PLASTIC DEGRADATION BY PROKARYOTES: A COMPREHENSIVE REVIEW

by

TOMMY TAYLOR

A THESIS

Presented to the Department of Biology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2024

An Abstract of the Thesis of

Tommy Taylor for the degree of Bachelor of Science
in the Department of Biology to be taken June 2024

Title: Plastic Degradation by Prokaryotes: A Comprehensive Review

Approved: *Stilianos Louca, Ph.D.*
Primary Theis Advisor

Plastic pollution is becoming a significant concern and has created a litany of ecological problems, many of which have adverse effects on human health. The nature of plastic materials ensure that they remain in the environment, undegraded, for upwards of hundreds of years and this is especially concerning given that much of the world's plastic remains in landfills – allowing for environmental degradation to release microplastics and other pollutants into the natural environment. For this reason, it has become increasingly necessary that alternative methods of eliminating or degrading plastic waste from the environment be elucidated. Microbial degradation - the degradation of plastic by microscopic organisms – is showing particular promise as an eco-friendly method of eliminating plastic waste. In particular, the degradation of plastics by prokaryotes is a promising new avenue of bioremediation but current information and insights into future applications currently remain underexplored and unknown. This is of special concern given that the number of microbial species predicted to be capable of degrading plastic is rapidly increasing. Thus, the goal of this project is to then synthesize much of the major known biochemical, genetic, and taxonomical information regarding the degradation of plastics by prokaryotes and explore some of the existing literature regarding bioremediation in order to provide a comprehensive resource for future investigation and development.

Acknowledgements

I would like to acknowledge Ph.D. candidate, Rob Porch, for his invaluable support and advice throughout the process of planning and writing this thesis. I would also like to acknowledge Dr. Stilianos Louca for his support and feedback throughout this process as well, and more broadly, for his consistent support while I was a member of his lab and contributing to the FAPROTAX2 project. Additionally, I thank my CHC representative Dr. Rebecca Altman for serving on my thesis committee and for all her unforgettable and valuable support in navigating the thesis process. This thesis would have not been possible without their feedback, nor would it have been possible if not for being granted the opportunity to work on that project in the first place.

I would also like to thank and acknowledge my mother, Dr. Cornelia Taylor, for her feedback on this thesis and for providing me with solid insight into my project and inspiring me to pursue a career in medicine. Lastly, I would like to acknowledge my friends and family for supporting me through this step in my academic career and I would especially like to thank and acknowledge my partner of four years, Aaron Urquilla-Figueroa, for his encouragement and moral support. This thesis would not have been possible without any of the above individuals and their continued support of my career and academic endeavors.

Table of Contents

List of Figures

List of Tables

Introduction

Environmental Impact of Plastic Pollution

The use of plastic has been a ubiquitous aspect of human life since its inception in 1907 [1]. The synthesis and manufacturing of plastic is one of the most significant components of the current global economy, and the millions of tons of plastic produced annually [2] are reflective of its importance and prominence in the global sphere. While there are a wide variety of plastics that are currently synthesized and utilized in modern manufacturing, single-use plastics – such as those utilized in food wrapping, bottles, and containers, are the biggest contribution to global plastic pollution by mass [3]. Much of this plastic is not recycled or incinerated, and thus ends up diffusing into the natural environment from plastic landfills. Transient plastic can then be broken down into its respective monomers by natural factors [4] and the resultant microplastics are then transported through the air and water – resulting in microplastic sedimentation and pollution at the highest [5] and lowest [6] points on earth.

Chemical Properties of Plastic

Plastics are synthetic or semi-synthetic polymers (a compound made up of many smaller molecules known as monomers). The composition of a given plastic is dependent on its intended functional use, but the general model for plastic composition describes a synthetic hydrocarbon (organic) polymer. The polymeric nature of plastic and the nature of their chemical bonds ensures that they are versatile, durable materials that are not quickly degraded [8], but this also hinders our ability to effectively remove them from the natural environment. An extenuating factor in the presence of plastics in the natural environment is the tendency of plastics to absorb and retain persistent organic pollutants - like dichlorodiphenyltrichloroethane (also known as

DDT), a toxic insecticide with severely negative environmental impacts [9]. The accumulation of these pollutants on plastic debris further perpetuates the impact that these organic pollutants are able to exert on the environment, and plastics in the marine environment, specifically, are often able to release these organic pollutants at a much higher magnitude [10].

Various additives - such as plasticizers, antioxidants, pigments, and heat stabilizers [11] - can be added to plastics to confer different functional properties. Plastic additives are not usually incorporated into the covalently bonded backbone of the polymer and are thus released into the environment following degradation of the plastic [9]. This is a growing concern, as these additives can accumulate in microplastics following degradation, and can be released into the environment with negative consequences for the natural ecosystem. The most commonly produced plastics, and therefore, the most likely to be present as environmental pollutants, are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PU), and polyethylene terephthalate (PET) [12].

In the context of degradation, it has become important to separate these plastics on the basis of the nature of their carbon backbone, with PS, PP, PE, and PVC being categorized as having a carbon-carbon backbone comprised solely of covalently bonded carbons, while PU and PET contain heteroatoms (any atom that is not carbon or hydrogen, such as oxygen or nitrogen) that facilitate the formation of hydrolysable amide and ester bonds – improving enzyme-substrate interaction through improved functional group chemistry. All of these characteristics of modernly synthesized plastics are fundamental to understanding their degradation – both biotically and abiotically.

Biodegradation of Plastics by Prokaryotes

Biodegradation of plastic refers to the depolymerization of plastic substrates and conversion of the produced intermediates into metabolites by living organisms. Prokaryotes (single-celled organisms belonging to the domains Bacteria and Archaea) are of particular importance in the process of biodegradation, due to the vast diversity of their metabolic properties and the often-rapid nature of their reproduction. While eukaryotic organisms, such as fungi, have been suggested to have potential as plastic degraders, bacteria currently serve as the primary organism for biodegradation due to their abundance in the natural environment.

Currently, there are many prokaryotes known to be capable of degrading various synthetic plastics. Further efforts are continuously being made to elucidate the potential of microbial plastic degradation. There has been remarkable progress made on this front in the past two decades, but there are many factors that limit our ability to fully understand this prokaryotic trait such as: inconsistencies in the definition of plastic biodegradation, discrepancies in the culturing and testing process to isolate these organisms, and the inability of many putative plastic-degraders to be cultured. This has especially limited the capacity to use plastic-degrading microbes for bioremediation (the biological removal of plastic waste from the natural environment).

Results

Plastic and Plastic Degradation

Plastics are synthetic polymers (a large molecule comprised of many subunits bound together to form a singular unit), and as a result of their ability to form long, cross-linked chains, possess great durability, versatility, and practicality as materials. Annual global plastic production has increased from two million tons in 1950 to 390.7 million tons in 2021 [19]. Much of this plastic (approximately 42% of global production) is used for packaging, and is comprised primarily of PET, PP, or PE plastics [20]. Despite the recyclability (in the case of PS and PE) or inherent capacity for natural degradation (in the case of PET) present in these synthetic materials, the overwhelming quantity of single use plastic that ends up in landfills around the globe has consequences for both local communities and the global ecosystem.

Despite the growing global dependence on plastic manufacturing, current methods of waste management and pollution reduction have not yet been able to make an effective contribution to mitigating the impact of plastic pollution on the environment. The release of harmful particles – either in the form of microplastics or the release of broken-down plasticizers - in the process of plastic manufacturing, use, storage, and recycling, as well as the accelerated rate at which plastics continue to be produced have hindered existing efforts to remove plastic waste from the environment, with approximately 58% of produced plastic entering the natural environment as pollutants [21]. Furthermore, current methods of recycling are not without their own ecological flaws, such as the release of volatile organic compounds and microplastics as a result of plastic waste incineration [22], the loss of plastic particles to the environment in the case of landfills [23], and the inefficient and uneconomic nature of reuse and recycling [19]. Additionally, the current and predicted rate at which plastic is produced far outcompetes the rate at which plastic waste can currently be mitigated [19], thus ensuring that plastic pollution and waste are going to continuously and unsustainably increase for the foreseeable future.

Biodegradation of Plastic

Given that plastic has and will likely continue to be a ubiquitous aspect of the natural environment, the discovery of organisms capable of degrading and utilizing the carbon structure of these polymers for growth and survival is increasing. The isolation of microorganisms, particularly bacteria, that are capable of degrading the plastic present in the environment has opened the door for an alternative to current methods of plastic waste mitigation and reduction: biodegradation.

Most accurately, the biodegradation of plastic refers to the breakdown and plastic as a carbon source by microorganisms into organic matter – such as carbon dioxide (CO_2), water, and other products of metabolism [24]. It is important to note that biodegradation is distinct from the process of biodeterioration, and a lack of consensus over the functional definitions of each has contributed to inconsistency in how plastic degradation has been observed and defined [28]. Modification of plastic waste by various abiotic processes – such as thermo-oxidation, UV irradiation, and mechanical erosion by the natural environment – is often a prerequisite step that initiates biodegradation, as the oxidative modification of the plastic material improves physical and chemical interactions of microorganisms with the substrate.

The first step in the process of biodegradation is the attachment of microorganisms to the surface of the polymer. In the case of many commonly produced plastics – such as PP, PS, and PE – the hydrophobicity of the linear carbon backbone hinders this colonization step, and it is for this reason that the abiotic degradation mentioned previously is essential for the biodegradation process. Upon binding to the surface of the polymeric material, the microorganism can then grow using the polymer as a primary carbon source through the secretion of extracellular enzymes that cleave the polymer chain and produce smaller carbon units that can be utilized as a carbon source by the associated microbes. The continued breakdown of the plastic polymer and metabolism of the produced carbon will eventually result in primary degradation of the polymer and will, over the course of months and/or years, lead to the ultimate degradation of the plastic polymer into microbial metabolites [24].

