



# Non Bio-degradable Plastic Eating Bacteria: A Review

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

In the 21<sup>st</sup> century, synthetic plastics are a fundamental part of the global economy and the utilization of non-bio-degradable petrochemical plastics such as polyvinyl chloride, polyethylene, polypropylene, polystyrene, and polyethylene terephthalate has increased (80%) worldwide in the last five decades since invention. Conventional petro-chemical plastics either splinter via abiotic factors or segregate and absorb biotic factors during the bio-degradation process however, non-biodegradable petrochemical plastics are resistant to degradation via carrying poisonous excipients. Therefore, the degradation process of non-bio-degradable plastics relies on micro-organisms such as *Ideonella sakaiensis* 201-F6, Phormidium, Lewinella, *Bacillus megaterium*, *Rhodococcus ruber*, *Serratiamarcescens*, *Enterobacterasburiae* YT1, and *Bacillus* sp. YP1 as advanced recycling operations only covers approximately 10% of petro-chemical plastic waste. The purpose of this review is to emphasize the source, and mechanism of different micro-organisms capable to decompose petrochemical plastics.

**Keywords:** *Enterobacterasburiae* YT1; *Ideonella sakaiensis*; non-bio-degradable plastics; polyethylene; polyethylene terephthalate.

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## 1. INTRODUCTION

Synthetic plastics manufacturing is one of the most rapidly expanding industries on the planet. In spite of the fact that plastics have been used in daily life for over a century, large-scale production began in 1950 [1]. The numerous properties of plastics such as ease of synthesis, robustness, and durability make them superior to other materials in many fields and have resulted in a 20-fold increase in production scale in the five decades since their invention, transcending 300 million tons per year and extending up to 335 million tons in 2015 [2-4]. Furthermore, it is expected that plastic production will almost quadruple by 2050 [2].

Non-bio-degradable petrochemical plastics such as polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) (Fig. 1.) account for about 80% of total worldwide plastic usage. Globally, 56 million tonnes of PET are among the 311 million tonnes of plastic estimated to be produced annually. Although plastics are a fundamental part of the global economy, the problems that come with their widespread applications must be addressed.

In 2019, PE (low, medium, and high density) accounted for 30% of the European plastic

demand, with PET and PVC each accounting for approximately 8 and 10% respectively (Fig. 2.) [5].

During the biodegradation process, the conventional petrochemical plastics either splintered under UV radiation, temperature, and pressure (abiotic factors) or segregate and comprehend by micro-organisms (biotic factors) attributed to high molecular weight, long-chain polymer structure, hydrophobicity, crystallinity and deficiency of complementary functional group of plastics [6,7]. Larger and micro granules of petrochemical plastics are resistant, carriers of toxic excipients, ubiquitous in marine or earthly habitats, and assembled in living organisms [8,9].

Advanced recycling efforts cover only a small portion of petrochemical plastic waste and result in lower-value goods that are downgraded. They rely on the addition of large amounts of the virgin polymer as well as substantial energy consumption. Alternatively, several micro-organisms have been proven to degrade non-bio-degradable plastics.

The purpose of this review is to emphasize the source, and mechanism of different micro-organisms capable to decompose petrochemical plastics.

