

## A Review on the Role of Microbes in Polyethene Degradation

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### Abstract

Polyethene is a polyolefin produced from polymerization of the olefin ethylene (C<sub>2</sub>H<sub>4</sub>). It is one of the most commonly used plastic and one of the most resistant to degradation. Its accumulation in the surrounding has caught the attention of many governments and researchers with attempts to come up with better disposal methods. This review focused on the role played by microorganisms in the degradation of polyethene. The references reviewed were obtained from journals and databases including PubMed, Google Scholar (<http://scholar.google.com>) and Science Direct (<http://www.science-direct.com>). We focused on data published from 2010 up to 2021. The findings obtained indicated that 19 genera of bacteria and actinomycetes and 5 fungal genera have the ability to degrade polyethene through secretion of extracellular depolymerases. The enzymes cleave polymer chains into low molecular weight fragments, which are then assimilated through the microbial cell membrane and mineralized. Microbial degradation is a sustainable and promising idea. However, there is need for more research to clearly determine the mechanism of enzymatic degradation, which will be useful in the development of novel biotechnological tools for degradation of a variety of plastic materials by microorganisms.

**Key Words:** Polyethene, Depolymerases, Degradation, Microorganisms.

### Introduction

The manufacture of plastic dates back to the 1950s. Since then, the sector has greatly grown and has become one of the biggest and most important sectors economically (Tudor et al., 2019). In fact, a world without plastics would actually seem unimaginable today (Geyer et al., 2017). Plastic is used in almost all sectors including packaging, car manufacturing, building and construction and agriculture due to its pleasant properties such as flexibility, inertness, durability, malleability, light weight and low costs (Chae & An, 2018). Despite these multiple uses, plastics cause high levels of pollution and leakage to the environment (Emblem, 2012). About 140 million tons of man-made polymers are manufactured yearly (Caruso, 2015). In 2015, the production of plastics globally was 322 million tons out of 6,300 million tons of wastes generated. About 79% of these wastes were disposed on landfills or was leaked to the environment, 9% was recycled and the remaining 12% incinerated (Europe, 2016).

The rapid increase and poor disposal methods of waste plastic is amongst the greatest challenges facing the globe today. This is mainly attributed to plastic resistance to degradation, their outstanding properties and importance in industry (Gewert et al., 2015). Plastics have unusual bonds and high number of aromatic rings in their structure which make them hard to break down in the environment (Pathak, 2017). Disposed plastic remain for many years in the environment without degradation, hence accumulate in the environment, resulting into serious environmental pollution with hazardous effects to both plants, animals and human (Dey et al., 2012). The problem of plastic pollution is a global problem which will continue if the production levels and consumption are not controlled. Proper procedures on plastic waste management and disposal need to be put in place to help curb the menace of plastic pollution (Löhr et al., 2017).

This review discusses the role played by microorganisms in polyethene degradation as

well as the limitations of the current methods used in degradation of polyethylene.

## Methodology

In conducting this review, systematic and comprehensive literature reports on the role played by microorganisms in polyethylene (PE) degradation were used. Empirical online searches were carried out using PubMed, Google Scholar (<http://scholar.google.com>) and Science Direct (<http://www.science-direct.com>). About 92.3% of the literature sources were from peer reviewed journals whereas 7.7% were from grey literature. This review focused on the impacts of plastic pollution in the environment, the current methods used for PE degradation and their limitations, role of different microbes in biodegradation, and the mechanism and role of microbial enzymes in PE degradation. Factors affecting plastic degradation, role of intestinal microbiome in PE degradation and the toxicity of polyethylene degradation products were also searched. Other articles and publications were obtained by tracking citations from other publications or by directly accessing journal websites. Scientific studies conducted from 2010 up to 2021 were accessed. The keyword combinations for the search were polythene, biodegradation, microplastic, enzymes, microorganisms and degradation.

## Findings and Discussion

### *Polyethene types, properties and uses*

Plastics can be categorized either as thermoplastics or thermosets (Lithner, 2011). Those that melt under high temperatures (heated) and harden when temperatures are lowered (cooled) are called thermoplastics. Their strength and thermal properties are determined by the branched or linear molecular structure. Thermoplastics are generally recyclable since they can be re-melted and reformed (Rajendran et al., 2015). They include polystyrene (PS), polypropylene (PP), polyethylene (PE), Polyvinyl Chloride (PVC), polyethylene terephthalate (PET) and polyamide (Rajendran et al., 2015). Thermosets are those plastics that undergo an irreversible chemical change through formation of a 3-dimensional network when subjected to heating. They are unrecyclable since it is

impossible to re-melt and reform them. They include unsaturated polyesters, polyurethanes (PUR), melamine, silicone and epoxy, phenolic, and acrylic resins (Ahmad et al., 2017).

