Plastic Pollution, Bangladesh, First-class Microbes and their Enzymes -*A Review*

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment of the requirements for the Bachelor of Science in Biotechnology

Bachelor of Science in Biotechnology
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Dedicated to my parents, my grandparents, my family and to all my teachers at all levels in my Education

Declaration

I hereby declare that I, Ashiqur Rahman Khan Chowdhury, wrote and submitted this thesis entitled "*Plastic Pollution, Bangladesh, First-class Microbes and their Enzymes -A Review*" under the supervision of Dr. Iftekhar Bin Naser, Assistant Professor, Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh.

I further certify that this thesis was written entirely by me and has not been submitted, in whole or in part, to any prior institution for a degree or certificate, unless otherwise indicated by reference or acknowledgment.

Student's Full Name & Signature:

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Ashiqur Rahman Khan Chowdhury

Approval

The thesis project titled "*Plastic Pollution, Bangladesh, First-class Microbes and their Enzymes -A Review*" submitted by Ashiqur Rahman Khan Chowdhury (ID: 15336001) has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Science in Biotechnology.

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Abstract

The invention of plastics has been generally seen as a benefit to contemporary living because of its light weight, great strength and multi-faceted use while being cheaper than other alternative materials. However, due to their minimal biodegradability, overconsumption, and widespread mismanagement, plastics have now become pervasive in all environmental compartments and are considered accountable for creating massive pollution to air, soil, and water bodies and endangering our planet's sustainability. Different characteristics of plastic polymers have been created to substitute materials such as wood, glass, and metals in a variety of applications. As a result, there has been an exponential rise in the production of plastic waste, which has subsequently been identified as a worldwide environmental concern. Plastic waste has had a negative impact on life on Earth, owing to its unwelcome build up in landfills, seeping into the soil, increasing greenhouse gas emissions, and other factors. Their effect on aquatic environments is much more harmful, since they induce entanglement, ingestion, and intestinal obstruction in aquatic species. Furthermore, plastics, particularly microplastics, have been shown to disrupt chemical interactions between marine species, to induce intrinsic toxicity via leaching, and to absorb persistent organic pollutants and pathogens. Bangladesh is no exception to this worldwide trend, but there has been some effort to evaluate the quantity of plastic waste and its consequences, which is required to properly combat this growing danger. The present techniques for removing these pollutants (incineration, landfilling, and recycling) are very expensive, unsustainable, and add to our environmental load. As a result, current research has focused increasingly on the ability of biological systems to breakdown synthetic polymers. In this respect, it has been shown that certain insects, bacteria, and fungus consume these polymers and transform them into ecologically beneficial carbon compounds. As a result, this review emphasizes the various functions performed by microbes in this process, based on current research. Actinomycetes, algae, bacteria, fungi, and their enzymes are discussed in terms of their involvement in increasing the breakdown of synthetic plastics, with an emphasis on their modes of action and possible enzymatic processes. Furthermore, key areas for further investigation, such as the manipulation of microorganisms through molecular cloning, modification of enzymatic characteristics, and metabolic pathway design, are highlighted, as well as the need for researchers to conduct a comprehensive study of plastic pollution in Bangladesh and for appropriate authorities to develop policies and regulations before it is too late.

Keywords

Plastic Biodegradation, Plastic Pollution, Microbes, Enzymes, Polyethylene (PE), Polyethylene-terephthalate (PET), Polyurethane (PUR/PU), Polypropylene (PP), Polystyrene (PS), Polyvinyl chloride (PVC), Nylon, Mechanisms, Factors, Bangladesh

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Introduction

Microorganisms have developed over millennia to convert and mineralize many chemicals, including plastics, and therefore play an essential role in the preservation of numerous environmental processes. They have been in the forefront of preventing bioaccumulation of different pollutants by consuming and recycling these chemicals into molecules that may be re-used by nature. As a result, microbial communities adapt to a variety of environmental obstacles by modifying their genomes to enable the integration of novel chemicals into their metabolic pathways, and hence into the biogeochemical cycle. Thus, microorganisms' capacity to adapt to the metabolism of various anthropogenic chemicals has been discovered to be based on natural selection of mutants with the required degradative enzymes but less specific substrate specificities and likely new metabolic pathways. However, the uncontrolled use of natural resources by humans has created unprecedented ecological disruption via the introduction of xenobiotics at a quicker pace than microbial adaptation and development. As a consequence, ecosystems' self-cleaning ability is being taxed, and pollution build up to dangerous levels has become the norm. Synthetic plastics, which are human-made polymers produced from petroleum, are at the top of the list of these ever-accumulating pollutants. Plastic materials have become essential in many areas of human endeavours during the past fifty years, displacing materials such as glass, metals, and wood in a variety of applications due to its cheap cost, durability, and high strength. As a result, in the past twenty-five years, worldwide plastic manufacturing has increased (Feil and Pretz, 2020). However, the vast bulk of the estimated 8.3 billion virgin plastics created so far have been single-use convenience items that have ended up in our natural ecosystems (Nielsen et al., 2020). Because of its unwanted build up in landfills, seeping into the soil, and increasing greenhouse emissions, this has had a negative impact on life on land. Plastics have been found to have negative impacts on the activity and variety of soil microbiota, as well as on soil organism reproduction (Lahive et al., 2019) and leaching in soil invertebrates in recent studies (Selonen et al., 2020). Plastic waste has been linked to the disturbance of marine creatures' endocrine systems (Jung et al., 2020), intestinal obstruction, and a false sense of satiety in aquatic animals (Paço et al., 2019). They've also been discovered to disrupt chemical communication in aquatic environments, induce intrinsic toxicity via leaching, and absorb persistent organic pollutants and infections (Barceló and Picó, 2019). Microplastics, which are plastic particles smaller than 5 mm in diameter, have gotten a lot of attention recently since they've been shown to cause irreversible harm in a variety of ecosystems (Lwanga et al., 2017). Microplastics have also been shown to clump together

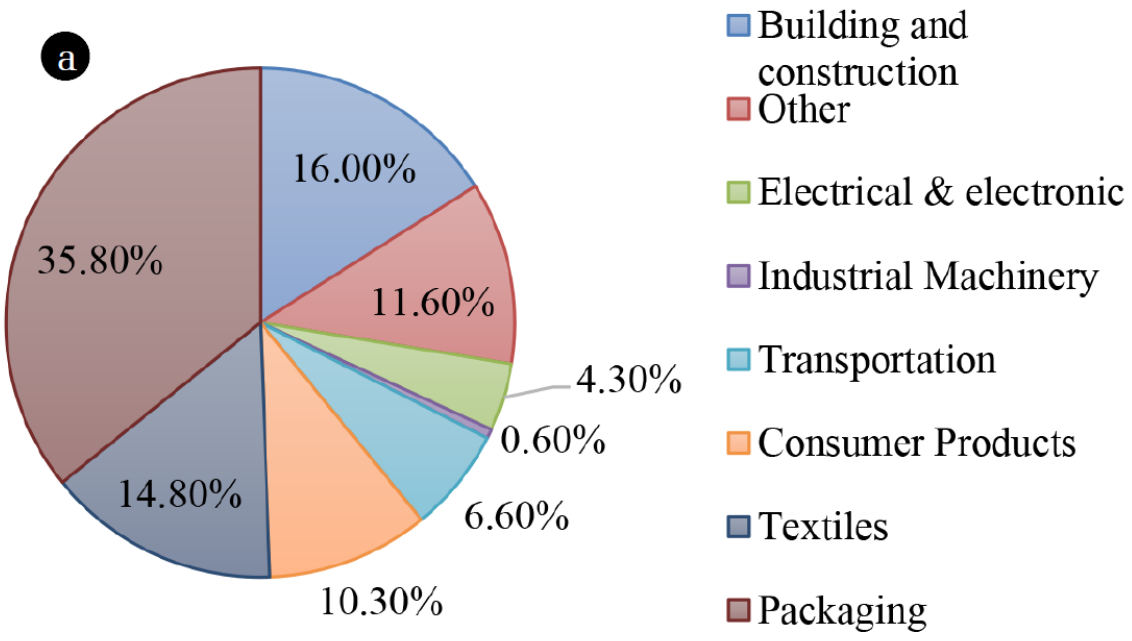
harmful substances in water, such as heavy metals and organic pollutants (Wang et al., 2019). Plastics and accumulated toxins may therefore infiltrate different food chains (terrestrial and aquatic) and ultimately make their way into the human body through trophic transfer of microplastics, presenting a number of possible health risks (Lwanga et al., 2017). Many environmental concerns have been raised as a result of industrial plastic production, particularly addressing the discharge of microplastics into the atmosphere and water systems. Industrial predecessors such as pellets, spherules, granules, discs, and other plastic raw materials are used to make a variety of microplastics (Lechner and Ramler, 2015). Mother liquors, organic halogenated solvents, washing liquids, and waste hydraulic oils are only a few of the harmful pollutants released into the environment by industrial facilities (Öncel et al., 2017). The bioaccumulation of plastic polymers in ecosystems is caused by their intrinsic properties that prevent breakdown, particularly their high molecular weight and crystallinity. Another stumbling block has been found as the lack of suitable functional groups required for oxidative reactions, since plastics are extremely hydrophobic and have stable functional groups such as alkane and phenyl (Devi et al., 2016). Incineration, recycling, landfilling, and the emerging usage of bioplastics are some of the current methods for reducing their environmental impact. However, each of these approaches has its own set of drawbacks. Incineration of various plastic polymers, for example, produces more hazardous and volatile wastes such as furans, dioxins, heavy metals, and sulphides, all of which are probable carcinogens (Verma et al., 2016). Plastic recycling has also been highlighted as being inefficient in terms of cost, since recycled plastics are more costly than virgin plastics (Gradus et al., 2017). Furthermore, "down-cycling" has been identified as a negative side effect of recycling, since recovered goods are often of lesser value and usefulness than virgin ones (da Silva and Gouveia, 2020). As a result, the use of biological systems as effective biodegradation alternatives for these refractory polymers has been a major focus of scientific research in recent years. The depolymerisation of these polymer chains by enzymes into intermediates with changed characteristics, enhancing their accessibility for cellular absorption, has been identified as the main process underlying the biodegradation of high molecular weight polymers such as plastic (Zhang et al., 2020). Different species, both higher and lower, capable of turning plastic polymers into simple molecules such as CO_2 and H_2O have been discovered, with insects receiving the most attention and microbes receiving less. The capacity of mealworms, superworms, and waxworms to consume, breakdown, and mineralize different plastic polymers has been shown in their larval stage, although with the assistance of their gut flora (Zhang et al., 2020). In addition to

symbiotic microorganisms that breakdown plastics in collaboration with insects, other microbes from diverse habitats have been proven to have biodegradative ability. As a result, the emphasis of this review is on current research into the functions of various microbes and their enzymes in the biodegradation of synthetic plastics. The method of action of these biodegraders, as well as the most important variables influencing plastic biodegradation, have been highlighted. The present and prospective use of biotechnological techniques in the alteration of different organisms and their enzymes for improved plastic breakdown is also discussed along with the plastic pollution status in Bangladesh. This study will serve as a valuable resource for academics and policymakers alike as they plot a new path in the microbial battle against plastic waste bioaccumulation.

Plastic Pollution

Pollution is defined by Landrigan et al. as undesired waste discharged into the air, water, and land as a result of human activities. It is the world's leading environmental cause of illness, resulting in early deaths, economic losses, the eroding of human capital, and ecological damage. Toxic metals, plastics, synthetic chemicals, agricultural runoff, and sewage are all part of the mix (Landrigan et al., 2020). Although the first commercial usage and large-scale manufacturing dates back to the 1950s, plastic has already become an inseparable element of human progress (Dorothy et al., 1999). Plastics are often produced from organic materials that are synthetic or semi-synthetic. Plastic is produced from cellulose, coal, natural gas, salt, and crude oil as basic ingredients, with petrochemicals accounting for the majority of industrial plastics (Hyat, 2015). Around 20 different kinds of plastics are used globally, according to European plastic producers' organizations (Plastics Europe, 2008). Polyethylene (PE), low-density and high-density polyethylene (HDPE/LDPE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), and polyurethane (PUR) resins, as well as polyester, polyamide, and acrylic fibres, are some of the most often used plastics (Plastics Europe, 2008). Plastic has a broad range of applications and is used in a variety of industries throughout the globe. The packaging sector is the largest user of plastics as it uses almost 36% of total global plastic output (Maria & Ieva, 2020). Surprisingly, this industry also contributes the most to global plastic waste output (Maria & Ieva, 2020). Plastic is appealing for various uses spanning from food packaging to electrical industries because of its cheap cost, durability, ease of manufacturing, lightweight, excellent thermal, and electrical insulation

(Richard et al., 2009; Anthony, 2015). However, the chemical link between the monomers responsible for plastic's longevity renders it resistant to many natural breakdown processes. Plastic waste does not degrade; instead, it accumulates in landfills and the ocean (Mark et al., 2011). Annually, more than 300 million metric tons of plastic are manufactured for different uses throughout the globe (Singh & Sharma, 2016), and plastic accounts for around 10% of the municipal trash stream by weight (Richard et al., 2009). Single-use plastics account for almost half of all manufactured plastic waste, mostly plastic bags, straws, stirrers, and takeout clamshells (NRDC, 2020). 25% of all plastics manufactured each year are burned, 20% are recycled, and the other 55% are directly discharged into the environment (Hannah and Max, 2018).



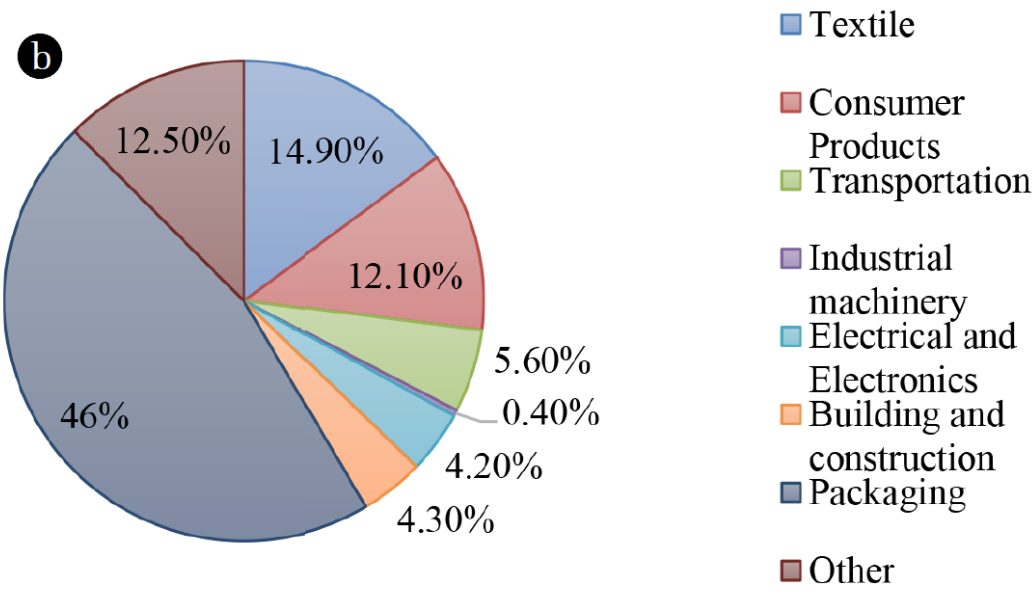


Figure 1 (a) Global plastic consumption by industry in 2018 **(b)** Global plastic waste output by sector in 2018 (Maria & Ieva, 2020).