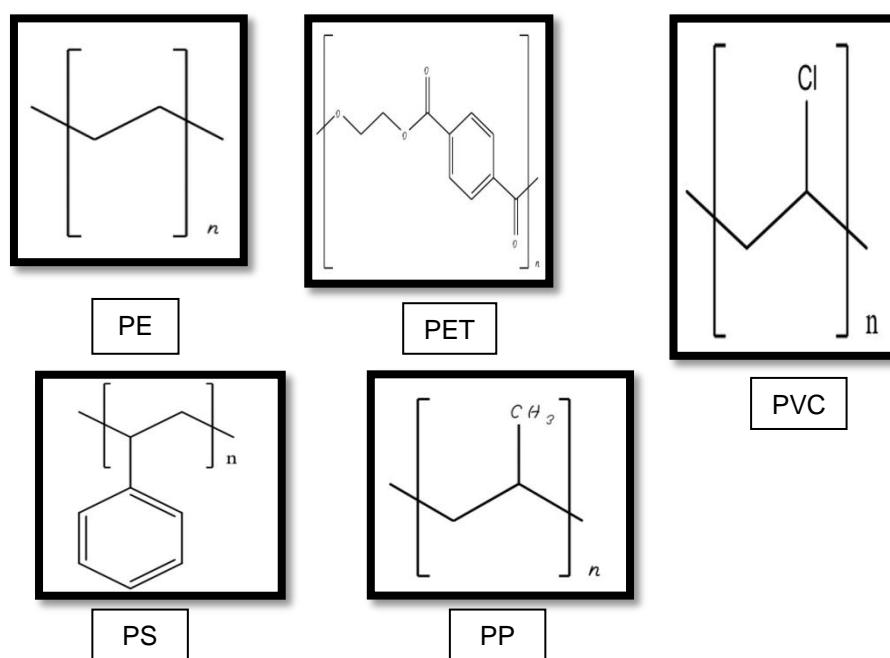


Fig. 1. Structure of non-biodegradable plastics

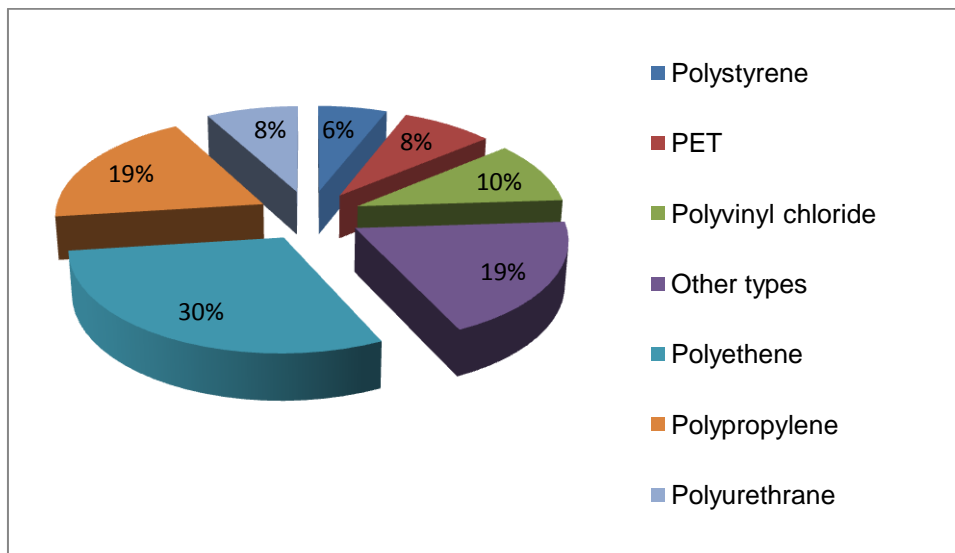


Fig. 2. Different plastics demand in Europe [5]

## 2. GENERAL MECHANISM OF MICROBIAL DEGRADATION OF PLASTICS

Degradation of plastic wastes, any physical or chemical change in polymers, is influenced by environmental factors like light, heat, moisture, pressure, or biological activity. Micro-organisms use polythene film as their main source of carbon and on the surface of polymer microbial bio-film is necessary for bio-degradation [10].

In the primary degradation of plastic, the main chain cleavage of polymer leads to the formation of low-molecular-weight fragments (oligomers), dimers, or monomers attributed to extracellular enzymes secreted from micro-organisms resulting in the formation of bio-film after colonizing polyethylene film's surface, which accordingly amplifies bio-degradation of the polymers. Cell surface hydrophobicity of the micro-organisms plays a significant role in the formation of this biofilm. It is commonly recognized that the generation of microbial biofilm on the substratum is essential for biodegradation [10]. Microorganisms can attach to surfaces of materials initially, but after that, they can generate massive bio-films that can change the physico-chemical characteristics of plastic films, such as functional ligands, molecular weight (MW) distribution hydrophobicity/hydrophilicity, crystalline structure, and surface morphology [11]. However, polymer catalyzes the hydrolysis at a rate noticeably faster than the deterioration caused by microbes, as per

measurements, which show that film surfaces acquire more hydrophilic following microbial attachment [12].