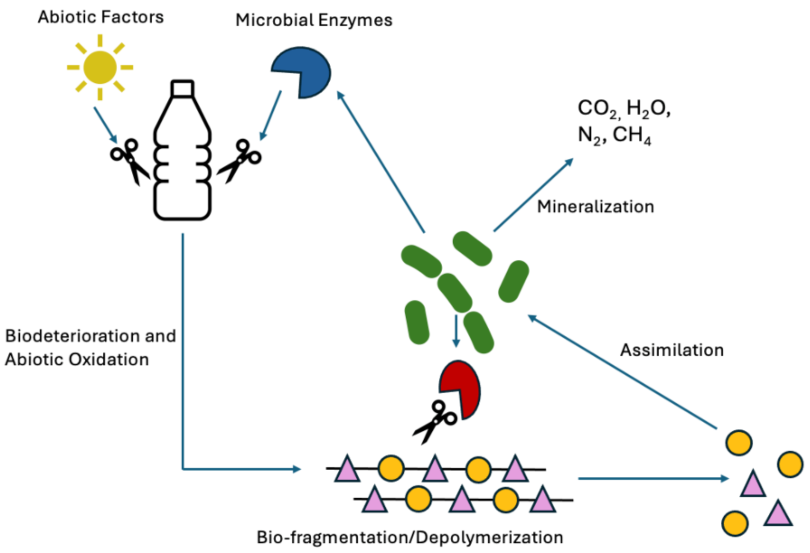


Figure 1. Schematic representation of microbial plastic biodegradation

Experimental Observation and Analysis of Plastic Biodegradation

The experimental observation and analysis of biodegradation can currently be accomplished through the use of several methods – all of which depend on chemical analysis of the plastic substrate following culturing with any microorganisms of interest. As explored in Kotova et al. in 2021, there are many ways to currently verify and determine plastic degradation by microbial organisms in an experimental setting. These methods, such as FTIR spectroscopy and radioactive carbon labeling, often depend on changes to the physical properties of the plastic, such as weight, integrity of the plastic surface, and the production of metabolic intermediates.

In either case, experimental determination of plastic biodegradation is difficult because many techniques are only able to quantify or measure one step in the multi-step process of biodegradation. That’s not to say, however, that the experimental determination of plastic biodegradation is impossible. Rather, a combination of techniques and careful application of the methodology employed are crucial to ensuring validity and accuracy of the obtained results. This is especially important when considering follow-up studies or further analysis of a given strain based on a putative assessment of plastic degradation.

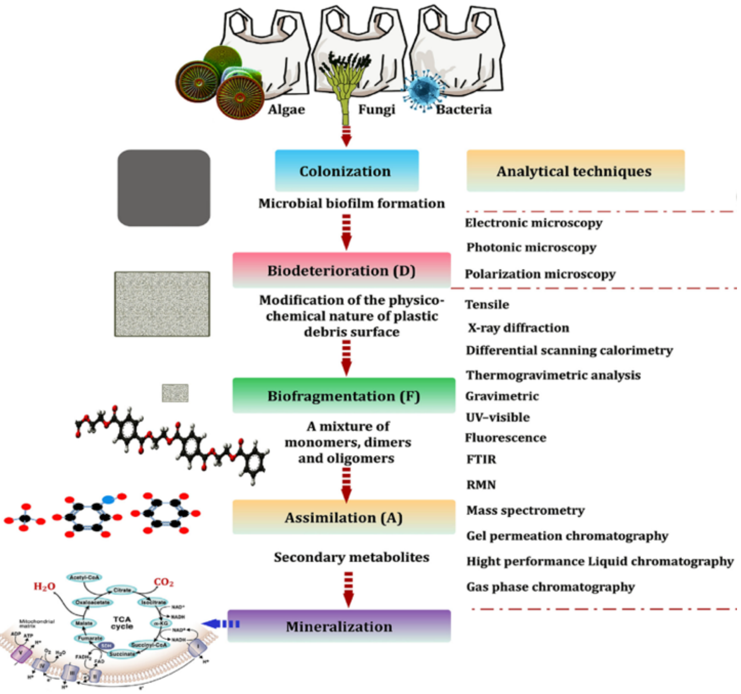


Figure 2. Common methods of validating plastic biodegradation

Adapted from [109]

Biodegradation of Plastics by Prokaryotes

The isolation of microorganisms capable of the biodegradation of synthetic polymers has been reported as early as the 1960s [29] with the isolation of a fungal species capable of degrading polyurethane-based plastics. Research on biodegradation of plastic by microorganisms has continued since then, and prokaryotic organisms capable of plastic degradation were discovered in 1971 when bacteria were observed to be capable of reducing the mass of a PVC sample, and the isolation of prokaryotic microorganisms capable of degrading similar plastics continued soon after with the discovery of a *Flavobacterium* species capable of degrading nylon isolated in 1975 [36]. Since then, more progress has been made in isolating and observing plastic-degrading capabilities in several other prokaryotic microorganisms across several levels of taxonomy. Microorganism capable of degrading the most abundant types of plastic available in the environment have been extensively isolated and described in the past 50 years, and the degree to which these organisms are capable of breaking down these plastics have varied greatly.

Plastic Type	Identified Organism(s)	References
PE	*Rhodococcus ruber, Bacillus cereus, Pseudomonas sp. E4, Pseudomonas putida IRN22, Pseudomonas putida LS46.*	[125], [126], [127]
PS	*Azotobacter beijerinckii* HM121, *Microbacterium sp. NA43, Exiguobacterium sp. Y12*	[128], [129], [130]
PVC	*Klebsiella sp. EMBL-1, Bacillus sp. AIIW2, Mycobacterium sp. NK0301*	[70], [131], [132]
PET	*Ideonella saikaiensis, Thermobifida fusca, Streptomyces sp. SM14, Humicola insolens, Saccharomospora viridis*	[18], [76], [77], [133]
PUR	*Bacillus subtilis MZA-75, Pseudomonas aeruginosa MZA-85, Comamonas acidovorans*	[134], [135]

Table 1. List of some organisms known to be involved in degrading plastics of different types

Plastic Degradation by Archaea

To date, no species belonging to the domain Archaea have been reported to completely degrade plastic. However, many Archaea have been identified as belonging to microbial communities that have colonized the surface of plastics in a range of environments [37]. While no species have been identified to play a direct role in the degradation of plastic, an archaeal PET-hydrolyzing enzyme was isolated in 2023, thus suggesting that Archaea may play some role in plastic degradation [38]. It has been speculated that Archaea likely currently contribute to the degradation of biodegradable plastics through the process of anaerobic digestion and utilization of the produced carbon intermediates and degradation products of bacteria [37], but this has not been confirmed experimentally and this role in the context of non-biodegradable plastics – which constitute the majority of existing plastic produced and wasted – remains to be determined. Thus, this review will be focused on the degradation of plastic by bacteria.

Biodegradation of Plastic With A C-C Carbon Backbone

Plastics with a C-C backbone (PE, PS, PP, and PVC) – derived from petroleum and petroleum-based sources - are highly recalcitrant (resistant to changes in integrity or composition) to degradation (both mechanical and chemical) and recycling due to their high hydrophobicity, crystalline structure, and high molecular weight. As a result, these plastics are resistant to many traditional methods of waste management and recycling – such as the breakdown of the plastic into monomers and resynthesis of a new plastic. Additionally, recycling is often hindered due to their incompatibility with traditional catalysts [39]. Such plastics are often only biodegradable following the addition of carbonyl groups to the carbon backbone as a result of oxidative damage – either from sun exposure (UV irradiation), temperature, or chemical oxidizing agents (i.e., free radicals) in the environment [40]. The crystalline structure and presence of amorphous regions in the plastic significantly hinders the ability for microorganisms to colonize the surface of the polymer and utilize it as a carbon source [41] while the tight packing, high molecular weight, and hydrophobicity all prevent the assimilation of the plastic material by microorganisms and hinder the chemical interactions that facilitate degradation [42]. Furthermore, many of the produced plastics contain plasticizers – such as antioxidants and stabilizers – that protect the plastic from environmental degradation [43].

There are, however, several prokaryotic organisms that are capable of producing enzymes that degrade plastics with a C-C backbone, albeit with less success than other recalcitrant plastics (i.e., PET). Presently, several redox enzymes are thought to be involved in assisting the partial degradation of various C-C backbone plastics, but organisms and enzymes capable of complete enzymatic cleavage and fragmentation of a C-C backbone plastic have not yet been discovered

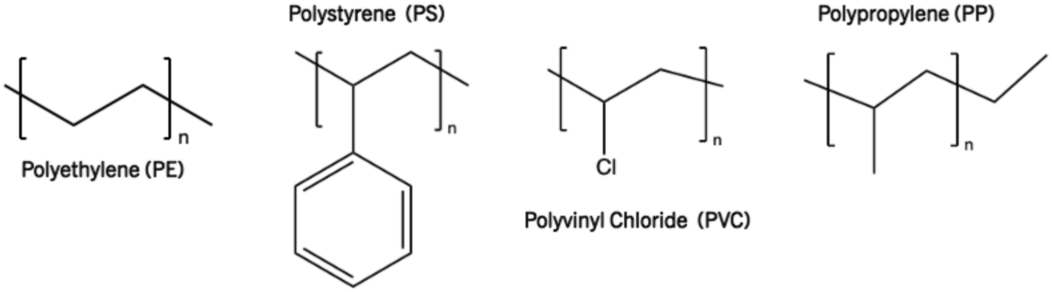


Figure 3. Chemical structure of C-C backbone plastics

Prokaryotic Degradation of Polyethylene (PE)

Polyethylene (PE) is a polyolefin thermoplastic and has the chemical structure of a straight chain alkane. PE is widely used in packaging for its highly recalcitrant properties – such as high hydrophobicity and high molecular weight. This recalcitrance, while ideal for its applications in the industrial and manufacturing context, has proven to be a contributing factor to its inability to be effectively degraded – either by natural processes or biological organisms [44].

As of now, there have been several studies done to determine whether biodegradation of PE by microorganisms is feasible. The introduction of hydrolysable functional groups through UV irradiation, chemical oxidizers, and thermo-oxidative processes is critical for prokaryotic biodegradation, and current models suggest that PE biodegradation by some prokaryotes is the result of a synergistic process involving abiotic, chemical priming of the polymer substrate and enzymatic breakdown [16,93]. It is important to note, however, that while abiotic deterioration is important in facilitating the biodegradation of PE, some prokaryotic species – such as a strain of *Serratia marcescens,* and two cyanobacterial strains (*Phormidium lucidum* and *Oscillatoria subbrevis*) - were shown to significantly reduce the weight of untreated PE in biodegradation studies [45, 46].