During the Covid-19 pandemic in 2020, the usage of single plastics surged by a factor of ten. Around 96 percent of individuals use various kinds of personal protective equipment (PPE), such as disposable masks and face shields, which are immediately discarded into the environment, posing a plastic pollution risk (BBC, 2020). These massive quantities of plastic trash pose significant health and environmental risks. Plastic waste, particularly microplastics, has a significant impact on the marine ecosystem because they infiltrate the food chain, causing animal deaths due to indigestion and stomach bloating. Microplastics may also be found in fresh water systems and can enter the human food chain (Stefan-Adrian et al., 2019). Incineration and landfilling are the most popular plastic waste management techniques used by nations such as the United States, Germany, Brazil, and India (Rohit and Biswajit, 2015). Plastics that are disposed of directly in landfills are often burned, releasing 10,000 g of dioxins/furans into the atmosphere each year, posing significant health risks such as headaches, nausea, heart disease, respiratory sickness, and reproductive disorders (Kadapakkam and Sukhman, 2020; CPCB, 2010). Bangladesh, with a population of 166 million people, is a rapidly growing nation. Bangladesh has a good economic development despite the constraints imposed by its dense population. It now contains over 3,000 small and large plastic businesses, and plastic was ranked as Bangladesh's 12th biggest export generating industry in fiscal year 2017-18. (Inspira Advisory & Consulting Limited, 2019). With fast growth, Bangladesh's per

capita plastic consumption has increased dramatically from 2.07 kg in 2005 to 3.5 kg in 2014 (Monjur et al., 2017), with a daily output of 3000 tons of plastic waste, accounting for 8% of total waste produced (Mahmudul, 2019). Figure 2 depicts the amount of plastic waste produced per person per day in various nations (Hannah and Max, 2018), whereas Figure 3 depicts the proportion of plastic waste directly discharged into the environment in various countries (Hannah and Max, 2018). Though Bangladesh's per capita plastic use is low in comparison to other industrialized and neighbouring nations, the proportion of mismanaged plastic wate in the world total is quite high (Based on 2010 data). Furthermore, between 2005 and 2014, per capita plastic use grew at a pace of 16.2 percent, compared to a global rate of approximately 25 percent (Monjur et al., 2017). The plastic industries industry is projected to be worth about USD 3 billion, with USD 2.2 billion being local and USD 0.8 billion being foreign, and is anticipated to grow in the future (Momtaz, 2014). This expanding industry is expected to generate a significant amount of plastic waste, posing a major environmental concern. Plastic waste, for example, obstructs the flow of water by clogging drains and causing flooding. The development of the Aedes mosquito, which kills thousands of people each year, is aided by stagnant water in drains. The build-up of plastic waste has a significant impact on the Bay of Bengal's marine ecosystem. A study revealed that 6,705 pieces of plastic waste were recovered from four sea beaches in Cox's Bazar, with 63 percent of them being plastic (Mehedi, 2018). These huge plastic wastes have the potential to stifle fish development and kill beneficial species. Furthermore, plastic waste in soil has a negative effect on soil biota, soil environment, and fertility, as well as the agricultural industry (Jing-Jie et al., 2020). On March 1, 2002, the Bangladesh government implemented a poly bag ban in order to reduce plastic pollution (Ram et al., 2018). It also provides a tax break for recycling plastics in order to encourage recycling and discourage the use of single-use plastics. Over the years, however, there has been virtually little progress.

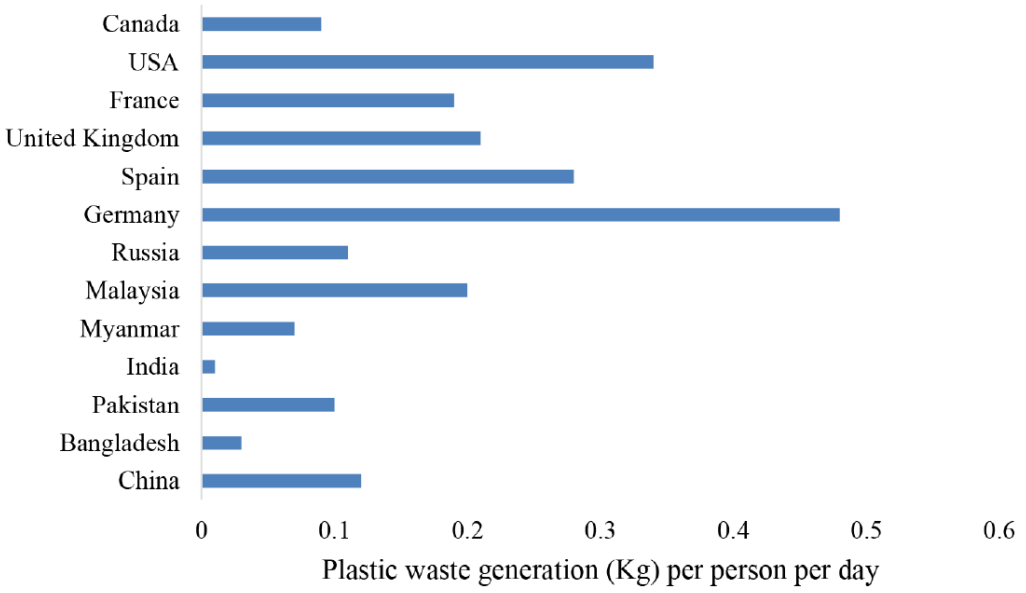


Figure 2: Amount of plastic waste produced per person per day in different nations (Hannah and Max, 2018).

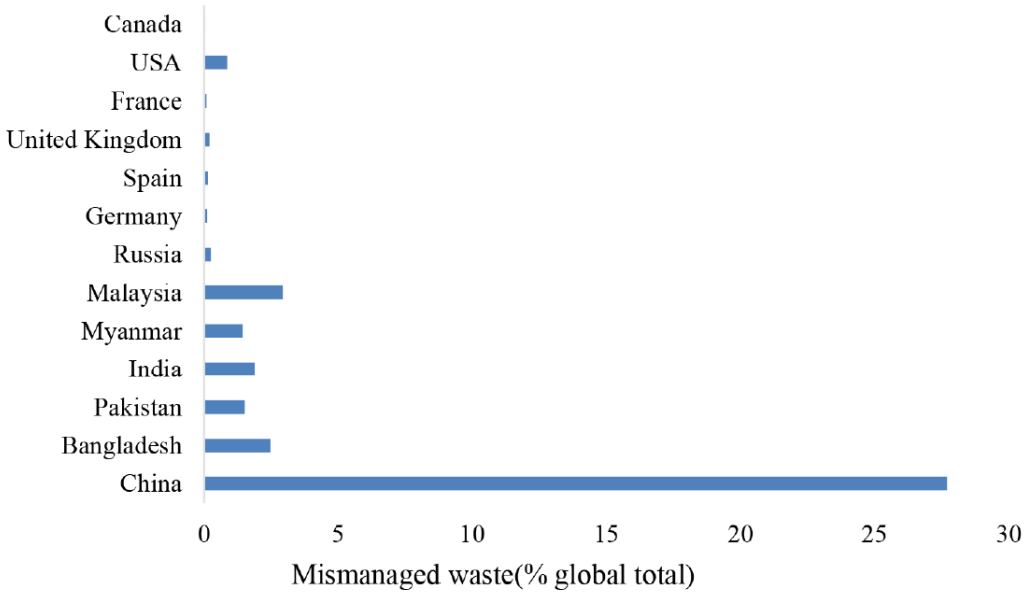


Figure 3: Contribution of various nations to worldwide mismanaged plastic waste (Hannah and Max, 2018).

Despite the fact that recycling is the most cost-effective and environmentally beneficial way to eliminate this massive plastic waste, recycling practices in Bangladesh are still in their infancy. Furthermore, rather of disposing of plastic waste in a suitable way for recycling, many choose

to dump it in open areas, along the roadside, in the river, or on the seashore (Monjur et al., 2017). The national use of plastics in 2014, for example, was 545,300 tonnes, while the plastic waste accessible for recycling was about 50,213 tons, which means that just 9,2% of total plastic used in the country was eligible for recycling (Khondaker, 2016). Landfilling or dumping of discarded plastics in water bodies such as channels, lakes, rivers, and even the sea, which eventually impact human health, is driven by high costs involved with recycling, a lack of accessible solutions, and knowledge of the consequences of plastic pollution (Monjur et al., 2017). Currently, Bangladesh's metropolitan regions produce 633,129 tons of plastic waste per year, of which 51 percent is recycled and the rest could save USD 801 million per year if recycled (Waste Concern, 2016). However, the two Dhaka municipal corporations, Dhaka North and Dhaka South, have lately prioritized the collecting of plastic from users, but the majority of it, along with other waste, is thrown in landfill. Bangladeshi plastic recycling businesses solely export recycled plastic flakes rather than producing any goods with a bright future (Mehnaz and Aditi, 2020). Furthermore, due to the high calorific value of plastic trash, which ranges from 20 to 46 MJ/kg, it has been estimated that 5115–11,760 MWh/d electricity may be produced from daily plastic wastes through gasification or incinerator energy recovery (Ohidul, 2015). The government has just recently begun construction of two waste-to-energy generating plants in Dhaka, one at the Aminbazar landfill and the other at the Matuail dump, to help make the city more livable and clean (Syed, 2020). In Bangladesh, there has been minimal attempt to evaluate the quantity of plastic trash in various environmental compartments, as well as the related environmental and human health effects. In light of the existing global and restricted regional research, this study seeks to depict the situation of plastic pollution in Bangladesh and the negative impact it has on the air, soil, and water, as well as public health.

Involvement of Microbes

Microbes of all types are at the vanguard of avoiding the bioaccumulation of different inorganic and organic chemicals in the environment, thus understanding their involvement in the biotransformation of xenobiotic molecules like plastic polymers is critical in contemporary biotechnology. The biotic component of synthetic plastic breakdown is mostly due to the activity of different microbial communities that have been identified as potential xenobiotic degraders based on their capacity to adapt to and utilize these compounds as growth and energy substrates. These organisms use their diverse enzyme systems to breakdown polymers into intermediates that may then be absorbed and metabolized to meet their energy requirements. Different actinomycetes, algae, bacteria, and fungi with the ability to biodegrade diverse plastic polymers have recently been studied in this respect. However, the pace at which different microorganisms degrade synthetic polymers seems to be rather sluggish, making the biodegradative process unsuitable for real-time commercial applications. Furthermore, this has led in the hunt for different additives to improve these materials' biodegradability (Selke et al., 2015), as well as the exponential development of bioplastics with higher biodegradability potentials (Thiruchelvi et al., 2020). Certain significant microorganisms and enzymes connected with plastic degradation are emphasized, as well as some their plastic biodegradation ability and features.

3.1 Actinomycetes

Actinomycetes are a complex genus of filamentous bacteria found in soil, plant tissues, and marine habitats. They're known for their metabolic flexibility and a wide range of biotechnological uses, including bioremediation, medicine and the food industry. Actinomycetes from several ecological zones, including the *Streptomyces* groups, *Rhodococcus ruber*, *Actinomadura spp*., and the thermophilic *Thermoactinomyces* species, have been isolated and shown to have considerable plastic biodegradative ability (Auta et al., 2018; Jabloune et al., 2020). Their ability to generate a broad range of hydrolytic enzymes as well as other bioactive metabolites has previously been emphasized (Gohain et al., 2020). These hydrolytic enzymes are one of the most important aspects in their capacity to thrive on a variety of plastic polymers and breakdown large molecular weight molecules into simpler ones. They're also known to generate extracellular polymers including dextran, glycogen, levan, and N-acetylglucosamine-rich slime polysaccharides, which help them adhere to plastic surfaces for microbial activity (Pujic et al., 2015). Biofilm development, like bacteria, has been

demonstrated to play a significant role in actinomycetal colonization of plastics (Gilan and Sivan, 2013). Using an esterase enzyme with a broad substrate specificity, *Streptomyces scabies*, a potato isolate, was found to degrade PET as well as other polymers such as p-nitrophenyl esters, cutin, and suberin (Jabloune et al., 2020). *Nocardiopsis sp*., an endophytic actinomycete isolated from hibiscus, was similarly shown to breakdown PE and fuel (Singh and Sedhuraman, 2015). In a microbial consortium, the efficacy of actinomycetal plastic degradation was also shown, with a significant proportion of actinomycetal species degrading polyurethane and other chemical additives (Gaytán et al., 2020).

3.2 Algae

Algae, both photosynthetic and heterotrophic, have been extensively researched for their important roles in bioremediation and other industrial uses. They are capable of removing both inorganic and organic contaminants from a variety of settings by collecting, adsorbing, or metabolizing them to safer levels (Hwang et al., 2020). However, compared to other microorganisms, just a few studies have been conducted on algae's ability to breakdown manmade plastic polymers. The majority of research has been on their application in the manufacture of green polymers. Despite the fact that many studies have shown that marine algae may absorb plastic items, this remains the case. Even in terrestrial environments, algae such as *Anabaena, Chlorella, Spirogyra, Nostoc, Oscillatoria*, and *Spirulina* have been seen colonizing various plastic surfaces, but there is no indication that they metabolized the polymers (Sarmah and Rout, 2018). Kumar et al. (2017) found that *Scenedesmus dimorphus, Anabaena spiroides*, and *Navicula pupula* can degrade both high and low-density PE, with *A. spiroides* being the most promising, decomposing 8.18 percent low density polyethylene after 30 days. *Spirulina sp*. was also able to biodegrade PET and PP, although the rate of degradation was considerably lower than that found in bacterial and fungal cells after 112 days in the research (Khoironi and Anggoro, 2019). These findings are not surprising, given that algae, unlike bacteria, use CO_2 from the atmosphere as their major carbon source and sunlight as their primary energy source (Dineshbabu et al., 2020). As a result, despite their ability to colonize plastic surfaces and assimilate microplastics, their metabolic pathways are not inclined to mineralize them, which is a major source of concern because this messed-up process has been identified as a pathway for plastic to bioaccumulate and find its way into the food chain (Hoffmann et al., 2020). However, a recent research has successfully used *Phaeodactylum tricornutum*'s exceptional ability as a genetic host and its low-cost growing conditions for the

biodegradation of PET. This was achieved by expressing the PETase gene from the photosynthetic diatom *Ideonella sakaiensis* in the photosynthetic diatom (Moog et al. 2019).