Polyethylene is a polyolefin (PO) produced by polymerization of the olefin ethylene ( $C_2H_4$ ). It is a thermoplastic with the general formula  $C_nH_{2n}$ , where n represent the number of carbon atoms in the polymer chain (Sangale et al., 2012). The most commonly used PE grades include high density polyethylene (HDPE), low density polyethylene (LDPE) and linear low density polyethylene (LLDPE) (Grover et al., 2015a).

The density and crystallinity of LDPE ranges between 0.915-0.935  $g/cm^3$  and 50 - 60%, respectively (Favaro et al., 2016). Except by strong oxidizing agents, LDPE is not chemically reactive at room temperature. It can tolerate heat of up to 95°C depending on the length of exposure (Sen & Raut, 2015a). It is tough and flexible but can be broken. LDPE has more branching than HDPE; it has weaker intermolecular forces, low tensile strength and high resilience. These properties make low-density polyethylene the most commonly used plastic especially for packaging purposes (Pramila & Ramesh, 2011a).




LDPE has a comparatively low density due to the few branches in the chain (about 2%), (Sen & Raut, 2015b). The most frequent types of LDPE include branched low density polyethylene (BLDPE) and linear low-density polyethylene (LLDPE) which vary in density, surface functional groups and degree of branching (Ghatge et al., 2020a).

LDPE has a broad range of uses which include making of packaging materials and plastic bags. It is also used as a barrier coating on textiles, paper and other plastics (Sen & Raut, 2015b). However, LDPE is not easily broken down after disposal due to presence of a hydrophobic backbone in its structure (Luckachan and Pillai, 2011).

HDPE is a thermoplastic with a density range of 0.941-0.967  $g/cm^3$  (Favaro et al., 2016). It has few branches hence more tensile strength and stronger intermolecular forces as compared to LDPE. It has excellent insulating properties and is widely used in industrial and daily applications such as making bottles, toys, films, utensils, pipe, wire and cable insulations (Kumar

et al., 2011). The properties of different polyethylene types vary (Table 1).

Table 1. Summary of Polyethylene types and their properties (Favaro et al., 2016)

Property	LDPE	LLDPE	HDPE
Density	0.915-0.935	0.910-0.925	0.941-0.967
Stress (MPa)	7-17	14-21	18
Melting ( $^{\circ}$ C)	106-112	121-125	130-133
Elongation (%)	100-700	200-1200	20-100
Elastic modulus (MPa)	102-240	100-200	960-1000
Structure			
Crystallinity (%)	50-60	35-60	>90

### ***Plastics pollution***

One of the major challenges that the world faces is the problem of plastic waste management. With the rapid increase and poor disposal methods, plastic wastes have over-accumulated in the environment causing detrimental effects to both flora and fauna.

These detrimental effects have forced many governments through their pollution control boards to develop strategies to minimize the use of plastic materials (Xanthos & Walker, 2017). For example, the Kenya Government through the National Environment Management Authority (NEMA), banned the use of polyethylene carrier bags in 2017 (Noor, 2020). In India, the usage and manufacture of polyethylene carrier bags with thickness below  $50\mu$  was banned by the Government of Maharashtra (Sangale et al., 2019). This has proved to be a good step especially in the Kenyan scenario, in reducing the volume of PE bags going to landfills and those that are carelessly dumped causing visual pollution and clogging the drainage systems. However, it is important to encourage the use of biodegradable plastics or polyethylene blends which pose no harm to the environment.

### ***Plastics on land***

Plastic pollution on land remains widely unexplored since more attention is on microplastics (MP) in aquatic environments (Machado et al., 2018). Even though plastic pollution is more in the marine ecosystem, more than 80% of these plastics were manufactured,

consumed and trashed on land (Bläsing & Amelung, 2018). This implies that plastic disposed on land causes both damage and contamination to the terrestrial environment and the same is transferred to the aquatic ecosystems. Presence of plastics in soil as a result of land filling or careless disposal affect plant root development and also reduce aeration in soil. The toxic chemicals in plastic may also be leached into water bodies (Grover et al., 2015b).

Littering of polyethylene and other plastic products cause visual pollution that affect sectors like tourism (Wachira et al., 2014). Plastic litter may clog drainage systems and block sewer systems offering favorable breeding grounds for disease transmitting vectors such as mosquitoes (Bardaji et al., 2020).

### ***Plastics in the aquatic environment***

Plastic polymers have been found all over the marine ecosystem (Wierckx et al., 2018). The presence of microplastics has been discovered in marine water, sediment and biota samples (Bour et al., 2018; Mintenig et al., 2017). They catch attention in marine environments due to their small size which is almost equal to the size of prey, or food particles ingested by marine organisms. They can therefore be easily mistaken as food by most marine organisms (Ryan, 2016). Their size make them bioavailable, which facilitate entry into the food chain at various trophic levels and bioaccumulation (Carbery et al., 2018). Analysis of environmental samples has shown presence of both secondary and primary MP (Mintenig et al., 2017; Phuong et al., 2016).