Many of the microorganisms that have been found capable of degrading PE have been eukaryotes (fungi), and this is predicted to be the result of fungi demonstrating increased affinity for the hydrophobic surface, increased resistance to the harsh living conditions mandated by biofilm formation on the plastic surface, and improved capacity to secrete the extracellular enzymes necessary to degrade the carbon backbone for eventual utilization as a carbon and energy source [48]. While these properties suggest that fungi are likely more efficient at degrading PE than bacteria, there have been over 100 organisms – comprised of members of both prokaryotic and eukaryotic taxa – isolated as being capable of PE degradation to date [49].

The first prokaryotic organism capable of PE degradation was discovered in 1991. As determined through observation of plastic substrate weight loss, *Streptomyces viridosporus* (a bacterial species already known for degradation of the plant polymer, lignin) was identified as an effective degrader of PE plastic [50]. Since then, progress in the isolation and discovery of PE-degrading prokaryotes has been made over the past twenty years.

In the context of PE biodegradation, it becomes necessary to make a distinction of which form of PE is capable of being degraded. The density of PE can vary based on whether the structure of the polymer forms a linear or branched (i.e. attachment of other hydrocarbon chains to the carbon backbone) conformation [54]. Low-density polyethylene (LDPE) is much more amenable to biodegradation, and many of the current studies on PE biodegradation have used either LDPE or medium-density polyethylene (MDPE) as the degraded substrate. High-density polyethylene (HDPE) is characterized by a linear alkane chain with minimal branching – thus increasing the strength of intermolecular forces and facilitating a highly crystalline structure. This makes HDPE significantly more resistant to biodegradation as it limits enzymatic interaction and prevents biofilm formation.

There have been several prokaryotic species isolated recently that were found to be intestinal symbionts of select species of mealworms, with an initial study published in 2014 revealing *Enterobacter asburiae* Y1 and *Bacillus* sp. YP1 – isolated from the gut of plastic-eating waxworms – were capable of forming a biofilm and reducing the hydrophobicity of a LDPE film following culture [57]. In the studies that followed, bacteria from the guts of wax moths (*Galleria mellonella*) [58] and yellow mealworms (*Tenebrio molitor*) [59] have been shown capable of degrading PE films/foams and LDPE films, respectively.

Given the recalcitrant nature of PE, the isolation of microbes capable of degrading untreated or abiotically oxidized PE is likely to continue at a slow rate, but the current state of identification and isolation of existing PE-degrading prokaryotes (and more importantly, their associated enzymes) shows promise.

Prokaryotic Degradation of Polypropylene (PP)

Polypropylene (PP), like PE, is a polyolefin thermoplastic consisting of a straight-chain alkane structure and a linear C-C carbon backbone. PP is structurally similar to PE, and its chemical properties are incredibly similar as well [60]. Like PE, the degradation of PP by microorganisms is greatly hindered by its chemical structure as its status as a highly hydrophobic, crystalline, and oxidation-resistive material makes degradation unfavorable. Despite this, some prokaryotes have been shown to have a limited capacity to biodegrade PP in both abiotically-degraded and untreated states.

Initial studies conducted in the early 1990s looking at the biodegradation of PP by microorganisms have yielded inconsistent results – with further analysis of the molecular products of weight loss experiments revealing that microorganisms are likely degrading the plasticizers (degraded as aromatic esters) added to PP during the polymerization process instead of the hydrocarbon structure of the plastic [61]. Later studies involving PP films incubated with prokaryotes revealed a more complete degradation of the PP hydrocarbon structure, but the studies remain unclear as to whether it was actually the long PP hydrocarbon chain was degraded and utilized or if it was degradation of the low molecular weight components of the PP film [16]. A study involving a strain of *Stenotrophomonas panacihumi* demonstrated that only the low molecular weight components of PP were effectively degraded [62] – providing further evidence for the assumption that the PP hydrocarbon chain remains unsuccessfully degraded. Furthermore, despite the potential for PP biodegradation demonstrated in the studies done thus far, there remains little information regarding the mechanism of biodegradation and there are not currently any enzymes known to be involved in this process. Thus, like PE and the other polyolefins, the biodegradation of PP remains poorly understood.

Prokaryotic Degradation of Polystyrene (PS)

Polystyrene is a polyolefin thermoplastic consisting of a straight chain alkane with a benzene ring substituted for one of the hydrogens in the carbon-carbon chain (figure 3). Similar to other polyolefins, PS has not traditionally been demonstrated to be an effective substrate for microbial degradation, but many prokaryotic species have been demonstrated to be capable of at least partially, if not fully, degrading PS films and materials [16].

Biodegradation of PS has been observed as early as the 1970s, with initial experiments in which PS with radioactively labelled carbon was incubated with soil and liquid enrichment cultures revealing evolution of radioactive CO_2 with the primary species involved in such degradation identified as being bacterial strains of the genera *Bacillus*, *Pseudomonas, Micrococcus,* and *Nocardia* [63]. Many studies have emerged in the past twenty years that have highlighted the capacity of prokaryotes to degrade PS. Similar to what has been observed in the case of PE degradation, analysis of the degradation of PE by insect and insect larvae has resulted in the isolation of several intestinal symbionts of mealworm (*Tenebrio molitor*) [64], superworms (*Zophabas morio*) [65], and wax moths (*Galleria mellonella)* [66].

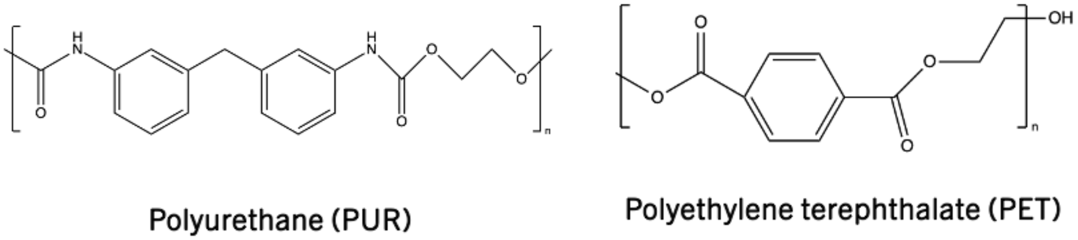
Prokaryotic Degradation of Polyvinylchloride (PVC)

Polyvinylchloride (PVC) is a thermoplastic consisting of a straight chain alkane in which one of the hydrogens has been substituted for a chlorine atom (Figure 2). PVC is an incredibly versatile material due to its highly durable, hydrophobic, and lightweight nature [60], but these properties, as is the case with all C-C backbone plastics, make it recalcitrant to microbial degradation.

Unlike the progress made in elucidating the biodegradation of similar C-C backbone plastics such as PE, PS, and PP, very little is known about the microbial capacity for PVC degradation. Some prokaryotic organisms have been reported to degrade PVC [67,68,69] but current research remains unclear, and in particular, the characterization of the metabolic and biochemical pathways to achieve such degradation is lacking. Recent studies, however, have shown promise in isolating PS-degrading microbes and prokaryotes, with a 2022 study revealing a bacterial symbiont of insect larvae to be fully capable of PVC degradation, and more importantly, that such insect larvae are then fully capable of growth through the energy derived from such degradation [70].

Degradation of Plastics with a Heteroatomic Carbon Backbone

Plastics that contain heteroatoms in their carbon backbone (PET and PUR) are markedly different from those with a carbon-carbon polymer backbone – with the starkest difference being the inclusion of hydrolysable amide and ester bonds in the structure of the polymer. As a result, these plastics are more susceptible to biodegradation as hydrolytic enzymes [71] produced by microorganisms are able to interact directly with the polymer backbone and fragment it for eventual assimilation and use as a carbon source. The ease of backbone cleavage, however, is often hindered by the presence of side groups that reduce the mobility of the polymer chain and modulate the crystalline nature of plastic to prevent microbial colonization and interaction [72].



Polyurethane (PUR)

Polyethylene terephthalate (PET)

Figure 4. Chemical structure of heteroatomic backbone plastics

Given the chemical structure of these plastics and the existing variety of hydrolytic enzymes that exists within the microbial metabolic repertoire, it is not surprising that much of the current work on biodegradation of plastic by prokaryotes is centered around the degradation of PET and PUR. In the past twenty years, there have only been a few bacteria (and some fungi) that have been shown to degrade PET and PUR into monomers or oligomers, but it is important to note however, that the metabolic and genetic architecture of such degradation has been characterized and elucidated to a greater degree than the degradation of C-C backbone plastics. For example, one enzyme responsible for the degradation of PET, *IsPETase*, has been the focus of several studies [78] and has even been adapted for uses in biotechnology and bioengineering [18]. In this way, it can be seen that the biodegradation of PET and PUR has proven to be a vital launching point for continued research into the interplay between this microbial trait and its implications on bioremediation.

Prokaryotic Degradation of Polyethylene Terephthalate (PET) ‘

Polyethylene terephthalate (PET) is a thermoplastic polymer consisting of a repeating unit of a benzene ring with two ester bonds (terephthalate group) attached to a two-carbon straight chain alkane (ethylene group). PET is a versatile material and is used in a wide variety of contexts, but the most prominent usage of PET is in the manufacturing of single-use plastic products [73]. As a result of its primary usage in single-use plastic production, PET plastic is often ubiquitous in the environment as waste and pollution. While the presence of ester bonds in the polymer backbone suggests that enzymatic degradation would be a favorable and efficient process, the chemical structure of the resultant polymer and the process of its manufacturing both impose serious limitations to effective degradation [71]. The aromatic terephthalate group present in the polymer backbone limits the mobility of the polymer chain – hindering interactions between microorganisms and the polymer substrate [74,75]. Furthermore, PET plastic is often subjected to industrial processing conditions that induce a high degree of crystallinity that inhibits interaction with colonizing microorganisms by shrinking the amorphous region - the region of the polymer most susceptible to enzymatic degradation [74,75].

As of now, there have been many hydrolytic enzymes – such as IsPETase, TfH, and Cut190 [16,18, 76, 77] - that have been demonstrated to degrade the polymer backbone of PET. The most prominent and studied prokaryotic species that has been proven to fully utilize PET plastic as a carbon and energy source (proven through detection of CO_2 as the final oxidation product of PET), is *Ideonella sakaiensis* [18]. Other species have been reported to be capable of PET degradation and have had their PETase-similar enzymes studied, such as *Thermobifida fusca, Humicola insolens,* and *Saccharomonaspora viridis* [16]. The identification of PET-degrading prokaryotes is especially vital given the growing amount of research looking at the biotechnological modification of PET-degrading enzymes to aid in bioremediation efforts.