3.3 Bacteria

Bacteria are known as the "engine" of the earth's nutrient cycle because they are at the forefront of nutrient transformation and cycling. Their function in decomposition, like that of other microbes, guarantees that carbon and nutrients are released from a variety of complex polymers, both natural and manufactured. They've been researched for their important functions in bioremediation, and they've been proven to breakdown a variety of materials, including antibiotics, metal compounds, petroleum, plastic, and other chemicals that have become popular in the Anthropocene period. Various bacterial species from the *Pseudomonas*, *Escherichia*, and *Bacillus* genera have been demonstrated to have considerable ability to breakdown plastic polymers using different techniques such as metagenomics, cloning, pure culture, and even computational methods (Gan and Zhang, 2019). These plastic-degrading bacteria have been found in a variety of biological settings, including dumpsites (Muhonja et al., 2018), recycling sites (Yoshida et al., 2016), landfills (Gaytán et al., 2020), the cold sea environment (Urbanek et al., 2018), and insects' guts (Ren et al., 2019). Studies have revealed that bacteria's ability to breakdown plastic is based on their inherent propensity to digest long-chained fatty acids, thus it's no surprise that Pseudomonas is the most well-studied bacterial genus when it comes to polymer degradation (Wilkes and Aristilde 2017). Biofilm production has been shown to play an important role in the bacterial breakdown of plastics, as it increases colony adhesion and persistence on the plastic surface (Puglisi et al., 2019). The ability of *I. sakaiensis*, a novel species isolated from a consortium of dumpsite bacteria, to degrade PET because it used the polymer as its primary source of energy and carbon, has been known to be one of the most significant findings on plastic degradation (Yoshida et al., 2016). Other common thermoplastics destroyed by *Pseudomonas aeruginosa*, *Bacillus megaterium, Rhodococcus ruber, Serratia marcescens, Staphylococcus aureus, Streptococcus pyogenes*, and other bacterial species include polystyrene and polycarbonate (Arefian et al., 2020; Ho et al., 2018). Similarly, microorganisms from other groups, such as *Bacillus*, *Pseudomonas*, and *Micrococcus*, have been shown to degrade various thermoset polymers, primarily polyurethane (Espinosa et al., 2020; Shah et al., 2008). Although most research focused on the biodegradability of single bacterial strains, in nature, bacteria typically work together as consortia, which has also been shown in many investigations (Lwanga et al., 2018; Shah et al.,

2008). As previously mentioned, a variety of extrinsic variables may influence the pace of microbial biodegradation in a negative or positive way, and some of these parameters have been investigated to speed up bacterial breakdown of various plastic polymers in vitro. Some bacteria, such as *Pseudomonas aeruginosa*, *Burkholderia seminalis*, and *Stenotrophomonas pavanii*, showed an improvement in the rate of PE breakdown when particular additives such as food-grade dye-sensitised nanoparticles and starch were added (Mehmood et al., 2016). Other pretreatment techniques have recently been demonstrated to substantially enhance the rate of bacterial breakdown of different plastic polymers, including anionic surfactant addition (Mukherjee et al., 2017), heat treatment (Savoldelli et al., 2017), and UV pretreatment (Montazer et al., 2018).

3.4 Fungi

Among all microorganisms, fungi and bacteria serve the most important roles in the preservation of biogeochemical cycles and vital nutrients on Earth. Different fungal species have been emphasized for their capacity to breakdown various plastic polymers based on their ability to use these synthetic polymers as their primary/sole carbon or energy source. In this respect, a diverse range of fungal strains from various classes, ecologies, and morphologies have been shown to destroy plastics. According to current research, the *Aspergillus* genus is the most important fungal group for synthetic plastic biodegradation. Some of the species are *A. clavatus* (Gajendiran et al., 2016), *A. fumigatus* (Osman et al., 2018), and *A. niger* (Usman et al., 2020). *Aspergillus* species have been isolated from various terrestrial environments and found to degrade PE, PU, and PP. Endophytic fungi isolated from various plants were shown to degrade PU to varied degrees in both solid-state and submerged fermentation conditions in a research (Russell et al., 2011). *Fusarium solani, Alternaria solani, Spicaria sp., Geomyces pannorum, Phoma sp., Penicillum spp*., and other fungi with considerable plastic degradability include *Fusarium solani, Alternaria solani, Spicaria sp., Geomyces pannorum, Phoma sp., Penicillum spp*., and others (Muhonja et al., 2018; Zhang et al., 2020). Furthermore, in contrast to most research that have concentrated on the potentials of pure cultures, many fungal consortia have been found to degrade different types of plastics in a synergistic manner, including PU (Cosgrove et al., 2007) and PE (Cosgrove et al., 2007, Sowmya et al., 2015). The functions of fungal enzymes, particularly depolymerases, have been emphasized in all of these investigations, as they have been in all biological processes. Furthermore, these enzymes' wide specificity, which enables them to break down a variety of polymers, is important (da Luz et al., 2019). Fungal hyphae's dispersion and penetrative capacity, as well as their ability to

produce hydrophobins for increased hyphal adhesion to hydrophobic surfaces, have been observed to be important factors in their initial colonisation prior to eventual depolymerisation (Sánchez, 2020). Pretreatment of various substrates involving different factors such as photo-treatment and temperature (Corti et al., 2010), acid pretreatment (Mahalakshmi and Andrew, 2012), and various additives (Jeyakumar et al. 2013; Sánchez, 2020) has demonstrated the enhancement of fungal biodegradation of plastics.

Table 3.1 Polyethylene degrading microbes

PE Degrading Microbes			
Strain	**Enzyme**	**Deg. Prod**	**Reference**
Rhodococcus ruber C208		x	Orr et al. 2004
Bacillus sphericus Alt; *Bacillus cereus BF20*		x	Sudhakar et al. 2008
Arthrobacter sp. GMB5; *Pseudomonas sp. GMB7*		x	Balasubramanian et al. 2010
Pseudomonas sp. E4		x	Yoon et al. 2012
Pseudomonas sp. AKS2		x	Tribedi and Sil et al. 2013
Bacillus subtilis H1584		x	Harshavardhan and Jha, 2013
Enterobacter asburiae YT1; *Bacillus sp. YP1*		detected	Yang et al. 2014
Serratia marcescens		x	Azko et al. 2015
Achromobacter xylosoxidans		x	Kowalczyk et al. 2016
Zalerion maritimum		x	Paco et al. 2017
Phormidium lucidum; *Oscillatoria subbrevis*		x	Sarmah and Rout, 2018
Alcanivorax borkumensis		x	Delacuvellerie et al. 2019
Phanerochaete chrysosporium	*manganese peroxidase*	x	Iiyoshi et al. 1998
Rhodococcus ruber C208	laccase	x	Santo et al. 2013
Pseudomonas sp.E4	*alkB gene*	x	Kim et al. 2012
Pseudomonas saeruginosa E7	*alkB1, alkB2 gene*	x	Jeon and Kim, 2016a
Acinotobacter bumannii		detected	Pramila and Ramesh et al. 2015
Arthobacter defluvii; *Bacillus amyloliquefaciens;* *Bacillus subtilis*		x	Thakur et al. 2012
Bacillus pumilus; *Bacillus subtilis*		x	Harshvardhan and Jha, 2013
Bacillus sp.		detected	Nowak et al. 2011
Bacillus sphericus		x	Sudhakar et al. 2008
Bacillus megaterium; *Bacillus subtilis;* *Bacillus cereus (MIX together)*		x	Abrusci et al. 2011
Bacillus amyloliquefaciens		x	Das and Kumar, 2013
Bacillus subtilis		x	Vimala and Mathew, 2016

Bacillus pumilus M27; *Bacillus subtilis H1584*		x	Harshvardhan and Jha, 2013
Brevibacillus borstelensis		x	Hadad et al. 2005
Brevibacillus		x	Nanda and Sahu, 2010
Chryseobacterium gleum		x	Jeon and Kim, 2014
Comamonas sp.		detected	Peixoto et al. 2017
Delftia sp.		detected	Peixoto et al. 2017
Kocuria palustris M16		x	Harshvardhan and Jha, 2013
Microbacterium paraoxydans		x	Rajandas et al. 2012
Pseudomonas sp.		x	Kathiresan et al. 2013
Pseudomonas aeruginosa		x	Jeon and Kim, 2015
Pseudomonas sp.		detected	Yoon et al. 2012
Pseudomonas sp.		x	Usha et al. 2011
Pseudomonas citronellolis		x	Bhatia et al. 2014
Pseudomonas sp.		x	Rajandas et al. 2012
Pseudomonoas aeruginosa; *Pseudomonas putida;* *P. siringae*		x	Kyaw et al. 2012
Pseudomonas sp.		x	Nanda and Sahu, 2010
Rhodococcus ruber		x	Gilan et al. 2004
Rhodococcus ruber		x	Sivan et al. 2006
Rhodococcus ruber		detected	Santo et al. 2013
Rhodococcus rhorocuros		x	Bonhomme et al. 2003
Rhodococcus rhorocuros		detected	Fontanella et al. 2010
Rhodococcus sp.		x	Nanda and Sahu, 2010
Rhodococcus sp.		detected	Koutny et al. 2009
Staphylococcus arlettae		x	Divyalakshmi and Subhashini, 2016
Stentrophomonas sp.		detected	Peixoto et al. 2017
Stentrophomonas pavanii		detected	Mehmood et al. 2016
Streptomyces sp.		x	El-Shafei et al. 1998

Table 3.2 Polyethylene-terephthalate degrading microbes

PET degrading microbes			
Strain	**Enzyme**	**Deg. Prod**	**Reference**
Thermobifida fusca	TfH		Muller et al. 2005
Humicola insolens; *Pseudomonas mendocina;* *Fusarium solani*	HiC; PmC; FsC		Ronkvist et al. 2009
Fusarium solani	LC-cutinase		Sulaiman et al. 2012
Saccharomonospora viridis	Cut190		Kawai et al. 2014
Idonella sakaiensis	*Is*PETase		Yoshida et al. 2016
Idonella sakaiensis	*IsPETase*		Wei et al. 2019a
Thermobifida fusca	TfCut2		Wei et al. 2019b

Table 3.3 Polyurethane degrading microbes

PUR degrading microbes			
Strain	**Enzyme**	**Deg. Prod**	**Reference**
Chaetomium globosum		x	Darby and Kaplan, 1968
Curvularia senegalensis		x	Crabbe et al. 1994
Geomyces pannorum		x	Cosgrove et al. 2007
Alternaria sp. PURDK2		detected	Matsumiya et al. 2010
Pestalotiopsis microspora		x	Russel et al. 2011
Aspergillus flavus		x	Mathur and Prasad, 2012
Cladosporium tenuissimum		detected	Alvarez-Barragan, 2016
Aspergillus tubingensis		x	Khan et al. 2017
Aspergillus sp. S45		detected	Osman et al. 2017
Penicillium sp.		x	Magnin et al. 2019a
Corynebacterium sp. B12; Pseudomonas aeruginosa		x	Kay et al. 1991
Comamonas acidovorans		detected	Nakajima-Kambe, 1995
Bacillus sp.		x	Ii et al. 1998
Pseudomonas flurescens		x	Howard and Blake, 1998
Pseudomonas chlororaphis		x	Howard et al. 1999
Bacillus subtilis		x	Rowe and Howard, 2002
Acinetobacter gerneri		x	Howard and Burks, 2012
Alicycliphilus sp. BQ1		detected	Ocegura-Cervantes, 2007
Bacillus pumilus		x	Nair et al. 2007
Pseudomonas chlororaphis		x	Gautam et al. 2007
Bacilus sp. AF8; Pseudomonas sp. AF9; Micrococcus sp. 10; Arthrobacter sp. AF11; Corynebacterium sp. AF12		x	Shah et al. 2008
Bacillus subtilis; Pseudomonas aeruginosa		detected	Shah et al. 2016
Pseudomonas putida		x	Peng et al. 2014
Bacillus safensis		x	Nakkabi et al. 2015ab
Aspergillus niger; Cladosporium herbarum		x	Filip et al. 1979
Staphylococcus epidermis		x	Jansen et al. 1991
Alternaria tenuissima		x	Oprea et al. 2018
Pseudomonas denitrificans; Pseudomonas fluorescens; Bacillus subtilis; Yarrowia lipolytica		x	Stepien et al. 2017
Curvularia senegalensis	esterase	x	Crabbe et al. 1994
Comamonas acidovorans	pudA	x	Akutsu et al. 1998
Bacillus subtilis	lipase	x	Rowe and Howard, 2002

Pseudomonas fluorescens	pulA	x	Ruiz et al. 1999b
Pseudomonas chlororaphis	pueA	x	Stern and Howard, 2000
Pseudomonas chlororaphis	pueB	x	Howard et al. 2001
Thermobifida fusca	LC cutinase; TfCut2; Tcur1278; Tcur0390	x	Schmidt et al. 2017

Table 3.4 Polypropylene degrading microbes

PP degrading microbes			
Strain	**Enzyme**	**Degradation Prod**	**Reference**
Pseudomonas stutzeri; Bacillus subtilis; Bacillus flexus		detected	Arkatkar et al. 2010
Phaenerochaete chysosporium; Engyodontium album		detected	Jeyakumar et al. 2013
Stenotrophomonas panacihumi		x	Jeon and Kim, 2016b
Aneurinibacillus aneurinilyticus; Brevibacillus agri; Brevibacillus sp.; Brevibacillus brevis		detected	Skariyachan et al. 2018
Bacillus sp. strain 27; Rhodococcus sp. strain 36		x	Auta et al. 2018

Table 3.5 Polystyrene degrading microbes

PS degrading microbes			
Strain	**Enzyme**	**Deg. Prod**	**Reference**
Xanthomonas sp.; Sphingobacterium sp.; Bacillus sp. STR-YO		x	Eisaku and Linn, 2003
Rhodococcus ruber C208		x	Mor and Sivan, 2008
Microbacterium sp. NA23; Paenibacillus urinalis NA26; Bacillus sp. NB6; Pseudomonas aeruginosa NB26		detected	Atiq et al. 2010
Rhizopus oryzae NA1; Aspergillus terreus NA2; Phaenerochaete chrysosporium NA3		detected	Atiq et al. 2011
Exiguobacterium sp. YT2		detected	Yang et al. 2015c

Azotobacter beijerinckii HM121	hydroquinone peroxidase	detected	Nakamiya et al. 1997

Table 3.6 Polyvinyl chloride degrading microbes

PVC degrading microbes			
Strain	**Enzyme**	**Deg. Prod**	**Reference**
Alternaria sp. TOF-46		x	Moriyama et al. 1993
Poliporus versicolor; *Pleurotus sajor caju*		detected	Kirbas et al. 1999
Aureobasidium pullulans		x	Webb et al. 1999
Aspergillus niger		x	Gumargalieva et al. 1999
Aurobasidium pullulans		x	Webb et al. 2000
Penicillium janthinellum		x	Sabev et al. 2006
Mycobacterium sp. NK0301		detected	Nakamiya et al. 2005
Chryseomicrobiumm imtechense; *Lynsinibacillus fusiformis;* *Acinetobacter calcoaceticus;* *Stenotrophomonas pavanii*		x	Latorre et al. 2012
Phanerochaete chrysosporium; *Lentinus tigrinus;* *Aspergillus niger;* *Aspergillus sydowii*		detected	Ali et al. 2014
Acanthopleurobacter pedis; *Bacillus cereus;* *Pseudomonas otitidis;* *Bacillus aerius*		detected	Anwar et al. 2016
Bacillus sp. AIIW2		detected	Kumari et al. 2018
Phanerochaete chrysosporium		detected	Khatoon et al. 2019
Pseudomonas citronellolis		x	Giacomucci et al. 2019

Types of Plastics and their Enzymatic Degradations

Plastic degrading enzymes have subsequently been divided into two wide groups, namely, extracellular and intracellular enzymes as other enzymes involved in biologic breakdown of polymers (Gu, 2003). However, the category most researched are extracellular enzymes, which have a large spectrum of reactivity, ranging from oxidative to hydrolytic (Glaser, 2019). They are primarily engaged in the depolymerisation of long carbon chains in plastic polymers to a combination of oligomers, dimers, and, in rare cases, monomers. These enzymes have been categorized as laccases, peroxidases, lipases, esterases, hydrolases, carboxylases, and cutinases because they function similarly to microbial laccases, peroxidases, lipases, esterases, hydrolases, carboxylases, and cutinases (Gan and Zhang, 2019). These extracellular enzymes are also thought to be engaged in heterogeneous reactions at the solid/liquid boundary, since they act on macromolecules present on the solid plastic's surface while in the liquid phase (Chinaglia et al., 2018). The surface functionalisation of hydrophobic plastic surfaces, the breakdown of plastic metabolic intermediates into monomeric units, and the ultimate mineralisation of the final monomeric intermediates are all mediated by different enzyme groups. The aerobic and anaerobic processes required to convert intermediates to molecules that may be absorbed by bacteria are carried out by a vast number of intracellular enzymes (Pathak, 2017). However, there is a scarcity of knowledge on the biochemical capabilities and structural features of plastic degrading enzymes. There have been many categorization systems for these enzymes; however, in this study, the plastic degrading enzymes are classified by the polymer they operate on, namely polyethylene, polyurethane, polyethylene terephthalate, polypropylene, polystyrene, polyvinyl chloride and nylon. Several common polymers may be effectively degraded using a variety of microbes and enzymes. This section is a list of some of them, with dates ranging from the invention of their usage to the current day.