Most marine species face the danger of entanglement and ingestion of plastic objects (Thiel et al., 2018). Juveniles are frequently ensnared in plastic debris resulting into severe injury during growth of the animal and restricting its movement. This may hinder proper feeding and sometimes even breathing (Sigler, 2014). Many different marine species such as sea turtles, fur seals, filter feeders, marine birds, sharks, cetaceans, bivalves, crustaceans, elasmobranchs, planktons and fishes have been found to be negatively impacted by plastic debris (Gall & Thompson, 2015; Hammer et al., 2012a). Plastic ingestion is most common in marine birds as they mistake the plastic objects for food (Poon et al., 2017). Once ingested, the plastics remain in the alimentary canal and can block the digestive tract, decrease secretion of digestive enzymes, reduce feeding stimuli and also cause reproduction problems due to reduced steroid hormone levels (Webb et al., 2013).

Marine organisms such as zooplanktons, sea birds, cetaceans, marine mammals, turtles and fish get entangled and easily ingest plastic items including bottle caps, cigarette lighters, fishing nets and plastic bags. Marine animals that get entangled in plastic debris may end up dying due to suffocation, drowning, starvation or strangulation (Hammer et al., 2012b). Very often, small whales, birds and seals drown and get entangled in ghost nets, hence losing their ability to escape predators and to catch food. It is not possible to approximate the quantity of plastic litter that end up in the ocean, however, it is worth noting that quantities are quite substantial (da Costa et al., 2016).

In addition, high levels of organic pollutants and other hazardous chemical compounds like polycyclic hydrocarbons (PAHs), organic pesticides and bisphenol A (BPA) among others, have frequently been detected in the marine plastic debris (Bardají et al., 2020; Camacho et al., 2019; Wright & Kelly, 2017; Van et al., 2012). The presence of these compounds greatly exacerbates the threats related to plastic debris ingestion by aquatic species. Biomagnification of these chemical compounds may pose direct risk to human health when they feed on marine food (Gallo et al., 2018).

### *Plastic disposal methods & their impact*

Currently, recycling, incineration and landfills are the most commonly used large scale plastic disposal methods (Deepika & Jaya, 2015; Webb et al., 2013). Each of these methods however, may cause either economic exploitation or damaging effects to the environment.

#### *Incineration*

Incineration of solid waste is an efficient method of waste management which reduces the use of landfills. Energy is produced during incineration which could be used as a fuel source to replace fossil fuels. This energy can also be used for electricity generation, heat and power (Al-Salem, 2019; Bardají et al., 2020). Energy recovery through plastic incinerating has many environmental benefits. It reduces the quantity of plastic waste, and destroys harmful chemical additives, foams, blowing agents and granules (Awasthi et al., 2017; Bardají et al., 2020).

However, incineration has disadvantages such as being expensive and production of toxic emissions that may cause health problems to humans and the environment. Burning of plastics produces soot and solid residue ash as by-products (Verma et al., 2016a). Soot is accompanied with smoke, polychlorinated dibenzo furans (PCDFs), volatile organic compounds (VOCs), particulate bound heavy metals, polycyclic aromatic hydrocarbons (PAHs), and dioxins which are carcinogenic and highly mutagenic (Ujowundu et al., 2016). The plasticizers added during manufacture of plastics are carcinogenic and may cause various cancers (Halden, 2010). Furans and dioxins produced during plastic combustion play a role in ozone layer depletion (Zhang et al., 2017; Verma et al., 2016b). Dioxins may also disrupt the activity of the human endocrine hormone hence raising health concerns (Casals-Casas & Desvergne, 2011).

#### *Landfilling*

Landfill is an old method of managing solid wastes. The major disadvantages of landfilling are space utilization and leaching of chemicals to soil and ground water (Awasthi et al., 2017). One major problem in landfills is secondary pollution, which results from leaching of pollutants and chemicals such as trimethyl-benzene, xylene and toluene. Additionally, estrogenic compounds including phthalate, bisphenol A (BPA), and

polybrominated biphenyls (PBB) may be produced (Grover et al., 2015b). These compounds are associated with health risks including diseases of the reproductive system and cancer of the prostate, ovaries and breasts (Verma et al., 2016b).

Plastics in dumpsites take about 3 centuries to naturally break down. Additionally, photo degradation break down plastics into very tiny toxic parts which eventually pollute water and soil (Grover et al., 2015b).

Landfilling is associated with limitations such as long time occupation of space which could otherwise be used for other activities such as agriculture (Webb et al., 2013). Generally, landfilling is unsustainable due to space requirement and also the release of harmful liquids and gases leading to secondary pollution (Awasthi et al., 2017).