Prokaryotic Degradation of Polyurethane (PUR)

Polyurethane (PUR) is the name for a class of thermoplastic polymers – all of which contain a urethane motif in their polymer backbone. The polyurethane motif that forms the polymer backbone contains both an ester and an amide bond – both of which are known to be susceptible to hydrolytic cleavage. PUR can be further classified into polyester PUR and polyether PUR – with polyester PUR containing multiple ester functional groups in addition to the urethane group and polyether PUR containing multiple ether functional groups. These differences in chemical structure each present unique challenges to microbial degradation and interaction with microbial enzymes and such challenges are thus reflected in the number of microorganisms reported to be capable of degrading either class of PUR plastics [16].

Degradation of polyester PUR has been observed in a wide range of bacterial species in the past twenty years - with many belonging to the genera *Pseudomonas* and *Bacillus*. With respect to polyether PUR, there have been only a handful of bacterial species known to degrade this class of PUR polymers. Bacterial degradation of polyether PUR was first observed in 1991, with experiments demonstrating that a strain of *Staphylococcus epidermidis* (*Staphylococcus epidermidis* KH11) from an infected catheter was capable of utilizing polyether PUR as a sole carbon and energy source [80]. In 2017, three more bacterial strains – *Pseudomonas denitrificans, Pseudomonas fluorescens,* and *Bacillus subtilis* - were all found capable of reducing the weight of polyether PUR films [81].

Phylogenetic Distribution of Plastic Degradation as a Prokaryotic Trait

The discovery of plastic-degrading prokaryotes is growing significantly – with advancements in isolation capabilities and increased interest in this microbial trait fueling this growing field of research. Given the growing interest and rate of progress in the isolation and identification of plastic-degrading prokaryotes, it is important to understand any potential and existing phylogenetic relationships and investigate whether the degradation of plastic is a conserved trait and whether the prediction of plastic-degrading capabilities based on taxonomic identity is possible. This is especially important given that microbial communities that form biofilms of the surface of plastic pollutants (i.e., the first step in the process of degradation), are incredibly taxonomically diverse [136, 137].

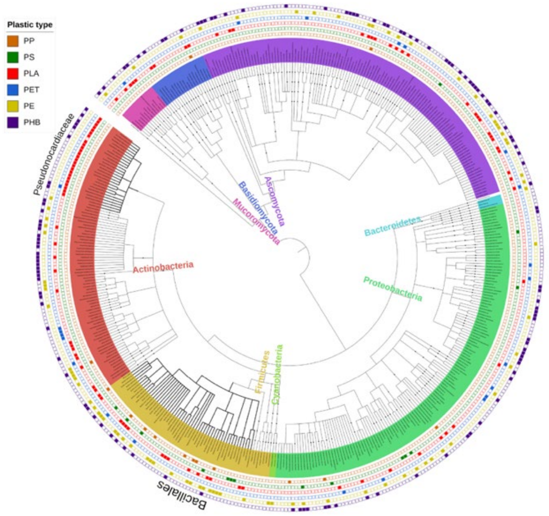


Figure 5. Phylogenetic distribution of plastic-degrading microorganisms

Adapted from [83].

In 2020, Gambarini et. al analyzed 1,451 publications (published up to April 2020) that reported microbial plastic degradation and performed an extensive analysis of the phylogenetic distribution of this trait. As of April 2020, 436 microorganisms - 286 of which were bacteria (65.6%) - were reported to degrade plastics [83]. These microorganisms demonstrate a striking amount of taxonomic diversity, with species reported for plastic degradation having been isolated from the phyla *Bacteroidetes, Proteobacteria, Cyanobacteria, Firmicutes,* and *Actinobacteria* [83]. At the level of genus, *Pseudomonas* spp. are the most frequently isolated plastic degrading bacteria (6.7%) and have been reported to be capable of degrading 35 different types of plastic [83].

The degradation of most plastics does not appear to be phylogenetically conserved. Only the degradation of PET plastic appears to maintain some degree of phylogenetic conservatism, with statistical analysis demonstrating a strong phylogenetic signal with clusters of both known and putative PET-degrading isolates residing in the phyla *Actinobacteria,* but the number of known isolates capable of fully degrading PET plastic is currently limited [83]. The most phylogenetically conserved type of plastic degradation is the degradation of the biodegradable polymer polylactic acid (PLA), but the biodegradable/biocompatible nature of this plastic is more amenable to microbial degradation – especially given its structural similarity to existing and long-degraded organic polymers like silk fibroin. Given the lack of phylogenetic conservation for the degradation of non-biodegradable plastics, it is likely that the degradation of synthetic plastics is a more recently acquired trait and an adaptation to the growing ubiquity of plastic pollutants in the natural environment [83].

Genomic analysis has revealed the presence of putative genes involved in the degradation of plastic in a wide breadth of microbial species, with 16,000 reported orthologs of plastic-degrading genes detected in the genomes of 6,000 microbial species. However, many of these species have not been reported and identified as plastic degraders. Furthermore, the presence of these orthologs occur across 12 different microbial phyla, but only seven of these phyla contain known plastic-degrading microorganisms. In any case, further research is necessary to expand upon and investigate the phylogenetic distribution of this trait [83].

Genetic Architecture of Prokaryotic Plastic Degradation

Advancements in sequencing technology have expanded the capability for identification and analysis of the microbial communities that colonize the surface of plastic in the environment. This, in combination with analysis of previously identified genes, has allowed for the estimation of plastic degradation capability and the identification of potential plastic degraders from many microbial communities on the surface of plastics based on identified, putative plastic-degrading genes [83]. These putative genes have been detected in the genomes of thousands of microorganisms [83], but whether these genes are actually translated into proteins that confer the ability to degrade plastic remains to be seen.

As of now, there have been no genes predicted to be involved in the degradation of several common types of plastic, such as PS, PP, and PVC, but there have been several genes that have been characterized as being partially involved in the degradation of PE, PET, and PUR as they encode known enzymes – such as cutinases, PETases, and alkane hydroxylases. The genes involved in the degradation of PE, for example, have been studied in the *Alcanivorax* spp. 24 and characterized as *alkB1* and *alkB2* (alkane hydroxylases), *almA (*alkane monooxygenases), and cytochrome P450 genes [84]. The PETase enzyme produced by *Ideonella sakaiensis* is encoded by the gene *cut1*[84], and the lipases produced by Moritella spp. JT01 for the degradation of PE is encoded by the gene *H39* [84]. Lastly, many of the esterases believed to be involved in the degradation of polyurethane (PUR) were found to be encoded by *pudA* [16], *pulA* [16], *pueA* [16], and *pueB* [16]. It is important to note, however, that the currently isolated genes are all related to novel enzymes involved in depolymerization, and this is likely due to the fact that the degradation of plastic often produces intermediates that can feed into known metabolic pathways (such as the catabolism of aromatic hydrocarbons in the case of PS) with already categorized and studied genes.

The continued investigation of genes involved in plastic degradation is vital because gene identification allows for the identification of putative plastic-degrading species based on sequence similarity, as seen in Gambarini et al. in 2021. Furthermore, the creation of a database in which the proteins, genes, and organisms known to degrade plastic can be uploaded – such as PlasticsDB [124] – could further expand the potential to putatively identify plastic-degrading enzymes and increase the existing body of knowledge regarding this prokaryotic trait. While the complete identification and elucidation of the genes involved in plastic degradation – especially degradation of plastics that are more recalcitrant to microbial attack - is likely not feasible within the near future, current advancements in sequencing technology have made it possible to make rational predictions.

Metabolic and Biochemical Architecture of Prokaryotic Plastic Degradation

The depolymerization of a plastic substrate is accomplished through combined chemical modification and cleavage facilitated by both abiotic (UV, thermal, and chemical oxidation of the polymer backbone) and biotic (the release of extracellular microbial enzymes that facilitate degradation and cleavage of the polymer backbone) methods. The release of monomers and oligomers following this depolymerization results in the assimilation of these compounds by the microbes that have colonized the surface of the plastic, and these assimilated degradation products are then utilized by the microbes and transformed for use in standard metabolic pathways – such as the TCA cycle and beta-oxidation.

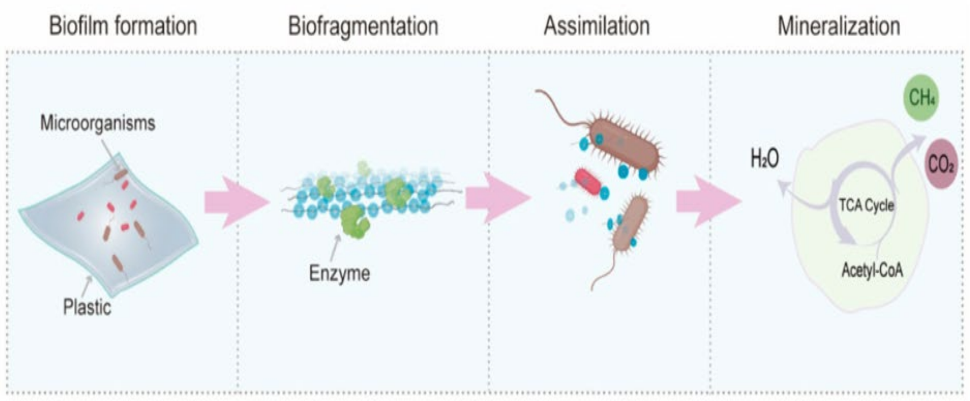


Figure 6. Schematic representation of the process of assimilation and mineralization

Adapted from [91]

The assimilation and subsequent digestion of the released degradation intermediates (organic matter) can occur aerobically (using molecular oxygen as the terminal electron acceptor) or anaerobically (using alternative electron acceptors such as nitrate), and in either case, the catabolism of these degradation intermediates results in mineralization [84]. This final step in the biodegradation process, known as mineralization, describes the release of metabolic byproducts in the form of mineral salts, CO_2, H_2O, and hydrogen sulfide/methane (in the case of anaerobic digestion).

The complete biodegradation process has been observed for all of the most commonly produced plastics, but the degree to which the enzymatic and metabolic architecture of such biodegradation is understood varies greatly. For many classes of plastics, classes of enzymes thought to be involved in the degradation of plastic, or at the very least, the depolymerization off plastic, have been studied and identified.