4.1 Polyethylene

Polyethylene or polythene, often abbreviated as PE, is a polymer with the chemical formula $(C2H4)_n$ that is generally made up of ethylene with different n values. Polyethylene comes in a variety of densities, from high to low, and is typically thermoplastic, although it can readily be converted to thermosetting. Polyethylene is mostly utilized in packaging, such as plastic bags, films, geomembranes, containers, and bottles, among other things. Polyethylene was first produced by German scientist Hans von Pechmann by accident in 1898 while researching

diazomethane (Pechmann, H., 1898). Polyethylene is a commonly used plastic polymer in today's world, accounting for about 100 million tonnes produced each year, or 34% of the global plastics industry in 2017. (Geyer et al., 2017, Plastics Europe, 2018).

Tsuchii et al. discovered in 1984 that *Acinetobacter sp* 351 could partially degrade low-molecular-weight polyethylene upon dispersion, but not high-molecular-weight polyethylene (Tsuchii et al 1984). Pometto later reported in 1992 that the PE portion of a heat-treated plastic blend was degraded by concentrated culture supernatants of lignocellulose degrading *Streptomyces* species exhibiting LiP activity (Pometto et al 1992). Iiyoshi et al. identified manganese peroxidase (MnP) as the major enzyme involved in the membrane degradation of high molecular weight PE by the lignin-degrading fungi *Phanerochaete chrysosporium* ME-446 (a white-rot fungus), *Trarnetes versicolor*, and the isolate IZU-154 from the hypnolignolytic fungi in 1998. Under nitrogen or carbon-limited growth circumstances, isolate IZU-154 exhibited the most substantial polyethylene degradation among the three lignin-degrading fungi, while *Phanerochaete chrysosporium* ME-446 could reduce the molecular weight and tensile strength of the polyethylene film (Iiyoshi et al., 1998). Tween 80, Tween 20, and CHAPSO are examples of surfactants that have been found to aid in the breakdown of PE by partially pure MnP. (Ehara et al. 2000, Iiyoshi et al, 1998). The genes encoding the most active MnP from IZU-154 have been discovered and described, albeit solely in terms of 2,6-dimethoxyphenol oxidation (Matsubara et al., 1996). Ei-Shafei et al. also looked into the capacity of fungus and *Streptomyces* strains to degrade degradable polyethylene in the form of discarded polyethylene bags containing 6% starch. Eight distinct isolated *Streptomyces* strains were used in the study, as well as two fungus, *Mucor rouxii* NRRL 1835 and *Aspergillus flavus* (Ei-Shafei et al., 1998). Yamada-Onodera et al. identified a strain of the fungus *Penicillium simplicissimum* YK capable of biodegrading polyethylene in 2001 (Yamada-Onodera et al., 2001). Later, Fukisawa et al. demonstrated that in the presence of a synthetic redox mediator, 1-hydroxybenzotriazole, which mediated the laccase enzyme's oxidation of non-phenolic substrates, a system composed of a fungal laccase from *Trametes versicolor* significantly reduced the molecular weight of a PE membrane (Fujisawa et al., 2001). Zhao et al. discovered in 2004 that a mixture of soybean peroxidase (SBP) and hydrogen peroxide may oxidize PE film surfaces and reduce surface hydrophobicity (Zhao et al., 2004). *Rhodococcus ruber* and *Brevibacillus borstelensis* are two other comparable degraders in laboratory conditions (Gilan et al. 2004; Hadad et al. 2005). Hadad et al. obtained from soil a *Brevibacillus borstelensis* strain that decomposed branching low-density polyethylene by using it as the only carbon and

energy source (Hadad et al. 2005). In 2007, Shah et al. investigated low-density polyethylene pieces buried in soil mixed with sewage sludge microscopically after 10 months and discovered fungal adhesion on the plastic's surface, suggesting that plastic may be used as a nutrition source (Shah, et al. 2007). *Fusarium sp.* AF4, *Aspergillus terreus* AF5, and *Penicillum sp*. AF6 were identified as the isolated fungal strains. The fungal strains' capacity to build a biofilm on polyethylene was ascribed to the surface's progressive reduction in hydrophobicity (Gilan et al., 2004). PE is structurally significantly different from the conventional substrates of laccases, phenolics, as described by Giardina et al. in 2010 (Giardina et al., 2010). However, since bacterial laccases are less extensively researched than fungal laccases, it's conceivable that the *R. ruber* laccase possesses unique characteristics that allow it to directly target PE. Otherwise, it's conceivable that *R. ruber* uses an as-yet-unidentified redox mediator, since these tiny molecules have been shown to significantly expand the substrate range of laccases (Giardina et al. 2010). Laccases may aid in the oxidation of the hydrocarbon backbone of polyethylene, according to Sivan et al. Other bacteria capable of degrading polyethylene include *Brevibacillus spp*. and *Bacillus spp*., where proteases are involved in the process (Sivan et al. 2011). In 2012, Yoon et al. revealed that an enzyme called alkane hydroxylase was discovered to be essential in the breakdown of low molecular weight PE by *Pseudomonas sp*. E4, and that its alkane hydroxylase gene alkB was implicated (Yoon et al., 2012). It was further verified by cloning three alkane hydroxylase genes in E. coli, alkB, alkB1, and alkB2 (Kim et al., 2012; Yoon et al., 2012; Jeon and Kim, 2015, 2016). These findings suggested that alkB, alkB1, or alkB2 played a significant role in polymer breakdown and recombinant protein assessment. In the presence of copper, Santo et al. showed that an extracellular thermostable bacterial laccase produced by *Rhodococcus ruber C208* causes oxidation and molecular weight decrease of UV-irradiated PE films (Santo et al., 2013). He also discovered that the laccase from *T. versicolor*, a common model enzyme, had no impact on PE (Santo et al., 2013). However, significant biodegradation without previous oxidation has been reported, such as by a *Pseudomonas* species degrading untreated PE (Tribedi and Sil 2013). In 2014, Sowmya et al. demonstrated that *Bacillus cereus* decomposed UV-irradiated PE, which was linked with a significant extracellular synthesis of both laccases and manganese peroxidases (Sowmya et al., 2014). Mukherjee and Kundu found that an extracellular lignin peroxidase (LiP) and a manganese peroxidase (MnP) from *Phanerochaete chrysosporium* MTCC-787 degraded a pre-oxidized high molecular weight PE sample after 15 days of incubation (Mukherjee and Kundu, 2014). In 2015, Jeon and Kim discovered an enzyme system in *P. aeruginosa* that included alkane hydroxylase, rubredoxin, and rubredoxin reductase, all of which were implicated in the

breakdown of low molecular weight PE (Jeon and Kim, 2015). Sowmya et al. discovered a Penicillium-derived laccase as possibly implicated in PE breakdown within this framework. Incubation of similarly pre-treated PE with a partly purified laccase and a MnP from *Penicillium simplicissimum*, on the other hand, resulted in just a little weight loss (Sowmya et al., 2015). Azeko et al. also observed the weight loss of untreated PE degraded by the *Serratia marcescens* strain (Azeko et al., 2015). Furthermore, strains of *Pseudomonas mediterranea* and *Bacillus megaterium* both degraded PE (LDPE and HDPE) and the relevant genes of interest were discovered, extracted, and heterologously produced in *E. coli* (Liu et al 2015). Wei and Zimmerman proposed in 2017 that some *Pseudomonas spp*. could breakdown petroleum-based plastics, such as plastic cups, polythene bags, and other LDPE-based materials, utilizing various enzymes. Furthermore, *Pseudomonas putida* is a well-known and well-characterized species that has served as a workhorse for genetic engineering research, thus this genus is of considerable relevance for low-density PE biodegradation in the future (Wei and Zimmerman et al 2017). Sarmah et al. (2018) demonstrated the capacity of two cyanobacteria, *Phormidium lucidum* and *Oscillatoria subbrevis*, to degrade PE (Sarmah et al., 2018). Laccases in fungi such as *Pleurotus ostreatus* have also shown substantial breakdown of PE, according to Gomez and Mendel (Gomez-Mendez et al., 2018). In addition, Xu et al. proposed in 2019 that oxidases or oxygenases may cleave the carbon-carbon bonds of polyolefins (PE and PS) via enzymatic cleavage based on quantum mechanics simulations. However, further research is needed to define the molecular roles of oxidases or oxygenases in the biodegradation of PE, such as the enzymes produced by the genes alkB, alkB1 or alkB2 (Xu et al., 2019). Laccases from fungi such as *Aspergillus flavus* have also shown substantial breakdown of PE, according to Zhang et al. in 2020. (Zhang et al., 2020). Yang et al. also isolated two bacterial strains from the stomach of the waxworm *Plodia interpunctella*, *Enterobacter asburiae* YT1 and *Bacillus sp*. YP1, and showed their capacity to degrade PE. These results suggested that waxworm bacteria might be a viable source for testing PE-degrading microorganisms in the future (Yang et al., 2014; Yang et al., 2015). Because abiotic oxidation has been demonstrated to play a key role in PE biodegradation, degradable PE formulations have been developed and promoted as more ecologically acceptable alternatives. These polymers often include weak spots in the polymer backbone (Bremer et al., 1982) or pro-oxidant additives (David et al., 1992), resulting in increased fragmentation and breakdown of the plastics. Polyethylene degradation is a combination of photo- and bio-degradation. Essential abiotic precursors are acquired first, either via abiotic oxidation (UV light exposure) or heat treatment. Second, chosen thermophilic

bacteria finish the biodegradation process by degrading the low molar mass oxidation products (Bonhomme et al., 2003).

4.2 Polyethylene terephthalate

Polyethylene terephthalate is a polar, linear polymer composed of repeated units of aromatic terephthalic acid and ethylene glycol joined together by ester bonds with repeating $C_{10}H_8O_4$ units (Webb et al., 2013, Gubbels et al., 2018). It is the most prevalent thermoplastic polymer resin of the polyester family and is often abbreviated as PET or PETE. Polyethylene terephthalate (PET) is primarily used in the manufacture of synthetic fibers for clothing and the textile industry, but it is also widely used in plastic bottles, containers for liquids and foods, thermoforming for manufacturing, and in combination with glass fiber for engineering resins, accounting for a significant portion of annual PET production. John Rex Whinfield, James Tennant Dickson, and the Calico Printers' Association of Manchester in England were the first to patent PET in 1941 (Whinfield, John Rex and Dickson, James Tennant, 1941). The trademark is now owned by DuPont Teijin Films US, a joint venture with a Japanese firm (TEIJIN: Trademarks). Because of its extensive use in packaging materials, beverage bottles, and the textile sector, worldwide PET output surpassed 41.6 million tons in 2014. (Research and Markets, 2015). PET is used mostly for synthetic fibers, over 60%, with bottle manufacturing accounting for approximately 30% of worldwide demand (Ji and Li Na, 2013). PET accounts for roughly 18% of global polymer output and is the fourth most-produced polymer after polyethylene, polypropylene, and polyvinyl chloride.

Müller et al. discovered in 2005 that the thermophilic actinomycete *Thermobifida fusca*'s TfH, a cutinase-like hydrolase enzyme, induced weight loss in low crystallinity PET films. This is the first study on the enzymatic breakdown of the inner bulk of PET films (Müller et al., 2005, Müller et al., 2006). In 2009, Ronkvist et al. used low-crystallinity PET films and high-crystallinity biaxially oriented PET films as substrates to demonstrate PET-hydrolyzing activities of three cutinases from different microorganisms, namely *Humicola insolens* (HiC, now named *Thermomyces insolens*), *Pseudomonas mendocina* (PmC), and *Fusarium solani* (FsC) (Ronkvist et al., 2009). However, according to Acero et al. (2011), all known PET hydrolases have low turnover rates (Acero et al., 2011), and the gene encoding these enzymes is similar to the polyester hydrolases of *Thermobifida* species (Herrero and Acero et al., 2011). The sole exception so far is a para-nitrobenzylesterase from *Bacillus subtilis* (Ribitsch et al.