### **Recycling**

The process of plastic recycling involves recovery, reprocessing and refining waste plastic to create new altered products (Vanapalli et al., 2019). It involves different processes that includes chemical, mechanical and thermal depolymerization (Garcia & Robertson, 2017). Plastic recycling can be classified into four types which include primary, secondary, tertiary and quaternary recycling. Secondary and primary recycling are collectively known as mechanical recycling. Tertiary recycling involves depolymerization of plastic polymer to its chemical constituents (Al-Salem, 2019). Energy recovery occurs during quaternary recycling (Singh et al., 2017). Crates, brush arms, non-food bottles, stationery such as rulers, speed bumps, and truck cargo liners are manufactured from recycled HDPE. Recycled LPDE are used to produce trash cans, non-food plastic bags and garbage can liners among others (Pohjakallio, 2020).

Recycling has been thought to be a better option compared to incineration and landfilling. However, recycling is relatively ineffective and may decrease the quality of the resulting polymer, the process is expensive and also emits toxic compounds resulting from melting waste plastic (Bardají et al., 2020; Webb et al., 2013). The colors, additives and stabilizers added to plastics during recycling make the recycled plastics more dangerous than virgin plastics. The volatile organic compounds have serious health effects

due to presence of many hazardous compounds which can either be cancerous or non-cancerous (Hahladakis et al., 2018a). Additionally, plastic recycling cannot be done more than thrice since each recycling decreases the strength of plastics. Some plastics such as multilayer and thermoset plastics are not recyclable hence creating disposal problems (Grover et al., 2015b). Recycling is an attractive method compared to incineration and landfilling. However, it is also considered much costly and inefficient due to presence of additives and other substances (Bardají et al., 2020).

### **Biodegradation of polyethene**

Microorganisms from over 90 genera have been found to have potential of plastic degradation (Ghosh et al., 2013a). Degradation by microbes is mainly caused by extracellular enzymes secreted by microorganisms (Karigar & Rao, 2011). Bioremediation of plastic waste is effective when microbial depolymerases attack and convert contaminants into harmless products (Karigar & Rao, 2011). It results from oxidation/ hydrolysis by enzymes secreted by microorganisms that cleave large polymer chains into shorter chain molecules such as monomers and oligomers, a process known as depolymerization (Das & Kumar, 2015; Pathak, 2017). These small sized molecules can cross the bacterial plasma membrane and can then be utilized as a carbon source (Mohan, 2011).

Polyethene degradation by microbes has been studied by many researchers and has become a great topic of interest. These studies have described PE degradation by bacteria, fungi, insects, algae and actinomycetes from sources such as garbage soil, marine water, compost soil, garden soil amongst others (Abraham et al., 2017; Bano et al., 2017a; Devi et al., 2015). The entire process of biodegradation involves four stages: biodeterioration, biofragmentation, bioassimilation, and mineralization (Montazer et al., 2020). Microorganisms need points of access in the PE structure to initiate the process of fragmentation. Initial oxidation of PE may occur in presence of environmental factors like chemicals, ultraviolet radiation (UV) and or heat without the microbial action. Some microorganisms are however, able to initiate the process of oxidation on their own through hydroperoxidation, a process called biodeterioration (Montazer et al., 2020).

This is followed by enzymatic cleavage where the polymer is broken down into low molecular weight monomers and oligomers through a process called biofragmentation (Urbanek et al., 2018 ; Bhardwaj et al., 2013). Hydrolase enzymes such as esterases and proteases catalyze the process of polymer break-down (Loredo-Treviño et al., 2012). Through bioassimilation the

fragmented polymer is taken up by microbes and mineralized into CO<sub>2</sub>, H<sub>2</sub>O, CH<sub>4</sub> depending on the conditions available (Khan & Majeed, 2019). Different steps are involved in polyethylene degradation (Figure 1).

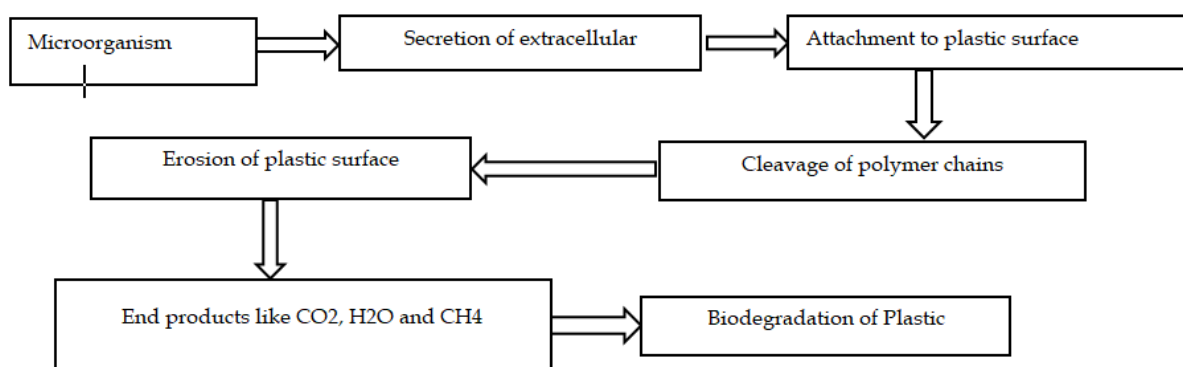


Figure 1. Mechanism of polyethylene biodegradation (Bhardwaj et al., 2013)