After bacterial inoculation, the geometry of plastic surfaces can be altered, and holes and voids have been seen on polymer surfaces [13,14]. Though film cultured with *Stenotrophomonas* sp. demonstrated a higher crystalline proportion, film incubated with *Comamonas* sp. and *Delftia* sp. exhibited a decrease in crystallinity [15]. Results on changes in the number-averaged and weight-averaged molecular weights of polymers appear to be in contradiction. For instance, some studies indicated that molecular weight dropped following bacterial inoculation, whereas other research asserted that it was accelerated following bacterial exposure [16].

Fourier transform infrared (FTIR) spectroscopy analysis can be used to assess the transition of functional ligands. The anaerobic or aerobic environment has an impact on the growth or shrinkage of functional groups. Some investigations have demonstrated an expansion in functional groups under aerobic conditions following microbial exposure however, other researchers have revealed the opposite results [17,18].

Due to the contradicting changes in PE film upon microbial exposure, attempts to reduce plastic pollution could be enhanced by improving our knowledge regarding the interaction between plastics and microorganisms.

### 3. MICRO-ORGANISMS DEGRADING NON-BIODEGRADABLE PLASTIC

Studies reported that pure strains of micro-organisms can decompose polymers and enables the assessment of metabolic processes and the impact of various environmental variables on PE degradation [19]. Though this strategy neglects the possibility that PE biodegradation could be the result of collective action between various microbial species in a natural environment, and that the activity of a single species could result in the deposition of intermediates or resurrected products with potentially higher toxicity that would hinder microbial growth [20]. One substance or a class of chemicals may accumulate temporarily or not degrade at all as a result of complex combinations of compounds having regulatory and inhibitive effects on biodegradation [21]. Microbial consortia, which frequently exploit the harmful metabolites produced by one bacterium as a substrate for the growth of another, can be used to get around this restriction [15]. Because of their collaborative arrangements, the bacteria in consortiums are more tolerant to the biodegradation of Polyethylene.

#### 3.1 *Ideonella sakaiensis* 201-F6

*Ideonella sakaiensis*, a Gram-negative, aerobic, non-spore-forming, rod-shaped bacterium, is obtained from the genus *Ideonella* and the family Comamonadaceae. It was found to culture and isolate with environmental samples adulterated with PET film, for example, sediment, soil, wastewater, and activated sludge in Modified lettuce and egg (MLE) medium [22]. Studies reported that this bacterial strain was demonstrated to grow on PET films exhibiting a low crystalline phase. Together, thermo-stable PETase and MHETase, two  $\alpha/\beta$ -hydrolase fold enzymes degrade PET in two steps (Fig. 3.) via mono-(2-hydroxyethyl) terephthalate (MHET), generating Terephthalic acid (TPA) and ethylene glycol (EG), the raw materials needed to synthesize polyethylene terephthalate (PET) again [23,24]. According to research, MHETase exclusively hydrolyzes MHET, not Bis 2-Hydroxyethyl Terephthalate (BHET), polyethylene terephthalate (PET), p-nitrophenyl (pNP) aliphatic esters, or aromatic ester compounds like ethyl gallate and ethyl ferulate, which are transformed by other enzymes from the tannase family, demonstrating

an incredibly limited substrate specificity [23]. Recent crystal structures of PETase ligand-bound validated the anticipated  $\alpha/\beta$ -hydrolase fold, explained target interaction, and even enabled the strengthening of catalytic characteristics or modulation of substrate specificity [25-29]. PETase from *Ideonella sakaiensis*, a PET-degrading esterase, exhibits significant growth at room temperature and on crystalline phase PET. Another enzyme, *Ideonella sakaiensis* MHETase, which is necessary for effective PET decomposition, has an unknown configuration [23]. There are several indicators of polyethylene terephthalate film degradation such as weight loss, CO<sub>2</sub> emission from PET catabolism, and surface morphological alterations in films. The change in surface topography and elevation of surface functional group numbers were determined by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) respectively [22].