2011). Following that, Sulaiman et al. discovered in 2012 that an LC-cutinase expressed by a gene from a metagenomic library of leaf-branch compost can effectively hydrolyze low-crystallinity PET packaging film (Sulaiman et al., 2012). Ribitsch et al. identified additional PET-degrading cutinases in 2013, including *T. fusca* DSM 44342 (Thf42 Cut1) and *Thermobifida cellulolysitica* (Thc Cut1 and Thc Cut2) (Ribitsch et al. 2013). Ribitsch et al. also found that fusing polymer and cellulose binding domains (Ribitsch et al., 2013) or hydrophobins (Ribitsch et al., 2015) improved cutinase adsorption to the surface of PET, resulting in greater hydrolysis product yields (Ribitsch et al., 2013; Ribitsch et al., 2015). Sulaiman et al. reported in 2014 that HiC hydrolyzed a low crystalline PET film nearly entirely, indicating that the crystalline portion of the PET film was also degraded. The thermostable bacterial LC-cutinase was found to hydrolyze low crystalline PET films as well (Sulaiman et al., 2014). Later research by Wei et al. showed that the enzymes involved in PET breakdown (PET hydrolases, tannases, and MHETases) are typical serine hydrolases like cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3), and carboxylesterases (EC 3.1.1.1). These enzymes have a conventional alpha/beta-hydrolase fold, with a serine, histidine, and aspartate residue in the catalytic triad (Wei et al. 2014a). Kawai et al. discovered that cutinase Cut190 from *Saccharomonospora viridis* AHK190 can hydrolyze low-crystallinity PET and PET-P. (Kawai et al. 2014). Barth et al. discovered four enzymes that act on PET in *Thermobifida* species, one from *Saccharomonospora*, and one from the phylum *Thermomonospora* in 2015. Ca2+ is often required for the thermal stability of these actinobacterial enzymes (Barth et al., 2015a; Barth et al., 2015b). He also showed a two-fold greater yield of breakdown products using a dual enzyme reaction system including a polyester hydrolase and the immobilized carboxylesterase TfCa from *Thermobifida fusca* KW3 (Barth et al., 2016). Later, Then et al. showed that the addition of divalent ions (Ca2+ and Mg2+) improved the thermostability of various PET-degrading esterases generated by the thermophilic actinomycete *Thermobifida fusca* KW3, enabling PET degradation (Then et al. 2015). In 2016, Yoshida et al. demonstrated that the bacteria *Ideonella sakaiensis* 201-F6 degraded amorphous or low-crystalline PET films at ambient temperatures. The bacteria also produced IsPETase, a PET-hydrolyzing enzyme (Yoshida et al., 2016). *I. sakaiensis* has been proven to be the most efficient organism for decomposing PET that has been discovered so far. ISF6-4831 and ISF6-0224 were identified as the genes encoding PETase and MHETase sequences, respectively (Yoshida et al. 2016). It was later discovered that at a mesophilic temperature of 30°C, the IsPETase had higher PET breakdown activity (Yoshida et al., 2016; Taniguchi et al., 2019). PET hydrolases were shown to breakdown low-crystallinity PET but not high-crystallinity PET in general

(Vertommen et al., 2005; Yang et al., 2016; Ronkvist et al., 2009; Wei et al., 2019b; Yoshida et al., 2016). The genome of *Ideonella sakaiensis* was sequenced further, and the enzymes PETase and MHETase were produced in *E. coli* and further characterized (Yoshida et al., 2016). Bilundo et al. later reported that truncating 71 N-terminal residues of a *Clostridium botulinum* esterase revealed a hydrophobic region that enhanced its adsorption to PET and hydrolytic activity (Bilundo et al., 2016). Perz et al. shown that protein engineering is a viable method for developing better enzymes for PET breakdown. The hydrolytic activity of the shortened esterase from *Clostridium botulinum* (Cbotu EstA) (Perz et al. 2016) was increased owing to the development of an exposed hydrophobic patch that was missing in the entire original enzyme and promoted its adsorption to the polyester (Bilindo et al. 2017). Wei et al. discovered that altering a critical amino acid residue involved in the interaction with a low molecular weight PET model compound may reduce TfCut2's susceptibility to product inhibition (Wei et al., 2016). Carniel et al. developed a reaction system including the fungal polyester hydrolase HiC and the Candida antarctica lipase CalB, which resulted in a 7.7-fold increase in terephthalate yield owing to the simultaneous degradation of MHET catalyzed by CalB (Carniel et al., 2016). Fungal cutinases, in addition to actinobacterial PET hydrolases, exhibited activity on PET substrates, according to Carniel et al. Cutinases from the phyla *Fusarium* and *Humicola* are the most well-known examples. In order to avoid the previously reported product inhibition by BHET and MHET, the latter was combined with the lipase CalB from *Candida antarctica* (Carniel et al. 2017). However, Haernvall et al. discovered a cutinase from *Pseudomonas pseudoalcaligenes* (PpCutA) and a putative lipase from *Pseudomonas pelagia* (PpelaLip) as possible enzymes working on polyesters in general using an in-silico genome mining method (Haernvall et al. 2017). Notably, the discovered bacterium belongs to *Pseudomonas pertucinogena*, a biotechnologically significant new species under the genus *Pseudomonas* (Bollinger et al. 2018). Wei et al. also discovered that polyesters with a high aromatic component ratio, such as PET, PBT, and PTT, are chemically synthetized using terephthalic acid, which is produced from crude petroleum (Wei et al. 2017). Various scientists later established the structure and function of PETase, mostly by comparing its 3D structure to that of comparable but better researched enzymes (Han et al., 2017; Austin et al., 2018; Joo et al., 2018). Austin et al. published the three-dimensional (3D) structure of *I. sakaiensis* PETase in 2018, and the overall structure was most similar to cutinases. It was discovered that a twofold mutation (S238F/W159H) shortened the enzyme's active site, making the protein more like a cutinase, as it resembled the enzyme from *Thermobifida fusca*, resulting in a better variation (Austin et al. 2018). Danso et al. also

discovered an extra disulfide link in the PETase of *I. sakaiensis*, as well as in structural models of functionally verified PET hydrolases. These results suggest that genes producing PET hydrolase are found in both marine and terrestrial metagenomes (Danso et al., 2018). Hajighasemi et al. reported on the functional screening of metagenomes and the characterisation of chosen enzymes in addition to the metagenome-derived PET enzymes mentioned above (Hajighasemi et al., 2018). In addition, Joo et al. utilized a rational protein design method to improve PETase catalytic efficiency by concentrating on the structure and behavior of the binding site rather than generating hundreds of random mutations and screening for favourable ones (Joo et al., 2018). Later, in 2019, Son et al. discovered that the PETase enzyme from *I. sakaiensis* 201-F6 was thermolabile, and that further modification of the PETase enzyme to increase its thermostability resulted in a novel variation that was 14 times more active than the original biocatalyst (Son et al., 2019). PETases link the polymer with the hydrophobic surface and the substrate-binding cleft, according to structural data provided by Palm et al. The lid domain of the enzyme nearly entirely confers substrate specificity and activity toward MHET (Palm et al., 2019). Wei et al., on the other hand, demonstrated that the recombinant *Thermobifida fusca* cutinase TfCut2 produced by *B. subtili*s was capable of degrading low-crystallinity PET films and two low-crystallinity PET samples from post-consumer packaging (Wei et al., 2019b). According to Shirke et al., thermostable PET depolymerases are required for effective enzymatic breakdown of PET. Glycosylation (Shirke et al., 2018) and rational protein engineering techniques, such as surface salt bridge optimization (Shirke et al., 2016), mutation of Ca2+ and Mg2+ binding sites (Then et al., 2015), introduction of disulfide bridge (Then et al., 2016), and stabilization of the β6-β7 connecting loop and extension of subsite IIc (Son et al., 2019), have all been used to enhance PET hydrolase thermostability. In 2020, Jabloune et al. revealed that a 25 kDa suberinase from *Streptomyces scabies* has exceptional stability and the capacity to hydrolyze PET to terephthalic acid, as well as other polymers (Jabloune et al., 2020). While the enzymes of *I. sakaiensis* are the most well-studied and investigated, additional enzymes and organisms have been discovered as effective PET degraders. Cutinases, esterases, hydrolases, lipases, and carboxylesterases are just a few of the enzymes that have been found to degrade PET (Danso et al., 2019; Ru et al., 2020, Jabloune et al., 2020).

4.3 Polyurethane

Polyurethane is made up of intramolecular urethane linkages that connect di-isocyanate or poly-isocyanate with polyol (Saunders and Frisch, 1964). Although the majority of polyurethanes are thermosetting polymers that do not melt when heated, they may also be thermoplastic. Isocyanates and polyols used to produce polyurethanes have two or more functional groups per molecule on average, which polymerize one after the other and are classified as alternating copolymers. The addition of aromatic ring structures to the polymer has an even greater effect on its physical and chemical characteristics. Polyether PUR and polyester PUR have varied characteristics depending on the polyols employed in the polycondensation process (Seymour and Kauffman, 1992). Polyurethanes are commonly utilized in the manufacturing of microcellular foam seals, gaskets, high-resilience foam seating, spray foam, rigid foam insulation panels, durable elastomeric wheels and tires like shopping carts, elevators, skateboard wheels, roller coasters and escalators, automotive suspension bushings, electrical potting compounds, high-performance adhesives, surface coatings, and segregated elastomeric wheels and tires. Polyurethanes were originally produced in 1937 by Otto Bayer and his colleagues at IG Farben in Leverkusen, Germany (Bayer et al, 1947). It ranks fifth among the most commonly manufactured synthetic polymers, with annual production exceeding 27 million tons (Plastics Europe, 2018).

Darby and Kaplan conducted the first study on microbial degradation of PUR in 1968 and discovered that seven fungi grew on the surface of solid polyester PUR. According to the studies, PUR biodegradation seemed to have been controlled by the polyol moiety. PUR is highly resistant to microbiological activity when made using polyether polyols, while polyester polyols are particularly vulnerable. Polyester-type polyurethanes (ES-PUR) were also shown to be more vulnerable to fungal assault than polyether-type polyurethanes (ET-PUR) (Darby and Kaplan, 1968). Pettit and Abbott later revealed in 1975 that the release of ammonia from the breakdown of urea units contributed to the degradation of polyurethane. The hydrolytic actions of microbial esterases could break the ester bonds of the urethane groups in a sequential manner (Pettit and Abbott, 1975). Filip et al., in 1979, found *Aspergillus niger* and *Cladosporium herbarum* growth in shake cultures using polyether PUR resilient foam as the only nutrition supply (Filip et al., 1979). Pathirana and Seal reported in 1983 that bacteria and fungi grew on the surface of soil covered polyester polyurethane and were tested in the lab for PU degrading ability (Pathirana and Seal, 1983). In the presence of PUR, Pathirana and Seal

also discovered that certain polyester-PUR degrading fungi generated extracellular esterases, proteases, and ureases (Pathirana and Seal, 1985). Later, in 1987, Bentham et al. extracted a variety of fungi from the surface of polyester PU foam, which they used as their only carbon source. The taxa *Emericella, Trichoderma, Aspergillus, Fusarium, Gliocladium*, and *Penicillium* were detected among the fungi isolates (Bentham et al. 1987). Papain and urease are two proteolytic enzymes that have been discovered to degrade medical polyester polyurethane, according to Phua et al (Phua et al. 1987). Tokiwa et al., in 1988, discovered that polyester polyurethanes were hydrolyzed using *R. delemar* lipase and hog pancreatic lipase (Tokiwa et al. 1988). Kay et al., in 1991, isolated and studied 16 distinct bacteria with the capacity to degrade PUR (Kay et al. 1991). Jansen et al. obtained a *Staphylococcus epidermidis* KH11 strain from an infected catheter and showed that it can use polyether PUR in the absence of organic nutrients (Jansen et al., 1991). Kay et al., later in 1993, discovered esterase activity in the culture supernatant of *Corynebacterium sp*. and observed that the ester bond hydrolysis caused PUR degradation (Kay et al., 1993). *Curvularia senegalensis, Fusarium solani, Aureobasidium pullulans*, and *Cladosporium sp*, all prevalent in soil, were shown to degrade ester-based polyurethane by Crabbe et al. in 1994. The esterase isolated from the fungus *Curvularia senegalensis*, which degraded polyester PUR, was shown to be capable of cleaving the ester linkages in the soft segments of polyester PUR (Crabbe et al., 1994). Furthermore, research found that a cholesterol esterase from bovine pancreas degraded ES-PUR and released hard-segment components (Santerre et al. 1994, Wang et al. 1997). Following that, in 1995, Nakajima-Kambe demonstrated an esterase isolated from *C. acidovorans* TB-35 degraded ES-PUR and produced diethylene glycol and adipic acid (Nakajima-Kambe et al. 1995). In 1998, Akutsu et al. isolated a cell surface-bond esterase from *Comamonas acidovorans TB-35* that could degrade PUR polyester (Akutsu et al., 1998). In this strain, Nomura et al. cloned the *pudA* gene, which encodes the polyester PUR degrading esterase (Akutsu et al., 1998, Shigeno-Akutsu et al., 1999, Nomura et al., 1998). It has been reported that the enzymatic hydrolysis of insoluble PUR polymer is a surface erosion process requiring efficient biocatalyst adsorption on the polymer surface prior to the polymer breakdown by the esterase enzyme (Akutsu et al., 1998). Degli-Innocenti tested enzyme activity against polyurethane in polyurethane-rhodamine agar plates, polyester-urethane (PEU), and polycarbonate-urethane (PCU) using purified polyurethanase enzymes, PueA and PueB (Degli-Innocenti et al., 1998). When Coomassie blue R-250 was introduced to bacterial cultures in mineral medium containing polyurethane, Howard et al., in 1999, listed the bacterial isolates for polyurethanolytic activity by clear zone development surrounding the bacterial colonies (Howard et al., 1999). Later on, purification of

a protease from *Pseudomonas fluorescens* (Vega et al., 1999), an esterase from *Comamonas acidovorans* (Allen et al., 1999), three esterases from *Pseudomonas chlororaphis* (Howard et al., 1999; Ruiz et al., 1999a), and a lipase from *Bacillus subtilis* (Rowe and Howard, 2002) were observed. All of the purified serine hydrolases listed above could hydrolyze emulsified polyester PUR. These enzymes were discovered to be a cell-associated membrane bound PU-esterase and an extracellular PUR esterase in these investigations. In the biodegradation of polyurethane, these two enzymes perform distinct roles. The cell-mediated access to the hydrophobic polyurethane surface is facilitated by the membrane-bound PU-esterase. The extracellular PUR-esterase then adheres to the polyurethane's surface. Bacteria may attach to the surface of polyurethane and hydrolyze the PUR substrate into metabolites as a result of these enzymatic activities (Allen et al., 1999, Howard et al., 1999, Vega et al., 1999). Additionally, a gene called *pulA* was cloned from *Pseudomonas fluorescens* that encoded an esterase (Ruiz et al., 1999b), and two additional genes, *pueA* and *pueB,* from *Pseudomonas chlororaphis,* encoded two other enzymes, both of which were lipases, were found to act on PUR (Ruiz et al., 1999b; Stern and Howard, 2000; Howard et al., 2001, Howard and Blake, 1998, Howard, 2007). In 2003, Barrat et al. discovered *Geomyces pannorum* to be the most common fungus, among the bulk of the organisms identified in soil for the breakdown of polyurethane, such as *Plectosphaerella, Nectria, Neonectria, Phoma,* and *Alternaria* (Barratt et al., 2003). After 6 months of soil burial of polyurethane film, Shah et al. recovered 5 bacterial strains and identified them as *Bacillus sp*. AF8, *Pseudomonas sp.* AF9, *Micrococcus sp*. AF10, *Arthrobacter sp*. AF11, and *Corynebacterium sp*. AF12 (Shah et al. 2007). Matsumiya et al. discovered in 2010 that the fungus *Alternaria sp*. PURDK2 can degrade polyether PUR foam by secreting urethane-bond–degrading enzymes (Matsumiya et al., 2010). The activity of serine and cysteine hydrolase in *Pestalotiopsis microspora* PU degradation was described by Russell et al. in 2011. Furthermore, it was found that the biodegradation activity of *Aspergillus niger* was very slow, with obvious indications of deterioration (Russel et. al 2011). *Fusarium solani, Candida ethanolica* (Zafar et al., 2013), and *Candida rugosa* (Gautam et al., 2007) have all been found as polyester PUR degraders in various investigations. While a lipase has been discovered as the main enzyme in polyester PUR metabolism in *C. rugosa*, no enzymes have yet been found in *C. ethanolica* or *F. solani*. *Cladosporium pseudocladosporioides, Cladosporium tenuissimum, Cladosporium asperulatum,* and *Cladosporium montecillanum* are among the *Cladosporium cladosporioides* species reported and three others were identified as *Aspergillus fumigatus*, *Penicillium chrysogenum* (Alvarez- Barragan et al., 2016) and *Aspergilus flavus* (Mathur and Prasad, 2012). The breakdown of PUR in *A. flavus* is thought to