Metabolism of PE

The degradation and metabolism of PE, a C-C backbone plastic as mentioned previously, has been studied extensively in recent years, and the metabolic pathway for the degradation of this plastic is better understood than its related C-C backbone counterparts. The biodegradation of PE, while still limited due to chemical and biological factors and incompatibilities, is heavily dependent on the alkane degradation pathway and the assimilation, transformation, and mineralization of polymer fragments created through abiotic oxidation [85].

The first step in the biodegradation of PE is the initial oxidation of the polymer to introduce functional groups on the hydrocarbon branches of the carbon backbone that are more amicable to attack by microbial enzymes. This initial oxidation step occurs either as a result of abiotic degradation and damage – such as UV irradiation – or as the result of biodeterioration (a process in which enzymes released by microbes colonizing the surface of the plastic are capable of directly oxidizing the plastic substrate) [86,87]. Following abiotic degradation, the now-oxidized polymer can undergo the process of bio-fragmentation and depolymerization as extracellular enzymes can now act upon the now-favorable substrate. Laccases and peroxidases (especially manganese peroxidases) are crucial to the production of alkane intermediates that can be enzymatically transformed and assimilated into the cell. Thermostable laccases isolated from PE-degrading strains of *Rhodococcus ruber* were shown to degrade PE in both the presence and absence of bacterial cells [88], and strains of PE-degrading *Bacillus cereus* have also been shown to release higher concentrations of extracellular laccases and MnP [89]. These enzymes have also been isolated from several fungal species capable of PE degradation [90], further highlighting their importance in the depolymerization of PE.

Alkane hydroxylases are another important class of enzyme known to be involved in the degradation of PE, due to their facilitation of the assimilation of the alkane degradation intermediates produced initially by laccases and peroxidases [91]. Alkane hydroxylases are enzymes that are capable of catalyzing the oxidation of linear alkanes and introducing a functional group (often a carbonyl) that facilitates assimilation in the cell [92]. Several PE-degrading prokaryotes have been found to produce alkane hydroxylases, and further analysis of enzymatic activity has demonstrated that such enzymes are capable of degrading PE – as evident by weight loss following culturing of PE substrate with isolated strains that secrete alkane hydroxylases [93]. Experiments in which the genes encoding these alkane hydroxylases (*AlkB*, *AlkB1*, and *AlkB2*) were recombinantly expressed in other bacterial strains have further confirmed their role in the degradation of PE [93,94].

The PE degradation intermediates produced by this depolymerization will undergo enzymatic hydroxylation of the alkane intermediate by alkane hydroxylases (monooxygenases) [91], and these modified oligomers can then be taken up by the cell through the use of dedicated alkane transporters. A recent transcriptomic study revealed that there are currently 19 putative proteins involved in such transport [85]. This step in the process of assimilation is also a metabolic bottleneck, however, as such transporters are likely limited in both their capacity to assimilate large PE fragments and their capacity to accommodate all the possible oxidation states of the alkane intermediates [85].

Following assimilation into the cell, many of the now fragmented components of the original polymer are converted by a series of enzymatic reactions into fatty acids which will be assimilated into the TCA cycle following beta-oxidation [92]. Alcohol dehydrogenases (Adh) usually act first to convert the alcohol functional group created by the alkane monooxygenases from their bond cleavage into a carboxylic acid [96]. The carboxylic acid group is then able to be acted upon by several enzymes – such as lipases and esterases – to produce of a molecule of acetic acid, a fatty acid intermediate, an alkane chain with a terminal carboxylic acid group, or a chemically similar intermediate. Any of these potential products can then be utilized in the process of beta-oxidation and further fed into the TCA cycle to provide energy to the cell – thus completing the transformation of a complex carbon polymer to microbial metabolites.

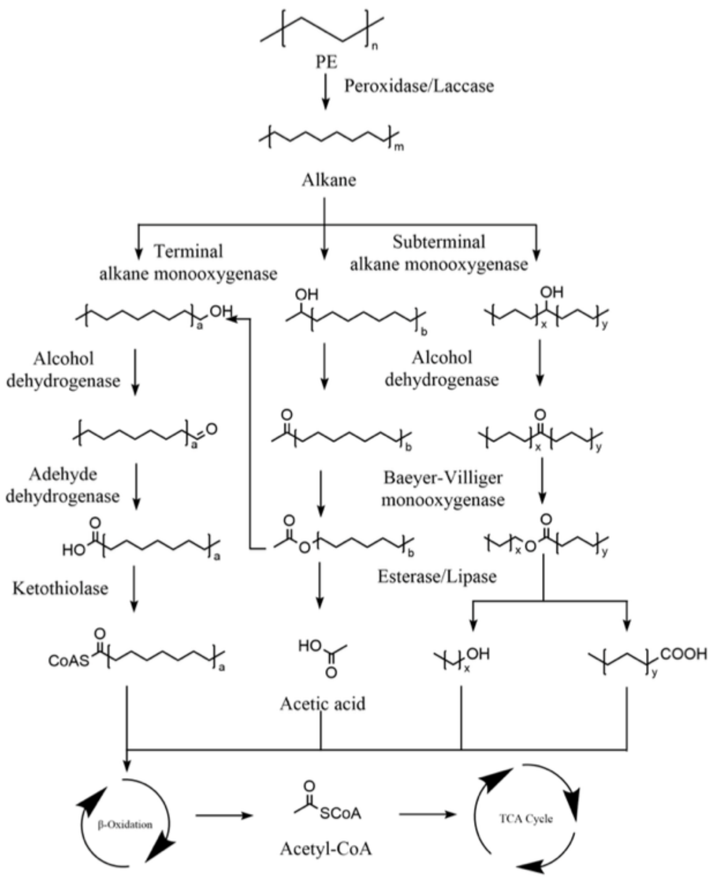


Figure 7. Metabolic pathways for the degradation of polyethylene (PE) plastics Adapted from [91].

Metabolism of PS

Unlike PE, the enzymatic and metabolic pathways through PS poorly understood. Especially difficult in elucidating the biodegradation of PS is the presence of an aromatic benzene ring as a substituent of the carbon backbone – thus facilitating the need for the metabolic capacity to degrade aromatic hydrocarbons in addition to the need to initially cleave the polymer backbone to release these aromatic intermediates. While some of the key enzymes involved in the degradation of PS have been identified, the full degradation pathway is currently unknown [91].

The initial step of depolymerization and the enzymes that facilitate the cleavage of the C-C backbone is significantly hindered as the bulky aromatic substituents present on the carbon chain inhibit favorable active-site/substrate interaction [92]. Some enzymes have been identified in the cleavage of the C-C backbone, however, with studies suggesting that this main-chain cleavage is facilitated by cytochrome P450 monooxygenases and alkane hydroxylases – similar to what has been observed in the degradation of PE. In the case of side-chain cleavage, initial studies have identified ring-hydroxylating dioxygenases as playing a potential role [95].

The mechanism for the further depolymerization and eventual mineralization of PS is dependent on the type of cleavage. Main-chain cleavage of PS results in the production of styrene and other aromatic hydrocarbons. The metabolism of styrene is well-documented and is thought to consist of a series of enzymatic reactions facilitated by a series of dioxygenases, hydrolases, and styrene oxide isomerases [96] that ultimately transform the initial styrene monomer to succinyl-CoA (which can then enter into beta-oxidation and the TCA cycle). In the case of side-chain cleavage, which produces PS oligomers instead of styrene monomers (or similar aromatic hydrocarbon monomers), it is believed that another series of enzymatic reactions convert the benzene substituent into various aromatic intermediates with the final result being the production of succinyl-CoA from benzyl-CoA.

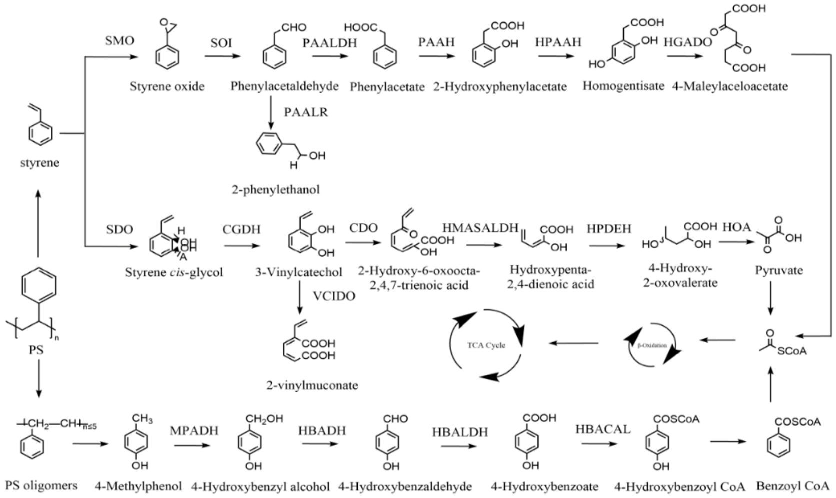


Figure 8. Metabolic pathways for the degradation of polystyrene (PS)

Adapted from [91].

Metabolism of PP

The mechanism through which PP remains metabolized remains to be fully understood. While there have been many organisms reported to be capable of degrading PP upon substrate incubation, there have not currently been any enzymes reported to be involved or capable of efficiently and effectively degrading PP plastics, and the overall mechanism through which these enzymes could assist in the degradation of this plastic is poorly understood [97,98]. PP is structurally similar to PE, and like PE, is often degraded much more efficiently following abiotic pretreatment (i.e., UV irradiation, thermal heating, etc.) and this is suggestive that the mechanism of PP degradation is likely enzymatically and metabolically similar to the degradation of PE. Further research is needed, however, to completely delineate and elucidate the metabolic and biochemical foundations of PP degradation.

Metabolism of PVC

Similar to the degradation of other C-C backbone carbons, the mechanism for the degradation of PVC remains poorly understood due to the enzymatic challenges posed by the hydrocarbon chain. The general mechanism for the metabolism of PVC begins with the general oxidation or fragmentation of the polymer chain, and the resultant polymers can be further depolymerized through catalase-peroxidases [99]. During the process of oxidation, some of the chlorine substituents are converted to hydroxyl groups (OH) and the remaining chlorine substituents can then be removed by dehalogenases – resulting in the formation of HCl and other chlorine compounds as waste [100], which poses a potential limitation to its effectiveness as a bioremediatory tool. Following the enzymatic action of the dehalogenases, the chemical transformation and eventual assimilation of the oligomers is similar to the PE biodegradation pathway – in which esterases, laccases, alkane hydroxylases, alcohol dehydrogenases, and monooxygenases break the compounds into intermediates of beta-oxidation and the TCA cycle. The subsequent assimilation of the PVC degradation intermediates is believed to be accomplished through the expression of several transporters, but further research is necessary to move beyond estimation [70]. Thus, more work needs to be done to investigate the properties of known strains and to further categorize the metabolic underpinnings of the biodegradation of PVC.