The speed of biodegradation also called biodegradation rate is dependent on the structure, chemical composition of polymer and abiotic conditions such as pH, temperature, microbial community present and moisture (Geyer, 2020). The main challenge to microbial colonization of plastic polymer as reported in cite literature here is high polymer hydrophobicity, compared to the hydrophilic surfaces of many microorganisms. Therefore, microbes with more hydrophobic surfaces can be more useful in initiating polymer colonization process hence improving the degradation process (Tribedi & Sil, 2013). Microbial degradation can be improved by finding out and improving growth conditions including temperature, moisture, pH, nutrient levels, carbon, nitrogen, oxygen and any other factor that may limit microbial growth and activity. This process is known as biostimulation (Adams et al., 2015; Kalantary et al., 2014). Furthermore, polyethylene (PE) biodegradation can be enhanced through addition of surfactants. Surfactants are surface active amphiphilic compounds that can reduce surface tension and interfacial tension of a solution (Duddu et al., 2015). Reduction of surface tension increases bioavailability of hydrophobic materials (Ahmad et al., 2017; Karlapudi et al., 2018).

Some microbes are able to produce biosurfactants on their own. For example, *Bacillus sp.*, *Bacillus licheniformis*, *Streptomyces coelicoflavus* among others (Duddu et al., 2015; Kavitha and Bhuvanewari, 2021; Mukherjee et al., 2018). However, nonionic surfactants such as Tween 80 can be added to a biodegradation system to improve PE degradation (Mukherjee et al., 2018). Surfactants are amphiphilic compounds that can reduce the hydrophobicity of PE hence increasing microbial attachment to the PE surface (Mukherjee et al., 2018).

*Pseudomonas* species are one of the most commonly cited bacteria that have potential to degrade PE and other polymers (Montazer et al., 2019; Wilkes and Aristilde, 2017; Ghosh et al., 2013b). *Pseudomonas sp.* AKS2 was found to carry out PE degradation through enzymatic activities and also through formation of biofilm (Tribedi & Sil, 2013). Formation of biofilm improves microbial adhesion to polymer surface due to better cell surface hydrophobicity, as compared to planktonic cells. *Pseudomonas sp.* AKS2 was reported to possess biodegrading ability against LDPE without prior oxidation by chemicals, UV radiation or thermal radiation (Tribedi & Sil, 2013). This suggests that *Pseudomonas sp.* AKS2 produces enzyme(s) that catalyse the cleavage of alkene bonds to carbonyl groups and/or

carboxylic acids. Alkane hydroxylase enzyme (alkB) from *Pseudomonas* has been found to play a key role in LDPE degradation (Yoon et al., 2012).

### Fungi in Polyethene biodegradation

The potential of fungi to degrade PE have been investigated in various studies (Sheik *et al.*, 2015; Ameen *et al.*, 2015; Pramila and Ramesh, 2011b). Their potential to degrade PE is associated with their ability to secrete enzymes and extracellular polymers such as polysaccharides, which are important in colonization of polymer surface (Esmaili et al., 2013). Fungi are important in polymer degradation because of their robust nature and for the wide variety of enzymes they secrete. Examples of these enzymes include cutinases, xylanases, lipases, esterase among others (Deshmukh et al., 2016).

A number of fungal species have been demonstrated to use PE as the sole carbon source. For example, Raaman et al., (2012) studied the biodegradation of LDPE polyethene carry bags under laboratory conditions. These authors isolated *Aspergillus niger* and *Aspergillus japonicus* Table 2. Other literature sources of polyethene degrading fungi

from plastic polluted soils at 37°C in 48 hrs. incubation. The effectiveness of these fungi on LDPE degradation was tested after 4 weeks incubation by weight loss analysis and Scanning Electron Microscopy (SEM). *Aspergillus japonicus* showed 12% weight loss while *Aspergillus niger* showed a weight change of 8% after the 4 weeks period. The same authors also reported pores on surface of the fungal degraded polythene from SEM analysis. In another study by Immanuel et al., (2014), LDPE and HDPE degrading *Aspergillus japonicus* and *Aspergillus terreus* from mangrove soils were incubated with pre-treated PE films for 45-60 days. Biodegradation rate was determined by weight loss analysis and by Fourier-Transform Infrared (FTIR). FTIR results indicated new carbonyl group after natural weathering, which decreased after microbial treatment. Decrease in carbonyl index ranged between 11.8-22.4%. This author also reported a reduction in weight of the PE films ranging between 10.70-22.54%. Several other studies that have investigated PE biodegradation using fungal isolates exist (Table 2).

