of reaction.

8.9 Biological analysis

Various biochemical assays, like protein analysis, fluorescein diacetate analysis, and adenosine triphosphate assays, can be used to evaluate the metabolic activity of cells in the biofilm and culture [75–77].

8.10 Molecular techniques

Though depolymerization of the polymer during degradation can take a more prolonged time, genetic engineering can be efficiently employed once the pathway of degradation is established. Genetic engineering can be utilized to obtain several desirable features in the process, such as reduction of reaction time, enhancement of regulatory mechanisms, modification of enzymes involved in the process, and making plastic easily amiable to microbial degradation. DNA/Protein sequences can be employed to assess the results obtained from genetic modifications to understand the correlation of genetic alterations with the process of biodegradation.

9. Value addition to biodegradation of plastics

The biodegradation process by microbial break down of solid matter can result in other essential substances. A nutrient-rich form of soil (Humus), CO₂, and methane gas are some of the crucial by-products of biodegradation that add value to the overall process. Methane, a potent greenhouse gas, can be a potential root of clean energy. CO₂, along with methane, can be employed to generate electricity using the internal combustion process. Methane harnessed during biodegradation limits its negative impact as a greenhouse gas since it is being used to generate a clean form of energy, electricity. Further, it also sidesteps the use of dwindling fossil sources for the generation of methane, which again adds up the value to the practice. Nevertheless, landfills can accommodate biodegradation of plastic waste while producing methane as a by-product that can be ultimately employed to obtain clean energy, makes the whole biodegradation process a crucial step towards a sustainable environment. Microbial communities can actively participate in the biodegradation process, where they break down the polymer by expressing different forms of the enzyme, reducing the time of decay from centuries to years (sometimes months, depending on the environment they are exposed to). Considering the amount of plastic disposed of in landfills every day, biodegradable additives (plasticizers) blended with biodegradable plastic can help smoothen the process of biodegradation while obtaining millions of megawatts of clean energy. Some of the new biopolymers can also be obtained from these environments, which can further add up to the more modern biodegradable plastics of microbial origin.

10. Challenges in plastic biodegradation

The current challenge involved in this area are as follows: (1) To gain a better understanding of the mechanisms of biodegradation of plastics by natural communities, (2) Capturing a well-defined list of species that colonize plastics and capable of

degrading them, and (3) Determining the molecular mechanisms involved in the degradation of different compositions of plastics. Currently, many researchers are contributing to the issue are attempting to answer these questions. A coupled DNA-SIP and high-speed pyrosequencing approach are already used to identify bacteria capable of degrading polycyclic aromatic hydrocarbons [78,79]. This approach is based on the isotopic labeling of plastics and the monitoring of their incorporation by bacteria to gain access to the functional community of "plasticlast bacteria," communities that are actively involved in the process of biodegradation.

Several attempts are being made in the field of research to develop techniques along with novel microbial communities that have the potential to alter the future of biodegradable plastic. A plastic manufacturer (Environmental Products, Inc.) has discovered a patented technology, TDPA™ (Totally Degradable Plastic Additive), that enables the degradation of common types of plastic under a monitored environment. The technology has been well-documented by researchers where it was found to be favorably working in the degradation of common plastics [80]. Thermal and photolytic pre-treatments can be applied to promote the abiotic degradation of low-density polyethylene (LDPE). The extent of biodegradation can be examined by monitoring the initial changes in molecular weight while analyzing other structural parameters as well. The degree of crystallinity, spectroscopic properties, and tensile strength are among several essential characteristics that can be assessed during the degradation process. Similarly, the biotic environment further helps in the overall degradation of polymers [80]. LDPE–TDPA™ oxidized samples showed 50-60 % of biodegradation during a specific duration (>18 months) while producing CO₂ in the process mediated by microbial communities of soil in a closed biometric flask. Research unveiled that the LDPE–TDPA™ formulations are found to effectively promote oxidation and biodegradation of PE in soil. Similarly, the discovery of an invertebrate species (insect larvae), which are reported with higher degradation rates, can reduce the waste polymer by ingestion followed by degradation in the gut, by expressing several enzymes is interesting and must be explored further. However, several areas are yet to be investigated concerning the cumulative time required for oxidation/biodegradation and the rate of degradation under varying environmental conditions.

Conclusions and future prospects

Various national and international research efforts have been encouraged in recent years, given the extent of pollution led by plastics in multiple environments. Understanding the mechanisms of biodegradation of plastics is still in its infancy. Though certain mechanisms have been observed in laboratory conditions, their study in a natural

environment remains largely unexplored. The molecular mechanisms of bio-deterioration, bio-fragmentation, bio-assimilation, and bio-mineralization of plastics are yet to be fully understood. The diversity of microorganisms associated with these different stages of biodegradation is also in a primitive stage. Understanding these processes is very important in order to define the biodegradation rates of plastics and to better predict the future of so-called "biodegradable" plastics. The primary site of plastic pollution is the landfills, which ultimately push the waste into the sea. The sea is the ultimate receptacle for all the waste produced on land. Though spreading awareness can limit the waste to some extent, it cannot be wholly relied upon, and a significant step towards excluding plastic waste from the environment needs to be taken. Replacing conventional plastic with bio-based and biodegradable plastic seems to be the only option to tackle this global environmental crisis. Nevertheless, polymer recycling technology based on the conclusions is a potential opportunity that has still to be tapped. Finally, it can be concluded that for environmental safety, we have to restrict or reduce the use of non-degradable polymers while simultaneously encouraging the development of more efficient biodegradable plastics that can replace the traditional plastic to make the planet earth a sustainable biosphere, again.

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