Metabolism of PET

Unlike the previously discussed plastics, polyethylene terephthalate (PET) is markedly different in its chemical composition due to the fact that its polymeric structure (the result of condensing ethylene glycol and terephthalic acid) consists of both an aromatic benzene ring and that its polymeric backbone consists of hydrolysable ester bonds. Several studies elucidating the microbial degradation and metabolism of PET as a carbon source have provided crucial insight into its mechanism of degradation. Although there are species-specific differences in the processing of PET intermediates (particularly ethylene glycol), the general outline and outcomes of the metabolic process remain the same across prokaryotic species capable of degrading PET.

The first step in the biodegradation of PET plastics is the hydrolytic cleavage of the ester backbone and this reaction is catalyzed by either by cutinases [78], which are naturally-produced ester-cleaving enzymes involved in the degradation of the natural polymer cutin (an aliphatic polyester) at the plant cuticle, or by cutinase-like enzymes such as PETase or TfH (isolated from *Thermobifida fusca*) [16,101]. PETases have been isolated from several prokaryotic species, and recombinant expression of the genes that encode these enzymes (*tfcut2* [101]*)* in other non-degrading microbes have further confirmed the PET-degrading capability of these enzymes [102, 103] This depolymerization results in the production of ethylene glycol, which can be directly metabolized through a series of enzymatic reactions into a molecule of acetyl-CoA and can thus enter the TCA cycle as a metabolic intermediate [91, 104]. Depolymerization also results in the production of terephthalate intermediates such as MHET and BHET. BHET is converted to MHET by the same PETase that initially cleaved the polymeric backbone, and MHET can be cleaved by a different enzyme, MHETase, into TPA and ethylene glycol [91, 105]. The ethylene glycol can then be metabolized as described above, and the TPA can be degraded into succinyl-CoA and acetyl-CoA by a series of enzymatic reactions following assimilation into the cell through use of a specific TPA transporter protein and passage through a TPA transporter [106]. The imported TPA can then be integrated into the TCA cycle following a series of enzymatic reactions – allowing for the production of cellular energy and mineralization of the plastic.

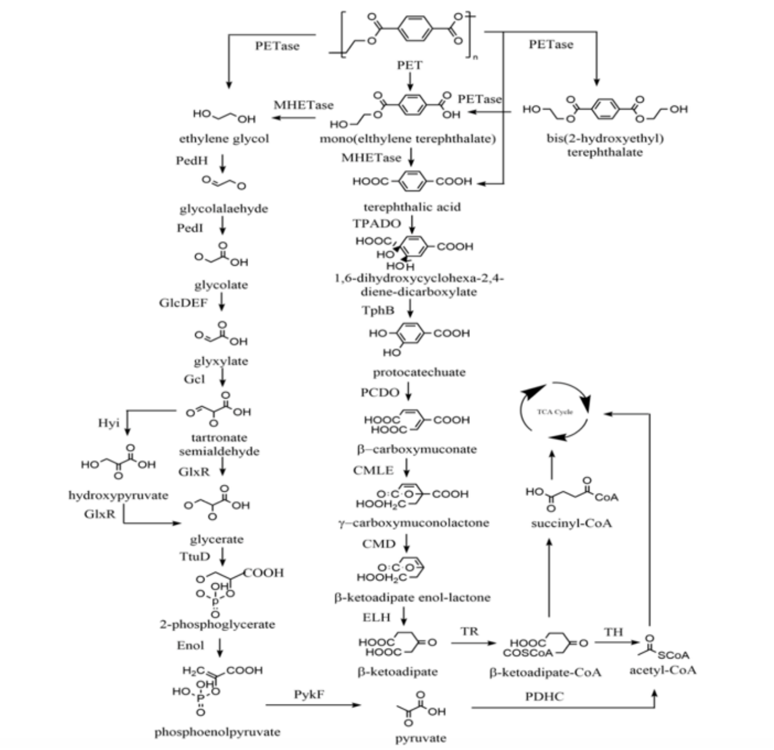


Figure 9. Degradation pathway for polyethylene terephthalate (PET).

Adapted from [91],

Metabolism of PUR

Polyurethane (PUR), like PET, contains a heteroatomic backbone with hydrolysable ester bonds and is therefore at increased susceptibility to microbial attack and degradation. Unlike PET, however, PUR is not found in abundance in the natural environment as a pollutant, and as a result, few strains (either prokaryotic or eukaryotic) have been isolated and identified as being capable of efficiently degrading PUR materials [16]. PUR is also unique due to the fact that it can be synthesized from a wide range of starting materials, and the variable "R" group can therefore affect the metabolic pathway needed for its degradation (Figure 10). Currently, polyester PUR has been shown to be efficiently degraded by microorganisms at a greater rate than polyether PUR, and as such, the degradation mechanism for PUR is largely based on the properties of polyester PUR.

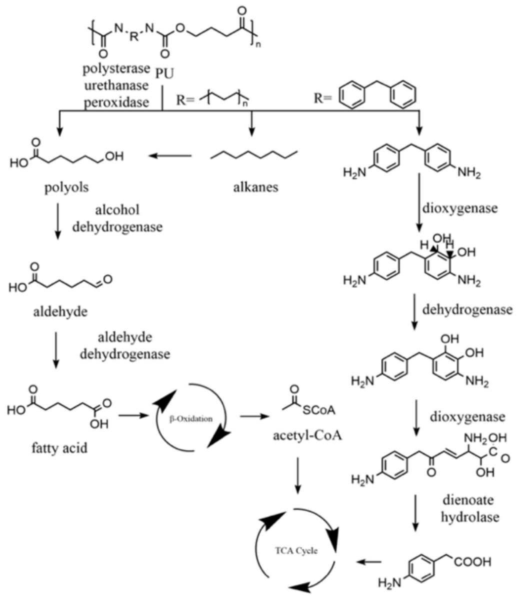


Figure 10. Degradation pathway for polyurethane (PUR).

Adapted from [91], [19], and [16].

The degradation of PUR begins with the depolymerization through the cleavage of ester and urea bonds in the PUR chain, resulting in the production of usable oligomer intermediates. This initial bond cleavage is accomplished by PUR poly-esterases, urethanases, and peroxidases [91]. Additionally, proteases, ureases, and esterases are thought to be vital to the initial step of depolymerization as they are capable of attacking the urethane, amide, and urea linkages present within the polymer chain to produce various nitrogen and oxygen-containing intermediates that can be further utilized and transformed for the generation of cellular energy [107].

In the case of PUR esterases and polyesterases, enzymatic transformation of generated polyols can result in the production of a fatty acid, which can then undergo beta-oxidation before being fed into the TCA cycle as acetyl-CoA. In some cases, these acetyl-CoA molecules can be converted into the natural polyester polyhydroxyalkanoate (PHA) following biochemical transformation and enzymatic attack by PHA synthase [108].

Intermediates containing the “R” group are also produced, and the metabolism of these degradation intermediates depends on the chemical structure of the R group. In the case of alkane R groups, the generated alkane can be oxidized to form a polyol intermediate that can then be metabolized, as previously mentioned. In the case of aromatic R groups, often present in PCL-based PU, the R group can be cleaved and transformed through a series of enzymatic reactions into an intermediate of the TCA cycle (acetyl-CoA) to generate cellular energy [16, 91, 108]. Given the diversity in the materials used to synthesis various forms of PU plastics, however, the potential for alternatives to the mechanisms described above are vast. For example, some species have been shown to utilize a mechanism of degradation involving the production of ethylene glycol as an intermediate (which is metabolized similarly to its role in the degradation of PET) [91], and other species have been shown to produce adipates as degradation intermediates from PCL-based PUR plastics [16].

Plastic Type	**Enzymes**	**Gene**	**Isolated From**	**References**
PE	Laccases	GenBankIDs: AII08809, AII11185, and A11221	*Rhodococcus opacus R7*	[88]
		Unnamed Sequence	*Rhodococcus ruber C208*	[148]
	Alkane monooxygenases	GenBankID: AYO90679.1	*Paenibacillus sp.*	[155]
	Cytochrome P450 monooxygenase	GenBankID: AII08421	*Rhodococcus opacus R7*	[139]
	Alkane hydroxylase	*AlmA* gene	Alcanivorax spp. 24	[154]
		alkB, alkB1, and alkb2 genes	*Pseudomonas sp. E4*	[94], [93]
PET	Para-nitrobenzylesterase	GenBankID: ADH43200.1	*Bacillus subtilis 4P3-11*	[150]
	PET Hydrolase	*Cut2* gene	*Ideonella saikaiensis*	[18]
		GenBank ID: CCK74972.1	*Oleispira antarctica*	[152]
	PET Lipase	GenBankID: A0A0K8P6T7	*Ideonella saikaiensis*	[153]
	Cutinase	GenBankID: BAO42836.1	*Saccharomospora viridis*	[77]
		GenBankID: ADV92525.1	*Thermobifida alba*	[152]

		GenBankID: ADV92527.1, ADV92526.1	*Thermobifida cellulosilytica*	[151]
		GenBankID: CBY05530.1, ADV92528.1	*Thermobifida fusca*	[151], [152]
		GenBankID: 4OYY	*Humicola insolens*	[150]
		GenBankID: ASA57064.1	*Vibrio gazogenes*	[139], [76], [77], [133]
PUR	PUR Esterases	*pudA*	*Comomonas acidovorans*	[140]
		pueB, pueA	*Pseudomonas chloroaphis*	[143], [141], [142]
		pulA	*Pseudomonas fluorescens*	[148]
	Polyester hydroylase	GenBankID: WP_088276085.1, WP_088276085.1	Pseudomonas aestusnigri	[149]

Table 2. List of known genes and enzymes involved in the degradation of major plastic types

Isolation of Plastic-Degrading Prokaryotes from the Natural Environment

While plastic-degrading microorganisms have been shown to exist across many different natural environments, plastic-degrading prokaryotes are most frequently isolated from sites that are likely to be in significant contact with plastic pollutants – such as landfills, soil (especially the soil surrounding plastic dumping sites and landfills), and the marine environment.