Synthetic biology techniques may be able to boost *I. sakaiensis*'s potential to decompose PET. These opportunities will spur the development of revolutionary environmental technologies and a new sustainable bioindustry, resulting in a brand-new "bio-economy" based on recycled and renewable resources rather than fossil fuels. In recycling and reuse, *I. sakaiensis* and its enzymes have enormous potential for PET breakdown.

#### 3.2 *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1

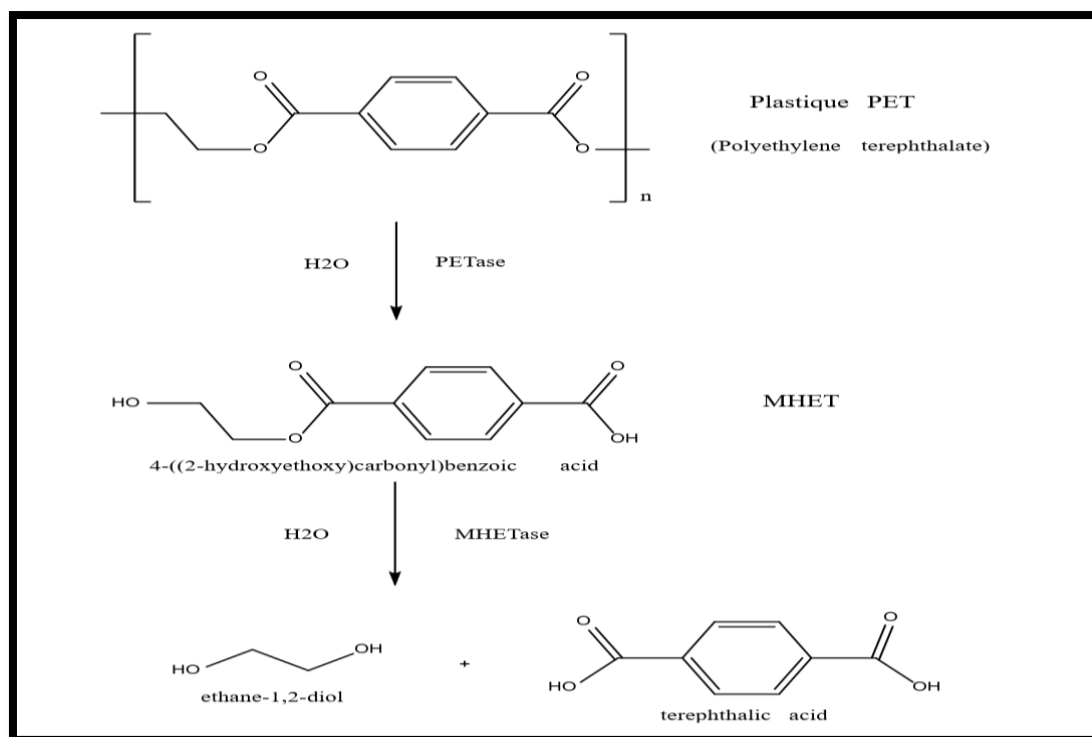
*Enterobacter asburiae* YT1 (Gram-negative) and *Bacillus* sp. YP1 (Gram-positive), facultative anaerobic, and potent PE-degrading bacilli, were found to be isolated from the gut of Indian meal moths or waxworms (the larvae of *Plodia interpunctella*). During incubation of the two bacterial strains on Polyethylene (PE) films for 28 days, viable bio-films were developed in a carbon-free basal agar medium by decreasing the hydrophobicity of the PE films detected by counting the number of cells colonizing on the PE film through a series dilution process. Several pits and cavities (0.3–0.4 m in depth) were detected on the surfaces of the PE sheets by using Scanning Electron Microscopy (SEM) and atomic force microscopy (AFM) whereas X-ray photoelectron spectroscopy (XPS) and micro-attenuated total reflectance/Fourier transform infrared (micro-ATR/FTIR) imaging microscope

confirmed the generation of carbonyl groups in the PE films. The remaining PE films had lowered molecular weights and electro-spray ionization mass spectrometry (ESI-MS) detected the release of twelve water-soluble offspring products confirming the degradation of PE films. It was found that suspension cultures of *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 (108 cells/mL) was capable to decompose about  $6.1 \pm 0.3\%$  and  $10.7 \pm 0.2\%$  of 100mg

PE films respectively after incubation of 60 days [30].