be caused by secreted esterases. Furthermore, certain B. subtilis and *Alicycliphilus sp*. isolates have been shown to be capable of degrading PUR (Shah et al., 2013; Rowe and Howard, 2002; Oceguera-Cervantes et al., 2007). Based on nuclear magnetic resonance (NMR) and infrared (IR) measurements, only *Pseudomonas sp.* lipase substantially degraded the additional PUR (Biffinger et al., 2015). In 2016, Gamerith et al. discovered that incubating a solid polyester PUR with a polyamidase from *Nocardia farcinica* coupled to the hydrophobic polymer binding module of the PHA depolymerase from *Alcaligenes faecalis* resulted in a higher yield of degradation products (Gamerith et al., 2016). *Pseudomonas protegens* strain Pf-5 degraded polyester PUR dispersions via a similar method. However, it was shown that PUR breakdown is tightly controlled by carbon catabolite regulatory mechanisms in this strain, and that both lipase genes, *pueE* and *pueB*, seem to be required for development on PUR dispersions (Hung et al. 2016). In 2017, Stepien et al., found one yeast and three bacteria capable of degrading polyether PUR sheets (Tecoflex®) (Stepien et al., 2017). Schmidt et al. discovered four polyester hydrolases that could degrade emulsified polyester PUR, namely, LC-cutinase, TfCut2, Tcur1278, and Tcur0390 (Schmidt et al., 2017). The promiscuous nature of the *Thermobifida*-derived cutinases may explain why cutinases, which are known to breakdown polyethylene terephthalate, also operate on PUR (Schmidt et al., 2017). Recent study on enzyme promiscuity suggests that lipolytic enzymes like cutinases are often promiscuous, converting up to 78 distinct substrates (Martinez-Martinez et al. 2018). According to Khan et al., *Aspergillus tubingensis* colonizes PUR and operates on the surface of polyester PUR films. However, no enzyme has been associated to PUR activity (Khan et al. 2017). In 2018, Oprea et al. studied the capacity of *Alternaria tenuissima* to degrade pyridine-based polyether PUR elastomers (Oprea et al., 2018). Magnin et al. recently revealed, through a hypothesized stepwise process, the synergistic effects of esterase and an amidase on the degradation of different PUR-compounds (Magnin et al., 2019). A waterborne polyester PUR dispersion was hydrolyzed and a solid polycaprolactone polyol-based polyester PUR was degraded by an esterase (E3576), which was chosen among 50 commercially available hydrolases (Magnin et al., 2019b). However, this esterase (E3576) was unable to breakdown polyester PUR films based on poly(hexamethylene adipate) diol, suggesting that the chemical structures of the polyol segments have a major impact on polyester PUR film biodegradation (Kim and Kim, 1998, Magnin et al., 2019b). Magnin et al., in 2020, also showed several enzymes from bacterial and fungal sources, including cutinases, esterases, lipases, laccases, peroxidases, proteases, and ureases, that could degrade PUR (Magnin et al., 2020). Using proximity ligation-based metagenomic analysis, Gaytan et al. discovered a number of enzymes involved in PUR

degradation in a group of microorganisms. Different dioxygenases, decarboxylases, dehydrogenases, transferases, ligases, hydrolases, isomerases, and peroxidases were shown to be responsible for the metabolism of diverse PU intermediates (Gaytán et al., 2020).

4.4 Polypropylene

Polypropylene, or PP, is a thermoplastic polymer made from the monomer propylene via chain-growth polymerization. It's an aliphatic hydrocarbon with a methyl group on each monomer of the polymer backbone, making it unique. Its characteristics are comparable to those of polyethylene, although it is somewhat tougher and more heat resistant. Plastic mouldings, stationery folders, packing materials, plastic tubs, non-absorbable sutures, diapers, and other items are often made of polypropylene. Polymerization of propylene was first shown in 1951 by Robert Banks and J. Paul Hogan of Phillips Petroleum (Stinson, 1987). From 1957 onwards, the Italian company Montecatini began mass-producing isotactic polypropylene (New Scientist, 2007). After polyethylene, polypropylene is the second most commonly manufactured commodity plastic. Polypropylene was valued $126.03 billion on the worldwide market in 2019 (Global Polypropylene Market Report, 2020).

In 1993, Cacciari et al. reported the attempted biodegradation of PP by cultures supplemented from sandy soils including PE wastes for the first time (Cacciari et al. 1993). The findings revealed that, apart from the PP itself, the plasticizers were susceptible to degradation by sandy soil microorganisms (Cacciari et al. 1993). In 2001, Mikulasova et al. created lignin-containing grafts and studied biodegradation by the fungus *P. chrysosporium* (Mikulasova et al. 2001). Artham et al. observed minimal alterations in the molecular level for their PP samples after one year in seawater in 2009. (Artham et al. 2009). The amorphous portions of PP were shown to be degraded by soil bacteria, according to Arkatkar et al. Since then, a variety of microorganisms have been investigated for their ability to breakdown PP in various environmental samples (Arkatkar et al., 2009). Arkatkar et al. recovered a *Bacillus flexus* strain from these PP samples later, in 2010. UV-treated PP films were incubated with a variety of *Bacillus* and *Pseudomonas* bacteria, including *B. flexus*, according to the research. PP, on the other hand, exhibited minimal change without pre-treatment (Arkatkar et al. 2010). Furthermore, three bacteria and two fungus strains isolated from a plastic-dumping site's soil were shown to be able to use PP as a carbon source for growth (Jeyakumar et al., 2013; Arkatkar et al., 2010). Later in 2013, Jeyakumar et al., using the fungi *Engyodontium album* and *P.*

chrysosporium, studied the effects of pre-treatment and metal ion pro-oxidant blending of PP (Jeyakumar et al. 2013). Jeon and Kim isolated a mesophilic strain of *Stenotrophomonas panacihumi* PA3-2 from the soil of an open storage yard for municipal solid waste in 2016, and found that it was capable of degrading two types of low molecular weight PP and one kind of high molecular weight PP (Jeon and Kim, 2016b). Auta et al. identified two marine bacteria from mangrove habitats, *Bacillus sp.* strain 27 and *Rhodococcus sp*. strain 36, which grew on aqueous synthetic medium containing PP microplastics (Auta et al., 2018). Many microbial communities have been found to biodegrade PP, including fungal species like *Aspergillus niger* and bacteria like *Pseudomonas* and *Vibrio* (Singh and Rawat, 2019; Sivan et al. 2011; Arutchelvi et al. 2008). Nonetheless, polypropylene (PP) is a significant polymer that is manufactured in greater quantities than most others (Karger-Kocsis et al., 2019). In stark contrast to their massive worldwide manufacturing pace, little accurate information on these essential polymers' enzymatic breakdown is available. Only a few studies have been published on polymer degradation based on weight loss and mixed species microbial communities (Cacciari et al., 1993; Iakovlev et al,. 2017).

4.5 Polystyrene

Polystyrene, abbreviated PS, is a synthetic aromatic hydrocarbon polymer with a phenyl ring connected to every second carbon atom of its backbone chain. It's well known for being used to make styrofoam. There are few studies on the biodegradation of polystyrene, although a few researchers have reported on the microbial breakdown of its monomer, styrene (Tsuchii et al., 1977). Polystyrene is used to make disposable cups, packaging materials, laboratory wares, certain electronic uses, protective packaging such as packing peanuts, jewel cases, for storage of optical discs like CDs and DVDs, lids, containers, bottles, tumblers, trays, models and disposable cutlery (Common Plastic Resins Used in Packaging, 2012). Because of its low weight, rigidity, and good thermal insulation, PS is widely utilized. Eduard Simon, a Berlin pharmacist, discovered polystyrene for the first time in 1839 (Blyth and Hofmann, 1845). In 2019, the worldwide polystyrene manufacturing capacity was 15.61 million metric tons (Fernández, 2021).

Guillet et al. utilized two kinds of 14C-PS as substrates for the first time in 1974 to demonstrate microbial breakdown of PS in both soil and activated sewage sludge (Guillet et al., 1974). The breakdown of PS by soil microbiota, 5 mixed florae and 17 lignin-degrading fungi was next

investigated using ^{14}C-labeled PS as a substrate. Apart from the mixed flora, scientists have attempted to identify PS-degrading microorganisms from other environmental samples (Sielicki et al., 1978; Kaplan et al., 1979). Milstein et al. discovered in 1992 that the white rot fungi *Pleurotus ostreatus, Trametes versicolor* and *Phanerochaete chrysosporium*, as well as *Gloeophyllum trabeum* the brown rot fungus, were linked to the depolymerization of polystyrene when coincubated with lignin (Milstein et al. 1992). Later, in 1997, Nakamiya et al. discovered that in a two-phase system, a purified hydroquinone peroxidase (EC 1.11.1.7) produced by the lignin-decolorizing bacteria *Azotobacter beijerinckii* HM121 was able to breakdown PS into low molecular components (Nakamiya et al., 1997). In 2003, Eisaku and Linn discovered three soil bacteria that might breakdown PS: *Sphingobacterium sp.*, *Xanthomonas sp.* and *Bacillus sp.* STR-YO (Eisaku and Linn, 2003). *Rhodococcus zopfii, Pseudomonas putida* and other gram-negative bacteria have also been shown to convert polystyrene, according to research. While the two-step process's general idea is interesting, it may not be practical on a broad scale (O'Leary et al., 2005; Ward et al., 2006; Savoldelli et al., 2017). Mor and Sivan discovered in 2008 that *Rhodococcus ruber C208* could grow only on PS, but that the degree of biodegradation was extremely low (Mor and Sivan, 2008). Atiq et al. identified three bacteria and three fungi from soil covered extended PS films that could attach and grow on PS but had low biodegradation rates of PS (Atiq et al., 2010; Atiq, 2011). The process of styrene breakdown in bacteria has been extensively investigated in *Corynebacterium, Pseudomonas, Rhodococcus, Xanthobacter*, and other bacteria (Tischler et al. 2009). In 2013, Tahir et al. discovered that *Lentinus tigrinus* could break down PS by an extracellular esterase (Tahir et al., 2013). Yang et al. found that new bacterial strains obtained from the intestines of insect larvae degrade PS, although the enzymes responsible have yet to be discovered (Yang et al., 2014, Yang et al., 2015). PS-degrading ability was also demonstrated in a wider variety of mealworms from 12 other sites across the globe using the same procedures, suggesting that PS degradation in mealworms is widespread (Yang et al., 2018). This finding prompted researchers to look into other insect species that may consume and degrade PS, such as superworms (*Zophobas atratus*) (Yang et al., 2019) and black mealworms (*Tenebrio obscurus*) (Peng et al., 2019). *Exiguobacterium sp*. YT2, a strain obtained from the stomach of *Tenebrio molitor*, was also shown to be capable of digesting PS (Yang et al., 2015c). In 2015, Krueger et al. discovered that the brown-rot fungus *Gloeophyllum trabeum* degraded the water-soluble PS counterpart polystyrene sulfonate significantly (Krueger et al. 2015).

In 2016, Mohan et al. discovered that PS degradation is caused by polymerases derived from *Pseudomonas* and *Bacillus* species (Mohan et al., 2016). Later, in 2018, Ho et al. identified enzymes involved in the metabolism of styrene, the PS monomer, before it enters the TCA cycle. Styrene monooxygenase, phenylacetaldehyde dehydrogenase, styrene oxide isomerase and phenylacetyl coenzyme A ligase have been identified as the enzymes responsible for these reactions (Ho et al., 2018). More bacteria have been identified from the guts of plastic-eating mealworms or superworms, according to new studies, and their potential for PS breakdown is currently being evaluated (Xia et al., 2019).

4.6 Polyvinyl Chloride

Polyvinyl chloride, often known as PVC, is a thermoplastic polymer. The monomers are usually organized head-to-tail, indicating that there are chlorides on alternate carbon centers. The polymers are linear and strong. PVC contains around 57 percent chlorine, and the presence of chloride groups in the polymer provides it significantly different characteristics than the structurally similar substance polyethylene. PVC is involved in the manufacturing of doors, pipes, windows, electrical cable insulation, plumbing, imitation leather, signage, flooring, phonograph records, bottles, inflatable products, non-food packaging, cards like bank or membership cards, food-covering sheets, and many other applications where rubber is replaced (Barton, 1932). After much research and testing, German scientist Eugen Baumann synthesized PVC for the first time in 1872 (Baumann, 1872). After PE and PP, PVC is the world's third most commonly manufactured synthetic plastic polymer, with about 40 million tons produced each year. Shin-Etsu Chemical of Japan is the world's biggest single manufacturer of PVC, with a worldwide market share of approximately 30% (Nikkei Asian Review, 2018).

In 1993, Mooriyama et al. discovered that fungus degraded a range of plasticized PVC bathroom goods, including bath-tub lids, shower curtains, and bath mats (Moriyama et al., 1993). Later, in 1995, Otake et al. discovered that a PVC sample buried in soil for 32 years had shown no signs of biodegradation (Otake et al. 1995). Kirbas et al. discovered in 1999 that PVC with a low molecular weight could well be subjected to biodegradation using white-rot fungus (Kirbas et al. 1999). PVC sheets buried in soil for 10 months were subjected to various fungal isolates in liquid culture for 4 weeks showed minimal biodegradation, according to Ali et al (Ali et al. 2014). Apparently, no enzymes capable of degrading PVC were found.

4.7 Nylon

Nylons are condensation polymers or copolymers produced by reacting difunctional monomers with equal quantities of amine and carboxylic acid, resulting in amides at both ends of each monomer. It's a general term for a group of synthetic polymers made up of repeating units connected by amide linkages (Clark et al, 2015). Nylon polymers offer a wide range of commercial uses, including flooring, clothing, and rubber reinforcements, as well as forms like moulded components for electrical equipment, automobiles, and other vehicles, and films, mostly for food packaging (Kohan, 1995). Nylon was the first commercially viable synthetic thermoplastic polymer, with DuPont starting research in 1927 and announcing it in 1938, just in time for the 1939 New York World's Fair (DuPont, 1988). Nylon output is expected to reach 8.9 million tons globally by 2020 (Business Wire, 2020).