Name	PE type	Reference
<i>Aspergillus niger</i>	LDPE	(Alshehrei, 2017; Raaman et al., 2012)
<i>Aspergillus japonicus</i>	LDPE	(Raaman et al., 2012)
<i>Aspergillus nomius</i>	LDPE	(Munir et al., 2018)
<i>Aspergillus terreus, Aspergillus fumigatus</i>	LDPE	(Abraham et al., 2017)
<i>Aspergillus sydonii</i>	LDPE	(Sangale et al., 2019; Zahra et al., 2010)
<i>Aspergillus fumigatus</i>	LDPE	(Muhonja et al., 2018a; Zahra et al., 2010)
<i>Trichoderma viridae</i>	LDPE	(Munir et al., 2018)
<i>Penicillium oxalicum</i>	HDPE/LDPE	(Ojha & Pradhan, 2017)
<i>Fusarium sp, Mucor sp</i>	LDPE	(Jyoti & Gupta, 2014)
<i>Penicillium sp</i>	LDPE	(Alshehrei, 2017)
<i>Aspergillus tubingensis, Aspergillus flavus</i>	HDPE	(Devi et al., 2015)

### Bacteria in polyethene degradation

A number of bacterial species from different genera have been shown to have potential of polyethene degradation (Muhonja et al., 2018b; Begum et al., 2015; Sharma et al., 2014). Previous

studies have determined PE biodegradation by bacteria isolated from garbage soil, compost soil, marine environment, mangrove soil and recently from guts of insects like the wax moth. Most of these potential candidate bacteria belong to the genera *Bacilli*, *Pseudomonas*, *Staphylococcus*,

*Rhodococcus*, *Streptococcus*, *Streptomyces*, *Brevibacilli*, and *Micrococcus* (Pramila et al., 2012; Harshvardhan & Jha, 2013; Singh & Bhatt, 2016). In a study conducted by Montazer et al., (2018), *Acinetobacter pittii* isolated from a plastic polluted

landfill was found capable of degrading UV-pretreated LDPE. Evaluation of biodegradation extent showed  $26.8 \pm 3.04\%$  gravimetric weight reduction. More examples of bacterial strains associated with PE degradation exist (Table 3).

Table 3. Polyethylene degrading bacteria

Name	Substrate	Reference
<i>Bacillus sp</i> , <i>Staphylococcus sp</i> , <i>Pseudomonas sp</i>	PE	(Singh et al., 2016)
<i>Staphylococcus aureus</i>	PE	(Archana et al., 2017)
<i>Arthrobacter sp</i> , <i>Pseudomonas sp</i> .	HDPE	(Balasubramanian et al., 2010)
<i>Streptomyces sp</i> .	LDPE	(Abraham et al., 2017)
<i>Pseudomonas sp</i>	LDPE	(Tribedi & Sil, 2013)
<i>Pseudomonas citronellolis</i>	LDPE	(Bhatia et al., 2014)
<i>Kocuria palustris</i> , <i>Bacillus pumilus</i> , <i>Bacillus subtilis</i>	LDPE	(Harshvardhan & Jha, 2013)
<i>Brevibacilli parabrevis</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas citronellolis</i>	LDPE	(Pramila et al., 2012)

### Actinomycetes in polyethylene degradation

Actinomycetes are a diverse group of Gram-positive branching bacteria. They possess unique mycelial structures and spore-forming abilities. Their colonies are hard and stick to agar, have soil-like odors and pale colors (Salim et al., 2017). Plastic degrading actinomycetes including *Rhodococcus ruber*, *Streptomyces sp*, *Microbispora*, *Actinomadura sp*. among others have been isolated from garbage soils, mangrove soils, plant tissues and marine environment (Duddu & Guntuku, 2015; Usha et al., 2011). Their ability to break down plastic polymers is mainly attributed to their ability to secrete hydrolytic enzymes like laccase, lipase, protease, xylanase, cellulase among others (Hari, 2019). Biofilm formation by actinomycetes also helps in surface colonization and degradation of plastic polymers (Amobonye et al., 2020).

A polyethylene and plastic cup degrading *Streptomyces species* isolated from garbage soil by Usha et al., 2011 was found to possess the greater biodegrading ability than other bacteria and fungi after 6 months incubation period (Usha et al., 2011).

In another study, a thermophilic *Streptomyces coelicoflavus* NBRC 15399<sup>T</sup> was isolated from oil

contaminated soil. A 30% weight loss was reported on the tested LDPE after four weeks incubation indicating the potential of this actinomycete in polyethylene degradation (Duddu et al., 2015). Polyethylene degradation has also been demonstrated in *Nocardioopsis sp*. isolated from *Hibiscus rosasinensis* leaves (Singh & Sedhuraman, 2015).

### Biodegradation by intestinal microbiome

Recently, several insect species have been reported to consume or degrade polyethylene by the help of microbes isolated from their gut. The larvae of meal moths, darkling beetles and wax moths have been reported to consume and degrade a various plastic polymers (Lear et al., 2021). For example, *Plodia interpunctella* larvae were reported to possess the ability to consume and crush polyethylene films in a study carried out by Yang et al., 2014. *Bacillus sp*. YP1 and *Enterobacter asburiae* YT1 were isolated from the gut of this worm and were reported capable of polyethylene degradation (Yang et al., 2014). *Galleria mellonella* larvae were reported to consume and metabolize LDPE (Cassone et al., 2020). In addition, this study also showed the ability of a gut bacteria (from the genus



*Acinetobacter*) to use polyethylene as the sole carbon source (Cassone et al., 2020).