### 3.3 Others

Studies reported the name of micro-organisms (Table 1.) that are capable to degrade non-biodegradable plastic but their mechanisms of degradation are still unknown.



**Fig. 3. Mechanism of PET degradation by *Ideonella sakaiensis***

**Table 1. List of micro-organisms involving biodegradation of plastic (degradation mechanism is not proven)**

Micro-organisms	Degraded plastic	Reference
Phormidium, Lewinella	Polyethylene terephthalate (PET)	[31]
Arcobacter Colwellia	Low-density polyethylene (LDPE)	[32]
Pseudophormidium sp., Phormidium sp.	Polyethylene (PE), Polypropylene (PP)	[33]
Proteobacteria, Bacteroides	Microplastic	[34]
Stanieria, Pseudophormidium	Polyethylene terephthalate (PET)	[33]
<i>Streptomyces scabie</i> (isolated from potatoes)	Polyethylene terephthalate (PET)	[35]
<i>Pseudomonas aeruginosa</i> , <i>Bacillus megaterium</i> , <i>Rhodococcus ruber</i> , <i>Serratia marcescens</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i>	Polystyrene (PS), Polycarbonate (PC)	[36,37]
<i>Aspergillus fumigatus</i>	Polyurethane (PU)	[38]
Phormidium sp., Rivularia	Polyethylene (PE), Polypropylene (PP)	[39]
<i>Aspergillus niger</i>	Polypropylene (PP)	[40]
<i>Nocardiopsis</i> sp. isolated from Hibiscus	Polyethylene (PE)	[41]

Moreover, in 2017, researchers from the UK and Spain discovered a specific type of caterpillar (*Galleria mellonella*) was able to break down PE at a pace that was comparable to any previously noted. It was shown that the wax moth caterpillars can degrade PE at a rate of 0.23 mg cm<sup>-2</sup> h<sup>-1</sup>, which is faster than the rate at which PETase can break down PET [42,43].

#### 4. CONCLUSION

This essay has highlighted relevant research on the capability of two bacterial strains to degrade synthetic plastic wastes. The general mechanisms of this biodegradation and the functions of the several enzymes involved have been clarified. The information on several bacteria with the potential to degrade plastic has, according to the already available literature, been based on pure culture isolates. This reveals unequivocally that the rich diversity of microorganisms present in many natural settings has not been fully utilized; in particular, no yeast species are capable of degrading plastic. The discovery of bacteria and biocatalysts with the potential for the biodegradation of plastic will be facilitated by the use of metagenomics, which ensures the exploration of both culturable and unculturable microbes. Moreover, other methods like genomics, transcriptomics, proteomics, and metabolomics can help in understanding the biological interactions that take place during the breakdown of synthetic plastics between genes, transcripts, proteins, metabolites, and outside environmental variables. Due to the interaction between the various microorganisms and their enzymes, it is also thought that using them in a consortium may increase the efficiency of plastic degradation. Although numerous enzymes that break down plastic have been discovered in a variety of places, little research has been done on their biochemical and structural characteristics. This information is important in order to explain the mechanisms underlying the biodegradation of resistant polymers. This knowledge will be helpful in the production of innovative plastic polymers with enhanced biodegradability, the design of microbial cell factories with better breakdown efficiency, and the modification of enzymes through protein engineering. Investigating whether various pre-treatment techniques and additives affect the microbial breakdown of synthetic polymers is also crucial since it is anticipated that using the right pre-treatment and additives would lead to better outcomes. There are many differences between the methodologies used to evaluate the

degradation efficiency in the various studies; as a result, developing and adopting a standard or universal methodology will greatly aid in the harmonization of data and the subsequent advancement of this field of study. More extensive research in this field is anticipated to soon lead to feasible biodegradation processes that can be created on a large scale, given the infinite potential of bacteria and their ongoing adaptation to the changing environment. Therefore, expanding the number of research is required to assess numerous potent candidates capable to degrade non-biodegradable plastics.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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