Trypsin enzymatic degradation of 6-nylon oligomers was first described by Ebata et al. in 1959. (Ebata et al. 1959). Tosa and Chibata discovered in 1965 that proteins, like natural silk, are polyamides in and of themselves. Different bacteria were reported to grow on various oligomers generated from nylon manufacturing in one of the earliest investigations (Tosa and Chibata, 1965). Tokiwa et al. discovered in 1979 that the sensitivity of CPEA to hydrolysis by *R. delemar* lipase reduced when the nylon blocks in copolyamide-ester chains were shortened and the nylon concentration increased (Tokiwa et al., 1979). *Flavobacterium sp.* strain KI72, which was recently renamed *Arthrobacter sp*. strain KI72, was one of the bacteria found thriving on these oligomer combinations (Tosa and Chibata, 1965; Takehara et al. 2017). Different hydrolases and aminotransferases involved in the early breakdown of oligomers of nylon and subsequent metabolism are encoded in the genomes of *Arthrobacter* isolates. Bacteria that use linear and cyclic 6-aminocaproic oligomers as sole carbon or carbon plus nitrogen sources have also been identified (Iizuka et al. 1967; Nonomura et al. 1974; Kinoshita et al. 1975). Furthermore, cyclic dimer hydrolase, as well as exogenous and endogenous 6-aminocaproic oligomer hydrolases, hydrolyzed the oligomers (Kinoshita et al., 1977). The genes for these enzymes are encoded on the pOAD2 accessory plasmid of *Flavobacterium sp*. strain K172, which assimilates 6-nylon oligomers (Negoro et al., 1980). The plasmid's entire DNA has been sequenced (Negoro et al. 1983, Okada et al. 1983, Negoro et al. 1992, Kakudo et al. 1993). Microorganisms such as *Flavobacterium sp.* (Kinoshita et al.1975) and *Pseudomonas sp.* NK87 (Kanagawa, 1989) have been shown to breakdown nylon 6 oligomers but not polymers in subsequent investigations. *Pseudomonas aeruginosa*, among other species,

was shown to be capable of converting oligomeric nylon in 1995. These findings show that *P. aeruginosa* and its developed strain PAO1 are capable of degrading 6-aminohexanoate linear dimers effectively (Prijambada et al. 1995; Tosa and Chibata et al. 1965). A 6-aminohexanoate dimer hydrolase and a 6-aminohexanoate cyclic-dimer hydrolase were found to have the most important enzymatic activity. However, some *Pseudomonas* species have been shown to use 6-aminohexanoate-dimers as their only carbon and nitrogen source (Kanagawa et al. 1993). Delguchi et al. discovered in 1997 that lignin-degrading white rot fungus IZU-154, *Phanaerochaete chrysosporium, Trametes versicolor* degraded high molecular weight nylon-6,6 membranes substantially under ligninolytic conditions with minimal glucose or ammonium tartrate (Delguchi et al. 1997). The enzyme, which was found to scrape the surface of nylon 6 and create deep horizontal grooves in the polymer, also degraded nylon-6 fibres (Delguchi et al. 1997). In 1998, Deguchi demonstrated that manganese enhanced nylon biodegradation, pointing to the fungi's lignin-modifying enzymatic system, particularly manganese peroxidase or MnP, as the agents responsible for nylon biodegradation (Delguchi et al. 1998). Furthermore, while the properties of a nylon-degrading enzyme purified from a culture supernatant of the white rot fungal strain IZU-154 were identical to those of manganese peroxidase, the reaction mechanism for nylon degradation was suggested to be different from that of standard manganese peroxidase. Regrettably, no gene or protein sequence has been discovered (Delguchi et al. 1998). In 2000, Negoro et al. discovered that three distinct hydrolases in *Flavobacterium* and *Pseudomonas* strains catalyze the metabolism of 6-aminohexanoate, a nylon intermediate product (Negoro et al. 2000). Under mesophilic and neutral to alkaline pH circumstances, these enzymes, known as 6-aminohexanoate-cyclic dimer hydrolase, endo-type 6-aminohexanoate-oligomer hydrolase, and 6-aminohexanoate-dimer hydrolase, worked together to convert the nylon intermediate to its monomer, 6-aminohexanoate (Negoro et al. 2000). For nylon, two biodegradation routes have been identified: hydrolysis and oxidative breakage of the polyamide bond. Oligomers have been used to study nylon hydrolysis extensively. *Flavobacterium, Arthrobacter, Pseudomonas,* and *Agromyces* strains all have enzymes that catalyze hydrolysis (Negoro et al. 2000; Ohki et al. 2005; Yasuhira et al. 2007). Negoro et al., further found that an endo-type nylon oligomer hydrolase and two nylon dimer hydrolases were encoded by the three genes *nylA, nylB* and *nylC* in Flavobacterium (Negoro et al. 2000). In 2012, Negoro et al. determined the crystal structure of the *Agromyces* oligomer hydrolase NylC and showed that a quadruple mutant could catalyze the depolymerization of polymeric nylon (Negoro et al. 2012). In soil and activated sludge, however, biodegradation of nylon-4 has been found to be a very quick and easy process (Hashimoto et al. 1994; Kawasaki

et al. 2005). Hashimoto went on to study the biodegradability of nylon 4 and nylon 6 blends in compost and activated sludge in 2002. The nylon 4 in the mix deteriorated entirely in four months, whereas nylon 6 remained unaffected (Hashimoto 2002). In 2007, Yasuhira et al. discovered enzymes in *Arthrobacter sp.* KI72 with characteristics comparable to those of the aforementioned enzymes (Yasuhira et al., 2007). The white-rot fungus *Bjerkandera adusta* and *P. chrysosporium* degrade nylon-6, and the polymer could be used as the sole source of nitrogen for mycelial development has also been observed (Friedrich et al. 2007; Klun et al. 2003). Yamano et al. discovered in 2008 that *Pseudomonas* bacteria generating hydrolytic exoenzymes, which were isolated from activated sludge, were involved in nylon biodegradation (Yamano et al. 2008). Sudhakar et al. observed in 2009 that a variety of marine microorganisms, including *Bacillus cereus, Vibrio furnissii, Bacillus sphaericus,* and *Brevundimonas vesicularis,* acted on nylon (Sudhakar et al. 2009). Takehara et al., in 2018, further stated that after the monomers have been hydrolyzed, several aminotransferases metabolize the monomers. Among the genes found in the draft genome of *Arthrobacter sp*. KI72 are nylD1 and nylE1, which are involved for secondary 6-aminohexanoate metabolism (Takehara et al. 2018a, 2018b). Fungal and bacterial sources of nylon-degrading enzymes have also been discovered (Nomura et al., 2001, Yamano et al., 2019).

Mechanism of Plastic Degradation

Microbes use biochemical transformation to break down complex substances into simpler ones. Changes in the physical characteristics of plastic polymers, particularly molecular weight decrease, loss of mechanical strength, and changes in plastic surface properties, indicate biodegradation (Ho et al., 2018). As previously mentioned, the goal of plastic biodegradation is to convert recalcitrant wastes into non-toxic lower molecular mass molecules that may be recycled into the biogeochemical cycle. Biodeterioration, biofragmentation, assimilation, and mineralisation are the distinct biochemical degradative routes involved in plastic biodegradation, and all of these processes are carried out via diverse enzyme activity and bond cleavage (Gu, 2003, Pathak, 2017). Figure 4 depicts a schematic depiction of the mechanisms involved in plastic biodegradation.

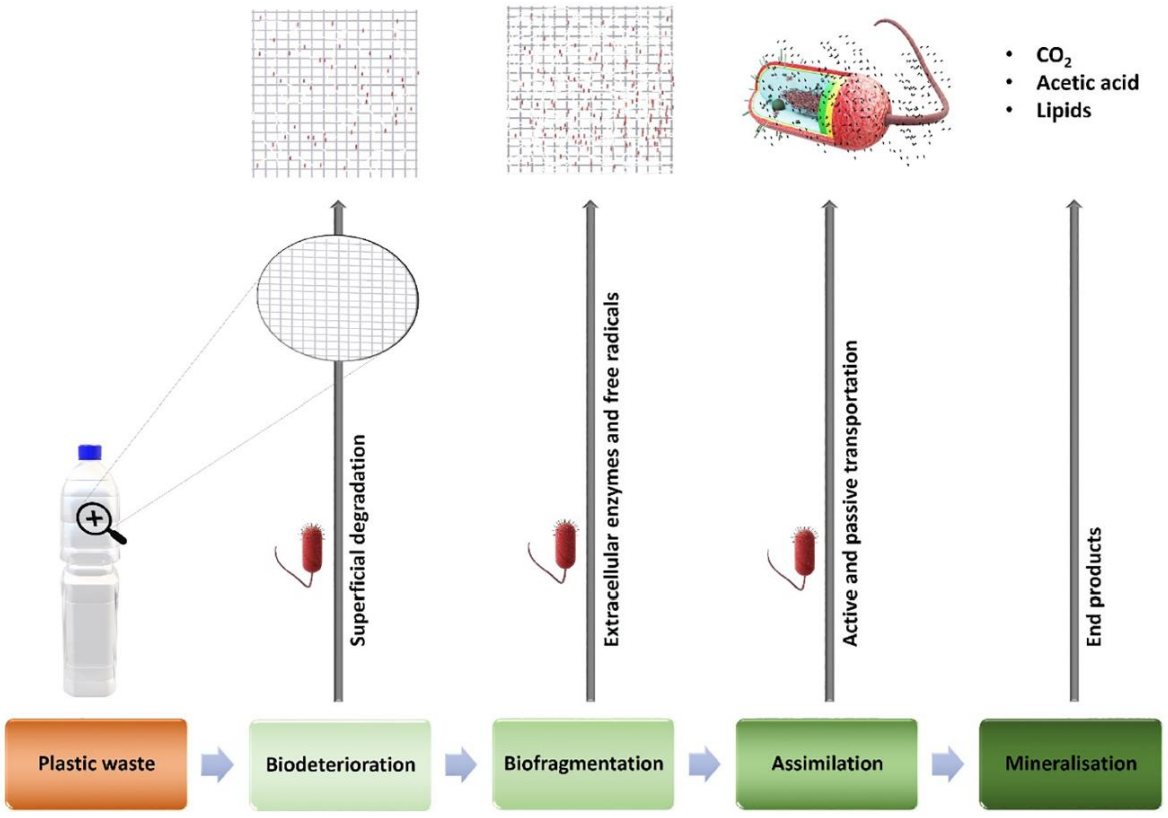


Figure 4: A schematic depiction of the mechanisms involved in plastic biodegradation (Abomonye et al, 2020).

5.1 Biodeterioration

The chemical and physical activities of microorganisms, or other biological agents, induce biodeterioration, which results in the surface breakdown of the plastic polymer as well as

changes in its chemical, mechanical, and physical characteristics (Anjana et al., 2020). Prolonged contact to external factors such as light, temperature, and chemicals in the environment enhances the changes seen in the polymers during biodegradation. The biodeterioration process is the first, and it begins with microorganisms adhering to and colonizing the polymer surface with the express purpose of decreasing the plastic materials' resistance and durability. As plastics are inherently hydrophobic, the addition of hydrophilic functional groups to plastic surfaces is often needed to enhance microbe adhesion (Nauendorf et al., 2016). Furthermore, it has been shown that the development of biofilms is required for polymers with greater surface hydrophobicity, such as polyethylene, in order to enhance the polymeric surface contact with bacteria (Schwibbert et al., 2019). In contrast to other bacteria in the planktonic phase, biofilm-forming bacteria such as *Pseudomonas* have been found to attach more firmly and destroy low-density polyethylene (Tribedi et al., 2015). Bacterial biofilms have been shown to shield microbial communities from external perturbations and increase their durability under various circumstances. Tribedi et al. (2015) also discovered that biofilm-promoting chemicals like mineral oil accelerated plastic biodegradation, while surfactants that inhibited biofilm formation slowed the degradative process. Fungal cells, on the other hand, have evolved to grow on almost every kind of surface known in nature, and their hyphae have been demonstrated to adhere to polymeric plastic surfaces (Sánchez, 2020). The fungi's adhesion to the polymer solids and subsequent development cause localised swelling, resulting in a polymer with substantially reduced mechanical characteristics. The bacteria continue to grow as soon as they adhere to the plastic surface, utilizing the polymers as their only carbon source. Other additions in the polymers, such as plasticisers that are more accessible and readily metabolized by the microorganisms, have been found to improve the adhesion and early development of these bacteria in studies (Ru et al., 2020). A *Pseudomonas aeruginosa* strain's ability to form and sustain active biofilms on polyethylene surfaces for two months was ascribed to its consumption of low molecular components in the polymer (Gupta and Devi, 2020). Exopolysaccharides have also been shown to have a role in the attachment and biodeterioration of plastic polymers by increasing biofilm adherence (Anjana et al., 2020). These extracellular polymers also function as surfactants, facilitating the transition between hydrophilic and hydrophobic phases, allowing microbial species to penetrate more quickly (Lucas et al., 2008).

5.2 Biofragmentation

The next stage, biofragmentation, is a depolymerisation process involving the catalytic cleavage of bio-deteriorated plastic polymers into smaller units by extracellular enzymes and free radicals produced by microorganisms (Jenkins et al., 2019). The biofragmentation process is thought to consist of two main reactions: polymer molecular weight decrease and oxidation of lower weight molecules. These reactions are required to allow microbial enzymatic systems to target smaller molecular weight molecules, which are generally unable to do so (Restrepo-Flórez et al., 2014). The glycosidic, ester, and peptide linkages inside the plastics are exposed to a hydrolytic nucleophilic assault on the carbonyl carbon, and these enzymes primarily catalyze the hydrolytic breakdown of the polymers. These hydrolytic processes take place in two ways: exo- and endo-attacks, each of which produce distinct products. While the former produces component oligomers or monomers, such as ethylene glycol and terephthalic acid, which the microbe may easily absorb into the cell, the endo-attacks' products must still be degraded before they can be digested (Pathak, 2017). *Rhodococcus rhodocrous* was able to degrade nearly all of the previously oxidized oligomers from polyethylene, demonstrating the importance of oxidation processes (Gravouil et al., 2017). Different inorganic and organic chemicals produced by microorganisms may potentially have a role in aiding the biofragmentation process, according to the study. Various inorganic compounds (ammonia, hydrogen sulfide, nitrites, thiosulphates, etc.) as well as organic acids (citric, fumaric, gluconic, glutaric, glyoxalic, oxalic acids, etc.) have been shown to scavenge cations from polymer surfaces, forming stable complexes that can cause surface erosion (Krause et al. The substantial change in the carbonyl index for the UV-irradiated polymer has also been inferred from earlier research, indicating a favorable connection between the rate of plastic degradation and photooxidation (Sen and Raut, 2015).