Three bacterial species *Microbacterium oxydans*, *Lysinibacillus fusiformis* and *Bacillus aryabhatai* were isolated by Montazer et al., 2021 from whole body extracts of *Galleria mellonella* larvae. He evaluated their potential to consume low-density polyethylene (LDPE) and obtain carbon from it invitro. He reported that these bacteria have potential to degrade LDPE (Montazer et al., 2021).

*Aspergillus flavus* isolated from the gut *Galleria mellonella* degraded HDPE microplastic particle into low molecular weight microplastic fragment after 28 days of incubation. Further analysis using Fourier Transform - Infrared Spectroscopy (FT-IR) showed presence of ether and carbonyl groups, which further proved PE degradation by the fungus (Zhang et al., 2020).

However, there is need for further studies to determine the enzymatic degradation mechanism in the guts of these insects. This information will be useful in the development of novel biotechnological tools useful in the biodegradation of a variety of plastic materials.

### Polyethene degrading enzymes

The type of chemical bonds present in plastic polymer determine the modification of polymers by enzymes (Wei & Zimmermann, 2017). Plastic polymer biodegradability greatly depend on presence or absence of hydrolysable functional groups in the polymer back bones (Restrepo-Flórez et al., 2014; Wei & Zimmermann, 2017). Plastic polymers that have hydrolysable functional groups can easily be depolymerized by microbial hydrolase enzymes including esterase, proteases and lipases (Bano et al., 2017a; Wei & Zimmermann, 2017; Wierckx et al., 2018). Plastic degrading enzymes are grouped into two; the intracellular and extracellular enzymes. The latter are the most studied and are said to be more reactive, and can carry out both oxidative and hydrolytic roles (Glaser, 2019; Amobonye et al., 2020; Ghatge et al., 2020b; Mohanan et al., 2020). They include the extracellular hydrolases and depolymerases. Extracellular depolymerases produce shorter polymer chains which can pass through the microbial plasma membrane and undergo subsequent chain cleavage and further metabolism (Dey et al., 2012). Hydrolytic cleavage occurs when an enzyme attaches itself

to the polymer surface catalyzing its breakdown (Banerjee et al., 2014). This cleavage results into low molecular weight oligomers, dimers and monomers which are then converted to H<sub>2</sub>O and CO<sub>2</sub> through the process of mineralization (Ghosh et al., 2013; Mohan, 2011). The monomers, oligomers and dimers are small enough to move across the cytoplasmic membrane where they are further exploited as carbon and energy source (Bano et al., 2017b). It is not yet clear how these molecules are metabolized inside the microbial cell. Some studies have however described that the low molecular weight molecules undergo oxidation in order to be transformed into carboxylic acid that can be metabolized through the tricarboxylic acid (TCA) cycle (Restrepo-Flórez et al., 2014). Enzymes which have been associated with PE biodegradation are laccases also called the blue copper oxidases, manganese peroxidase (MnP) (Bardají et al., 2020; Restrepo-Flórez et al., 2014), hydroxylases and reductases (Amobonye et al., 2020). The blue copper oxidases are so called because they have copper in their structure (Bardají et al., 2020). Addition of copper ions to cultures of *Rhodococcus ruber* C208 containing PE increased by 75%. FTIR analysis of the PE films indicated an increase in carbonyl peak. They reported a reduction of the molecular weight of the PE, indicating enzymatic oxidation by laccase (Santo et al., 2013).

### Factors affecting biodegradability of polymers

The main factors affecting polymer biodegradation are exposure/environmental conditions and polymer characteristics. Polymer characteristics are divided into chemical and physical characteristics and include features like shape, size, morphology, molecular weight, additives, hydrophobic and hydrophilic characteristics (Su, 2013). Exposure conditions are classified as biotic and abiotic. Abiotic factors include ionizing radiation, temperature, pH and moisture which affect the rate of hydrolysis reaction (Glaser, 2019). All these are important in influencing polymer surface colonization by microorganisms.

The molecular weight of a polymer can limit microbial colonization since the process is dependent on the surface properties that allow the microorganisms to attach (Restrepo-Flórez et al., 2014). Polymer crystallinity is important for microbial attachment since microorganisms

attach only on to the amorphous sections of the polymer surface (Glaser, 2019).

Additives such as pro-oxidants or starch can be used to improve polymer biodegradability. They are low molecular weight organic chemicals that can provide a starting point for microbial colonization. The presence of these additives influence the types of microorganisms colonizing the surfaces of these polymers (Ammala et al., 2011; Corti et al., 2010).