The most frequently encountered source of isolation is soil [16], and this is likely due to the high accumulation of plastic waste and the facilitation of microbe-plastic interaction as a result of plastic pollutants – especially small, fragmented components of plastic and microplastics – that can permeate the soil and facilitate greater interaction with the microbial consortia within it. Such interactions are facilitated to a larger extent in soil regions that contain high amounts of plastic waste such as landfills and plastic waste dumping sites. For example, *Ideonella sakaiensis*, a PETase producing bacterial species, was isolated from a PET recycling facility [18].

The marine environment is another abundant source of plastic-degrading prokaryotes, and several plastic-degrading organisms have been isolated from bodies of water that have been polluted with plastics [145]. Microorganisms in the marine environment are capable of forming compositionally diverse biofilms on the surface of floating plastics near the surface of the water and degrade the plastic through many of the pathways discussed previously [144]. Additionally, studies have found that the composition of the biofilms formed on plastic particles in the ocean are diverse, and that they are often enriched with hydrocarbon-degrading bacteria [146] – hinting at the potential for eventual plastic degradation.

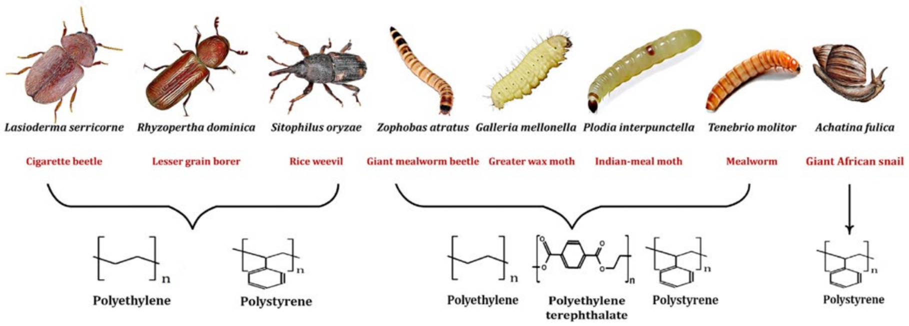


Figure 11. Eukaryotic organisms shown to be capable of consuming and degrading synthetic plastics

Adapted from [108].

Many plastic-degrading prokaryotes have recently been isolated from the guts of several invertebrates and their larvae. Waxworms (larvae) and wax moths, superworms (*Zophobas morio*), mealworms and adult yellow mealworm beetles(*Tenebrio molitor*) have all been found to contain bacteria within their gut that are capable of degrading synthetic plastics [58, 59, 61]. More importantly, the presence of these plastic-degrading microbes in the gut confers the ability to directly consume plastic to the organisms – thus greatly expanding the potential for biological plastic degradation and offering insight into the mechanisms through which other forms of plastic waste (i.e., microplastics) can be degraded within other organisms. This is reflected in the fact that current studies looking at the impact of microplastics have revealed that their growing environmental presence has resulted in potential degradation capabilities in the gut microbiota of several other organisms - including humans [109]. Thus, the gut microbiome is a promising source of potential plastic-degraders and future research into its plastic-degrading capability has significant implications for health, medicine, and more broadly, the bioremediation of plastic pollutants.

Extremophilic environments – environments defined by extreme, harsh conditions that prohibit the survival of most organisms (i.e., highly acidic, highly basic, high/low temperatures, and high levels of salinity) – are another potential source of plastic-degrading prokaryotes and these environments have remained poorly investigated within the context of plastic degradation. The isolation of extremophilic plastic-degraders is especially important given that the extreme growth conditions can often result in advantageous modifications to the chemical structure of plastic that increase its degradability. For example, thermophilic (heat-loving) bacteria would possess a significant advantage in the degradation of certain plastics, such as PET and PE, that undergo physical changes upon heating, such as increased mobility of the amorphous (enzymatically favorable) regions, that favor plastic degradation. Several thermophilic bacteria have been isolated and proven to be capable of degrading PET and polyethylene at temperatures ranging from 50°C to 60°C [110], and thermostable variants of the enzymes produced by these organisms have proven to be markedly efficient at degrading plastic. In particular, psychrophilic bacteria are another class of extremophiles with great potential for plastic degradation, especially given that psychrophiles comprise a significant proportion of marine microorganisms. As mentioned previously, the marine environment is a source of massive interest in the context of plastic degradation, and further examination of the microorganisms that inhabit it is essential. Outside of the marine environment, psychrophilic bacteria from alpine and arctic soils have been found capable of degrading PUR – highlighting the potential for bacteria from diverse environments to degrade plastic and underlining the widespread impact of plastic pollution in the global environment [110].

Given the growing rate at which plastic pollutants are entering and will continue to enter the natural environment, the prevalence of plastic substrates in the environment and the resultant amount of contact with prokaryotic organisms will, over time, result in the continued proliferation and discovery of microorganisms who have adapted to use plastic as a carbon and energy source. Currently, improvements in sampling capabilities and increased, extensive screening or observation of potential isolates are needed to further elucidate the extent to which this microbial trait has been observed and distributed and to greater understand the ecology and composition of the plastisphere (the global community of microbes that have colonized the surface of plastics).

Plastic-Degrading Prokaryotes and Their Potential Role in Bioremediation

Bioremediation

Given the growing impacts of plastic pollution on the natural environment and its organisms, there is an increasing need for more effective and sustainable methods of waste removal and management. An alternative to traditional methods of plastic waste management is bioremediation: the use of biological organisms to degrade or detoxify environmental pollutants. Bioremediation has been proven to be an effective option for the removal and mitigation of environmental pollutants in the past – with hydrocarbon-degrading prokaryotes proving to be especially useful in reducing the environmental impact of oil spills [15, 111,112]. The development of bioremediation technologies utilizing plastic-degrading microbes is essential for clean and effective methods of waste management methods – especially given that some prokaryotes are capable of degrading the toxic additives and contaminants present in many types of plastic [113].

Potential Bioremediation Applications of Plastic-Degrading Prokaryotes

The isolation of plastic-degrading microorganisms has opened the door for the bioremediation of plastic pollutants, and much of the research surrounding plastic-degrading microorganisms has been through the lens of their potential applications to remediating the impact of plastic waste on the environment. The simplest form of bioremediation would likely consist of large-scale culturing and replication of prokaryotic species that possess the enzymatic machinery – either naturally or through recombinant expression - capable of metabolizing a given type of plastic. These organisms can then be released into the natural environment at a location dense in plastic pollutants, with the goal for these organisms to degrade pollutants completely into metabolic byproducts like CO_2. While under ideal conditions, this model would ensure that prokaryotes act effectively as bioremediation tools, there are significant limitations and metabolic bottlenecks present in the application of this model to natural environments.

The current state of our understanding and the present capabilities of these organisms are not fully compatible with large scale efforts of bioremediation. To date, no large-scale efforts to utilize prokaryotes for bioremediation have been conducted. The conflicting interaction of various factors related to the strength and recalcitrance of the polymer and factors related to the abiotic and biotic degradation of the polymer [114] has thus far prevented the direct use of plastic-degrading microbes and has confined their bioremediation possibilities to the realm of speculation and theory. Furthermore, in its current state, the degradation of plastic – even with the use of engineered enzymes – is too slow and inefficient to effectively serve as a bioremediation tool, and manipulation of the environmental conditions to facilitate degradation are equally ineffective and potentially harmful [91, 107, 114].

Further research is needed to completely understand the metabolic and enzymatic processes that accompany the observed microbial degradation of plastic and to optimize their application to bioremediation. Some current models and preliminary studies have outlined the potential for alternative methods of bioremediation, such as *ex situ* bioremediation (the use of isolated sites in which plastic waste and microbes are combined) [115] and the biochemical engineering of existing enzymes [78, 84, 103, 105], but the future of bioremediation remains dependent on the rate at which advancements in our understanding of the full molecular mechanisms of degradation can be made.

Biotechnology and Prokaryotic Plastic Degradation

Biotechnological Approaches to Prokaryotic Plastic Degradation

Currently, the rate at which plastic-degrading enzymes and microbes are identified is incompatible with any practical applications or goals. For this reason, a biotechnological approach is predicted to hold significant promise in serving as a bridge for the transition of natural enzymes to effective waste management tools.

Genetic engineering of known enzymes – especially the well-characterized hydrolytic enzyme PETase – have demonstrated that the efficiency and efficacy of plastic-degrading enzymes can be augmented. Further studies have also demonstrated that such augmented enzymatic machinery can be functionally expressed in other microorganisms – opening the door for the specific, efficient microbial plastic degradation and the targeted modification of microbial consortia composition to better fit the ecological needs of the natural environment [116]. Several studies utilizing biotechnology have demonstrated successful engineering of plastic-degrading enzymes, with thermostable PETases [117] expanding the environmental range of potential degradation, and genetically engineered polyester hydrolases demonstrating a four-fold increase in the efficiency of plastic degradation [118]. Protein engineering has yielded similar results, with mutant PETase variants demonstrating improved kinetics and degradation efficiency compared to the normal enzyme [119]. Additionally, the development of genome editing technologies, such as CRISPR-Cas9, Zinc finger proteins, and TALENs, ensure that the genetic architecture required for plastic degradation can be conferred to non-degrading microbes – expanding the microbial potential for plastic degradation through increasing the availability of capable microorganisms [114].

It is important to note, however, that genetic engineering of plastic-degrading prokaryotes can result in negative secondary effects in the form of dilution of the gene pool [114] and the transfer of modified genetic material within adjacent microbial communities and within the plastisphere. The use of genetically engineered microorganisms would thus likely require additional modification to gene expression – perhaps through editing by CRISPR-Cas9 and the development of auto destructive or self-regulating genetic circuits – to prevent gene pool dilution and horizontal gene transfer. More broadly, the use of biotechnology rooted in genetic engineering of plastic-degrading enzymes and the production of genetically engineered strains, while a vital step in biologically degrading plastic waste, should be highly regulated and monitored to before widespread application.