5.3 Assimilation

At the assimilation step, the smaller molecular weight molecules generated during biofragmentation are carried into the cytoplasm of the bacterium. Although the absorption of plastic molecules through various microbial membranes has not been well studied, it is thought that the process, like that of hydrocarbons, includes both active and passive transportation. *Pseudomonas sp*. DG17 has been found to take up octadecane, a degradative component of plastic polymers (Shahnawaz et al., 2019), through facilitated passive transport mechanisms at higher concentrations, and energy-dependent active transportation at lower concentrations

(Hua et al., 2013). Furthermore, during the first oxidation of alkenes, numerous membrane-bound monooxygenases have been shown to be widespread in alkene-assimilating bacteria (Durairaj et al., 2016). The transfer of these compounds into the cytoplasm for subsequent processing has also been demonstrated to be facilitated by several membrane transport mechanisms. In a *Comamonas* species, a particular transporter has been identified as being responsible for the inward transfer of terephthalic acid, a hydrolytic product of polyethylene terephthalate (PET) (Hosaka et al., 2013). Porins have also been demonstrated to be capable of transporting polyethylene glycol, a plastic degradative product, into the cytoplasm for bioconversion (Duret and Delcour, 2010). Different transporters, mostly belonging to the major facilitator superfamily and the ATP binding cassette family of proteins, were shown to be increased in *Rhodococcus rhodocrous* when absorbing polyethylene oligomeric intermediates in a research by Gravouil et al. (2017). It was also suggested that certain transporters, such as a discovered transport protein with NADH dehydrogenase activity, may play a dual function in intermediate trafficking and oxidation.

5.4 Mineralisation

Once these plastic derivatives have been effectively delivered into the cells, they are subjected to a series of enzymatic reactions that result in their total breakdown into oxidized metabolites such as CO2, N2, CH4, and H2O. (Ho et al., 2018). Techniques such as isotopic tracking and the measurement of CO2 emission using Strum's approach have shown the full mineralisation of polymeric polymers (Yang et al., 2020). Alternately, the intermediates may be sent via several chemical routes. For example, it has been suggested that polyethylene breakdown occurs through the production of acetic acid, which may then enter the Krebs cycle via acetyl-CoA synthesis or be channeled towards lipid formation (Wilkes and Aristilde, 2017). Similarly, succinate, another Krebs cycle intermediate, is produced in *Pseudomonas sp.* via esterase activity on polyethersulfones breakdown (Tribedi and Sil, 2014). Styrene, the monomeric unit of the recalcitrant polystyrene, is primarily oxidized to phenylacetate, which is subsequently incorporated into the Kreb's cycle as phenylacetyl coenzyme A for full metabolism, according to the well-elucidated biodegradation of styrene (Ho et al., 2018). TPA 1,2-dioxygenase (TPADO) and 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate dehydrogenase (DCDDH) metabolize internalized terephthalic acid to generate protocatechuic acid (PCA) as the end molecule, according to studies on *I. sakaiensis*. PCA undergoes a series of enzymatic processes involving PCA 3,4 dioxygenase and dehydrogenase to produce 2-pyrone-4,6-dicarboxylic acid,

which is then channeled into the Kreb's cycle as pyruvate and oxaloacetate before being mineralized to CO2 and H2O. (Yoshida et al., 2016). The mineralisation phase may be aerobic or anaerobic, but it needs the activity of a number of enzymes, including esterases, lipases, cutinases, peroxidases, and laccases in both cases (Alshehrei, 2017).

Factors affecting Degradation of Plastics

Microbial biodegradation of plastic polymers in nature and under controlled circumstances is influenced by a number of variables that may be classified into three categories: polymer properties, environmental factors, and chemical factors. By increasing the surface area, hydrophilicity, and lowering the molecular weight, these characteristics mainly serve to promote future microbial activity.

6.1 Polymer characteristics

As polymers must be carried across the cellular membrane to be metabolized, the rate of microbial breakdown has been shown to decrease with increasing molecular weight. As a result, smaller polymer units, such as monomers, dimers, and oligomers, are more easily degraded and mineralized, as shown by *Rhizopus delemar* lipase (Tokiwa et al., 2009). The morphology of the polymer, which comprises the degree of branching, crystallinity, and physical shape, has a major impact on the rate of degradation of plastic polymers. Microbial breakdown is less absorbed in plastic polymers with a greater percentage of side chains and therefore enhanced branching. According to studies, the non-crystalline part of polymers is more vulnerable to enzymatic degradation because it is more loosely packed and accessible, resulting in an inverse connection between crystallinity and degradation rate (Devi et al., 2016). Under the same circumstances, a research comparing the half-lives of various plastic polymers using pseudo-zeroth-order kinetics found that the specific surface degradation rate of polyethylene, a more crystalline polymer, was 9.5 μm year-1 compared to 1105 μm year-1 for PET (Chamas et al., 2020). Tm, the melting temperature of the particular polymer, has a significant impact on its microbial breakdown rate. It has been found that the Tm and biodegradation rates have an inverse connection. Tm of plastic polymers, on the other hand, is affected by changes in enthalpy of melting (H) and entropy of melting (S), as indicated in the equation: $Tm = \Delta H/S$ (Tokiwa et al., 2009). Although no evidence exists to link the glass transition temperature (Tg) of synthetic plastics to microbial attack, it is thought that the

structural changes that occur at this temperature may likely increase microbial attack (Lucas et al., 2008). The amount of early microbial colonisation of plastic polymers is increased as the material's hydrophilicity increases, while the activity of extracellular enzymes is thought to be reduced as the material's hydrophobicity increases. Because of their enhanced wettability, hydrophilic surfaces have higher surface energy and lower contact angles with water, facilitating microbial adhesion to the polymer surface and speeding up breakdown (Chamas et al., 2020). As a consequence of environmental weathering variables such as UV exposure, the presence and development of polar functional groups in plastic polymers has been shown to result in a reduction in contact angle with water and therefore an increase in hydrophilicity. Molecular dynamic simulations were used to show the impact of polymer hydrophilicity/ hydrophobicity, with highly hydrophobic polymeric polypropylene having the least biodegradative potential compared to comparatively highly hydrophilic nylon (Min et al., 2020).

6.2 Environmental factors

As the complexity of the surrounding environment have a significant influence in the kinetics of biodegradation, microorganisms rely heavily on the initial solo or synergistic action of various environmental factors on the polymers. Because each habitat has distinct features, the pace of microbial activity will differ in a dry environment, humid air, a landfill, compost, the marine environment, and so on. Bond scission has been shown to be aided by factors such as light, heat, moisture, pH, and biological activity. They also have an impact on structural homogeneities and the development of new functional groups (Siracusa, 2019). The presence of moisture in the environment will aid in the miniaturization of plastic polymers by increasing their solubility as well as the rate of hydrolysis. As a result, more chain scission will occur, increasing the sites of microbial action on the polymer chains, resulting in enhanced biodegradation, as demonstrated by Chamas et al. (2020), who found that degradation in the marine environment is significantly higher than on land when all other factors are held constant. Different plastic polymers have been shown to be electromagnetic radiation sensitive due to their ability to absorb the stronger portion of tropospheric solar radiation. These synthetic polymers tend to absorb high-energy UV light, which causes their electrons to become more reactive, resulting in oxidation and scission (Brebu, 2020). At high temperatures, plastic has also been found to degrade thermally. Temperatures in landfills have been predicted to reach 100 degrees Celsius, a situation that increases degradation rates if enough moisture and oxygen

are present for following thermal-oxidative and hydrolytic breakdown processes (Hao et al., 2017). The disorderliness in the polymeric structure caused by the rise in atom kinetic energy leads to molecular scission of the components of the long-chain backbones (Ray and Cooney, 2018). This causes chemical interactions between the various components, resulting in changes in the polymers' physical and optical characteristics. Thermal degradation impacts polymer molecular weights, ductility and embrittlement, initial color, cracking, and other properties. C-C and C-H bond scission starts polymer breakdown via a sequence of reactions involving free radicals after the other environmental variables have had their impact (Devi et al. 2016).

6.3 Chemical reagents and additives

By influencing the functional groups as well as hydrophilicity/hydrophobicity, the presence of chemical reagents in polymeric structures (additives) or the surrounding environment may either activate, inhibit, or catalyze the biodegradative process (Fotopoulou and Karapanagioti, 2017). Additives have recently been added to polymers to act as pro-oxidants, flame retardants, and pro-degradants, among other things. Some additives have been found in studies to reduce plastic recalcitrance during later reprocessing or degradation (Aldas et al., 2018). Others function as microbial inhibitors; one noteworthy example is dibutyl tin dilaurate, a very toxic polymeric addition in PU that has antibacterial properties, inhibiting microbial activity on the polymer (Cregut et al., 2013). Furthermore, it has been shown that the presence of a less complex carbon source in the environment affects microbial activity on plastics (Mehmood et al., 2016). This action is mediated by catabolite suppression, as shown by the fact that when glucose was removed from the medium, degradation of a polyethylene derivative by *Pseudomonas* strain increased by 80% (Tribedi et al., 2012). Biodegradation, on the other hand, was aided by the inclusion of biodegradable additives such starch (Mehmood et al., 2016) and palmitic acid (Jayaprakash and Palempalli, 2018), which provided microorganisms with a food source. The addition of OH- group radicals to polymer surfaces by oxidative agents such as hydrochloric acid, hydrogen peroxide, sulphuric acid, and nitric acid has been demonstrated to enhance biodegradation (Moharir and Kumar, 2019). Surfactants such as Tween 80 and sodium dodecyl sulphate, on the other hand, have been shown to accelerate microbial breakdown by increasing the hydrophilicity of polymer surfaces (Ghatge et al., 2020). The inclusion of additives that significantly stimulated the oxidation of the polymer was attributed to the considerable increase in mineralisation rate and carbon fixing seen during the biodegradation of polyethylene (Jakubowicz, 2003).

Various effective efforts have been made to modify the genes encoding for these enzymes utilizing various molecular methods in order to raise production levels, simplify product recovery, and improve the activity of various plastic degrading enzymes. However, the majority of recombinant plastic degrading enzymes have been found to be cloned from *Pseudomonas* species. *P. fluorescens* ST, for example, was utilized as a source of polystyrene catabolism genes. Gene expression in the host system revealed epoxystyrene isomerase, monooxygenase and epoxystyrene activity in the gene products (Marconi et al., 1996). Vega et al. (1999) cloned a polyester polyurethane (PUR)-degrading enzyme from the same species, as well as another species of the same genus, *Pseudomonas chlororaphis*, that showed exceptional activity against Impranil (Howard et al., 2001). *Pseudomonas sp*. E4 was discovered to be a promising polyethylene degrader. Following that, its alkane hydroxylase gene was expressed in *E. coli* to create a heterologous enzyme capable of mineralising the tested low molecular weight polyethylene into CO2 (Yoon et al., 2012). A polyester hydrolase, designated as a type IIa PET hydrolytic enzyme, was also cloned and expressed in *E. coli* from *P. aestusnigri* (Bollinger et al., 2020). PE, amorphous PET and bis(2-Hydroxyethyl) terephthalate were all degraded by the recombinant enzyme, however commercial PET bottles were not. Site-directed mutagenesis, on the other hand, enhanced the enzyme's ability to breakdown films from PET bottles (Bollinger et al., 2020). Many research have been conducted on *Ideonella sakaiensis'* extraordinary capacity to substantially break down PET. In this respect, the PETase gene from the bacterium has been the subject of a considerable amount of genetic manipulation research. The PETase gene from *I. sakaiensis* has been successfully cloned and expressed in a variety of host systems, including *Phaeodactylum tricornutum* (Moog et al., 2019) and *E. coli* (Joo et al., 2018). The identical PETase gene was expressed in *E. coli* BL21 (DE3)-T1R to overcome the challenging enzyme recovery, instability, and solubility problems encountered with the wild type organism (Seo et al., 2019). Apart from *I. sakaiensis*, recombinant enzymes with substantial PETase activity have been cloned from a variety of microorganisms. One of these genes was obtained from a marine actinomycete strain, *Streptomyces sp*. SM14, and expressed in *E. coli*, resulting in an extracellular enzyme with a native signal peptide sequence identical to the original enzyme (Almeida et al., 2019). Another *actinomycete*, *S. scabies*, was used to clone an enzyme that can metabolize PET as well as other natural and synthetic substrates (Jabloune et al., 2020). Recently, the expression of an algal PETase gene, derived from the green microalgae *Phaeodactylum tricornutum*, was described in *Chlamydomonas reinhardtii*,

a faster growing and ecologically beneficial green algae (Kim et al., 2020). MHETase, a tannase-like enzyme that has been found to act in tandem with PETase for the entire metabolism of PET, has also been cloned from *Ideonella sakaiensis* and produced in *E. coli* in order to thoroughly investigate the enzymatic breakdown of PET (Janatunaim and Fibriani, 2020). Several protein engineering methods have also been used to boost the enzymatic activity of different plastic degrading enzymes. Km value was reduced considerably and *I. sakaiensis* activity was increased by more than a hundred percent using site guided mutagenesis (Ma et al., 2018). Other polymeric polymers, such as polyethylene-2,5-furandicarboxylate (PEF), have been designed to metabolize the enzyme's substrate selectivity (Austin et al., 2018). The capacity of a cutinase from *Thermobifida cellulosilytica* to degrade PET has similarly been shown to be increased 16-fold by covalent fusing of its gene with hydrophobins (Ribitsch et al., 2015).

Conclusion

The biodegradation of synthetic plastic wastes by microorganisms such as actinomycetes, algae, bacteria, and fungus has been widely studied in this article. The main processes of this biodegradation, as well as the roles of the many enzymes involved, were clarified. The information on various microorganisms with plastic degrading potentials has been based on pure culture isolates, according to the existing literature. This clearly shows that the great variety of microorganisms found in various natural environments has not been fully explored. The use of metagenomics, which allows for the study of both culturable and unculturable microorganisms, will aid in the identification of microbes and biocatalysts that have the ability to degrade plastics. Other -omic techniques, including as genomics, transcriptomics, proteomics, and metabolomics, will also help in understanding biological interactions that occur during synthetic plastic breakdown between genes, transcripts, proteins, metabolites, and external environmental variables. Because of the synergy between the microbes and their enzymes, it is also thought that using a consortium of microorganisms would result in higher efficiency in plastic breakdown. Although numerous plastic degrading enzymes have been discovered from various sources, their biochemical and structural characteristics have not been well investigated. These details are required to get a better understanding of the processes behind the biodegradation of refractory polymers. This knowledge will be helpful in the creation of new plastic polymers with enhanced biodegradability, as well as the modification

of enzymes via protein engineering and the design of microbial cell factories with higher degradation efficiency. The study of the effects of various pre-treatment techniques and additives on the microbial breakdown of synthetic polymers is also crucial, since it is anticipated that using the right pre-treatment/additives would result in better outcomes. There were many differences across studies in terms of techniques for evaluating degrading efficiency; therefore, establishing and adopting a standard or universal strategy would go a long way toward data harmonisation and, as a result, the development of this field of study. Furthermore, it has been shown that Bangladesh's excessive use of plastic has a major impact on the environment and public health. Given the inexhaustible potentials of microbes and their constant adaptation to changing environments, more in-depth research in this area is expected to soon result in viable biodegradation processes that can be developed on a commercial scale and help policymakers make effective policies to reduce plastic pollution.

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