Abiotic factors including ionizing radiation, temperature, pH and moisture affects the rate of hydrolysis reaction. Increased temperature and moisture speeds up the hydrolysis reaction rates and microbial activity (Haider et al., 2019). In high-moisture environments, there is an increase in hydrolysis reaction which increases chain scission leading to an increase in the available sites for microbial attachment hence faster degradation (Chamas et al., 2020). Photo degradation reduces the number of average molecular weight, which provides greater accessibility to the polymer chain by moisture and microorganisms (Rabek, 2012). Among biotic factors, extracellular enzymes produced by microorganisms have active sites with different shapes and hence more able to biodegrade certain polymers. Smaller molecules are more accessible to microbes than larger ones. Low molecular weight portions are taken into the cells and then converted into metabolites (Khan & Majeed, 2019).

### **Toxicity of polyethylene degradation products (PEDP)**

The finished plastic is non-toxic but the monomers that are used in the production of the parent polymers can be toxic. Toxicity of plastic products is as a result of additives and plasticizers such as fillers, stabilizers, reinforcements, adipates, phthalates and colorants (Andrady & Rajapakse, 2016). These are usually mixed with the polymers to help in improving both the physical (mechanical, thermal, etc.) and chemical properties of the polymers (Hahladakis et al., 2018b). These chemicals can leak out of the product in traces causing toxic effects. The products of polymer degradation vary depending on the polymer type, degradation mechanism, presence of impurities and exposure conditions such as temperature and oxygen (Lithner, 2011). Various

studies have investigated the effect of polyethylene biodegradation on both plants and animals. For example, the toxicity of PE degradation products was tested on sorghum and fish in a culture supernatant containing *Bacillus cereus strain VASB1/TS* and *Lysinibacillus fusiformis strain VASB14/WL*. The toxicity test on sorghum revealed a decrease in germination index and inhibited elongation. However, no death of the fishes was recorded (Shahnawaz et al., 2016). In another study by Aswale, 2010, moderate reduction in seed germination was recorded when studying toxicity of PEDP on soybean, sunflower, groundnut and safflower seeds using culture filtrate. Toxicity test was also done using Chironomous larvae and there was no mortality reported (Aswale, 2010).

Studies by Das and Kumar on the toxicity of degradation products produced by LDPE degrading microorganisms including *Bacilli sp*, *Aspergillus sp* and *Fusarium sp* on *Cicer arietinum* and *Vigna radiate* showed a significant germination rate and seedling growth. They concluded that PEDP do not harm the environment and can be used to promote plant growth (Das & Kumar, 2013).

Based on the above studies, there's no clarity on the toxicity of PEDP since some studies reveal moderate toxicity and others report no toxicity. Therefore, there is need for more research in this area to determine whether PEDP can be safely disposed into the environment or not.

### **Challenges and opportunities in polyethylene biodegradation**

Many researchers have studied microbial degradation of PE. Despite all these studies proving the potential of microbes in PE degradation, this has not been made possible in reality such as in dump sites and landfills (Montazer et al., 2020). Furthermore, most commonly used methods to evaluate changes in biodegradation such as change in tensile strength, formation of holes, cracks and biofilms, fragmentation, change in color, and surface roughening do not give conclusive evidence of complete degradation. Instead they indicate microbial activity on PE indicating a stage in biodegradation (Bardají et al., 2020; Gnanavel et al., 2012). There is no standardized protocol and procedure for studying PE degradation. Different researchers use different experimental

procedures, conditions and even different kinds of PE when performing degradation assays which makes comparison of results almost impossible (Montazer et al., 2020).

### Conclusion

Polyethylene is one of the most common and frequently used plastic worldwide due to its pleasant properties. Its total elimination is neither feasible nor desirable since it offers many applications in industry and in day to day life. The over-accumulation and detrimental effects of PE and other plastics in the environment have caused some governments to take the step of banning usage of some plastic materials especially the polyethylene carrier bags. This to a good percentage has helped in reducing PE pollution in the respective countries. However, PE pollution is still evident because what was already disposed still remains undegraded in the dumpsites and in landfills, not forgetting those that are still being used and disposed. Therefore, it is important to encourage use of biodegradable plastics to replace the non-degradable ones. Microorganisms have been proven to have potential of PE degradation without causing more harm to the environment. Microbial degradation is very promising. However, the mechanism of biodegradation is not clearly understood. There is need for more research to clearly determine the mechanism of enzymatic degradation which will be useful in the development of novel biotechnological tools useful in degradation of a variety of plastic materials by microorganisms. It's also important that more research be done to determine the mechanism of biodegradation in the guts of insects such as *Galleria mellonella*.

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### Author's Contributions

Beryle Atieno Okoth, Huxley Mae Makonde, Carren Moraa Bosire, Jeophita Mwajuma and Cromwell Mwititi Kibiti conceptualized the review and co-authored the manuscript. Cromwell Mwititi Kibiti, Huxley Mae Makonde

and Carren Moraa Bosire critically reviewed the manuscript. All authors have read and approved the manuscript.

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