Biotechnological Applications of Prokaryotic Plastic Degradation

There are currently several other promising biotechnological applications of prokaryotic plastic-degraders, that will likely emerge among bioremediation as potent tools following future research. For example, the production of bio-surfactants/bio-emulsifiers by microbes able to colonize hydrophobic substrates (such as synthetic plastics) can be manipulated with biotechnology to facilitate increased solubility of plastic pollutants and improve the interaction of microbes with the plastic substrate [120].

One example of a potential biotechnological application of plastic-degrading prokaryotes is the use of microbial enzymes – such as PETase – to aid in the recycling of traditional plastics. While similar to the process of bioremediation, the microbial enzymes in this case would not be facilitating the complete degradation and elimination of the plastic from the environment and would instead be focused on removing the secondary effects of traditional methods of recycling – such as harsh chemical processing and unwanted degradation products [19, 20, 121]. The use of depolymerizing enzymes to aid in the recycling and upcycling process would significantly mitigate the impact of these traditional methods and would serve as a biotechnological steppingstone to sustainable modifications to unsustainable practices as well as large-scale bioremediation efforts.

Biotechnology has the potential to dramatically transform the landscape of prokaryotic plastic degradation, and it can do so as both a catalyst for needed improvements in biochemical capacity and as a mediator for the introduction of sustainability to traditionally unsustainable methods. As the molecular mechanisms involved in plastic degradation continue to be unraveled, so too will the biotechnological potential of this vital prokaryotic trait. Further research is needed to truly harness the capabilities of plastic-degrading prokaryotes to develop effective waste management strategies and develop additional promising solutions to the impact of plastic pollution and its environmental unsustainability.

Discussion

Limitations to Existing Applications and Knowledge

The Isolation of Plastic-Degrading Prokaryotes

The ability to isolate and identify plastic-degrading prokaryotes is a significant limitation to our capacity to fully understand this microbial trait, and more broadly, to discover novel enzymes and use these enzymes in the context of bioremediation. It is estimated that less than 2% of bacteria on the planet are culturable in a laboratory setting, and less than 1% of the isolated species capable of plastic-degradation are capable of being cultured and studied using conventional methods [122]. Advancements in metagenomic sequencing, however, have allowed for the identification of putative plastic-degrading capabilities of large microbial communities, but further classification and categorization of microbial plastic degradation mechanisms requires isolation and culturing – processes that are currently not suited for such an investigation.

Mechanistic and Metabolic Understanding of Plastic Degradation

Currently, much of the research surrounding plastic-degrading prokaryotes is centered around the isolation and identification of species proven to be capable of degradation, rather than the identification and characterization of the metabolic and chemical pathways through which such degradation is accomplished. Much of the work surrounding the metabolic and enzymatic pathways involved in degradation are based on estimates and reconstructions based on information from the transcriptome and genome, with some information being provided from proteomic analysis. It is also important to note that some degradation pathways are hypothesized based on the degradation of chemically similar intermediates – such as the proposed degradation pathway for the styrene produced from PS – but the potential for novel pathways of degradation that have not yet been discovered is high.

Additionally, there is a lack of standardization of how plastic degradation is quantified and determined experimentally. As mentioned previously, the degradation of plastic is determined through a variety of ways – such as weight loss, FTIR, and detection of degradation products – but each method is characterized by its own set of limitations and the results of each are only capable of proving certain aspects of the degradation process. The use of a combination of methods has been suggested [26,28] to alleviate the ambiguity present in how plastic degradation is defined experimentally, but in any case, a standardized approach to experimentally determining the degradation of plastic is needed to further our understanding and, more importantly, to aid in the development of practical applications of plastic-degrading species.

Observation and Understanding of In Vivo Biodegradation

The formation of a biofilm with other microbes is an important first step in the colonization of the plastic substrate and the subsequent degradation of the plastic as it is used as carbon source. This poses a significant limitation to our understanding of plastic degradation, however, as the isolation of specific organisms capable of degradation eliminates the potential to observe the often-synergistic effects occurring within the biofilm that facilitate degradation. While some studies have demonstrated plastic degradation through co-incubation of plastic with a sampled microbial consortium, the discovery of novel enzymes and specific pathways remains hindered due to the strain isolation required.

Furthermore, plastic-degrading prokaryotes that are able to be cultured are often studied under conditions that are markedly different from the environment from which they were sampled. For example, initial studies demonstrating the capacity for prokaryotes to degrade PET plastics revealed that such degradation occurred most efficiently and prominently at temperatures near the glass transition point of the plastic, which is approximately 20°C – a temperature significantly high for the environments from which these organisms are often isolated (soil and bodies of water). Additionally, the plastic substrates often used to experimentally determine plastic degradation are often pre-treated using UV irradiation, thermal heating, photo-oxidation, or a combination of these factors, and while this is often meant to replicate the abiotic degradation often present and required for natural degradation, it also limits the ability to extrapolate the observed results to the real-world environment. This is especially critical given that the degree to which the plastic can be effectively pre-treated in an experimental setting is often greater than what is observed in nature, and this discrepancy can lead to the overestimation of plastic-degrading capability [28].

Efficiency of Prokaryotic Plastic Degradation

Aside from the present limitations in our understanding of microbial plastic degradation, there exist several limitations to the use of already-discovered plastic-degrading microorganisms and their enzymes for the purpose of bioremediation and other practical applications – a prominent objective given the growing problem of plastic pollution. For one, the degradation of plastic by microorganisms can often result in the production of microplastics – especially given the inefficiency observed in the degradation process – and this can further exacerbate the consequences of plastic pollution as microplastics are significantly harder to remove from the environment and can have lasting negative impacts on many organisms and ecosystems including humans. Microplastics can often serve as carriers for potentially harmful chemical compounds that are released from plastics, and the degradation of plastic by microbes is a potential method of releasing these chemicals. While some early research examining the role of bacteria in colonizing and degrading microplastics in the marine environment [37, 55, 120] has revealed the potential for a biological remediation of this effect, it would likely not be capable of offsetting the production of microplastics, especially by other bacteria.

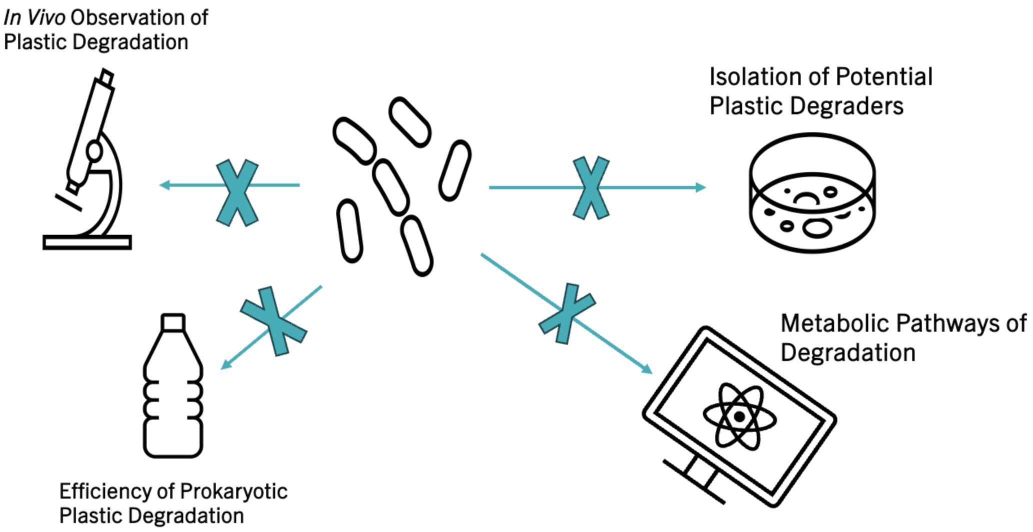


Figure 12. Visual of the major gaps in knowledge and limitations that exist within the context of prokaryotic plastic degradation

Limitations of This Review

In addition to the acknowledged limitations of the information presented in this review, there are also limitations in the scope of this review itself. The primary limitation of this literature review is that the degradation of the majority of the major plastic types is covered in detail, but the degradation, synthesis, and impact of each plastic type contains many complexities and additional information that could not have been reasonably included in this thesis. Similarly, the combined number of plastic-degrading prokaryotes across all plastic types is upwards of 100, and the number of putative degraders likely approaches 1000. For this reason, providing detailed information on all identified – both experimentally and putatively – plastic degraders is outside of the scope of this review, but effort was made to provide pertinent information about well-categorized and relevant isolates for each plastic type discussed.

Future Directions

Despite these limitations, the field of research regarding prokaryotic plastic degradation is emerging and promising. The resolution and investigation of key unanswered questions – many of which stem from the aforementioned limitations – is vital to the advancement of this field. These unanswered questions lay the foundation for the future of research into this prokaryotic trait, and it's important to consider these questions as research and isolation of these organisms continues in the near future.

What Role Might Genome Editing Tools Like CRISPR Play in This Process?

With recent advancements in genome editing technologies, such as CRISPR, the question of whether such technology can be used to edit the gene sequences of plastic-degrading enzymes to improve efficiency or confer the ability to degrade plastic to other organisms becomes increasingly important. However, given the limited amount of information available with regards to the genes involved in plastic degradation and the fact that this process is often the result of many enzymes (some of which are responsible for the degradation of other unrelated intermediates), the role that genome editing may play in altering the efficiency and capabilities of plastic-degrading prokaryotes remains unanswered. While further research into the practical benefits of genome editing would be vital to eventually bridging the gap to a bioremediatory future, it is important to note that the effect of introducing modified plastic-degrading genes also remains underexplored. Further investigation into the downstream effects of such genomic editing needs to be conducted first, so that answering this question is done so effectively and appropriately.

What Role do Prokaryotes Play in the Degradation and Production of Microplastics?

Microplastics are less than 5mm fragments of plastic and are an environmental pollutant that pose potential health risks to humans and other animals [147]. Microplastics are of increasing environmental concern given the scale at which they pollute the environment, and the inability for modern methods of waste mitigation to contain or remove them from the environment. The question of whether prokaryotes produce microplastics during the process of degrading plastic is vital to our understanding of the effectiveness and efficiency of their role. If microplastics are produced in significant quantities as a result of microbial degradation, then this could pose a significant problem as it would further contribute to growing concerns regarding current state of microplastic pollution. Inversely, if it was discovered that other prokaryotes are capable of colonizing and degrading these microplastics, it would open the door to further combatting the problem of plastic pollution at a scale that is not currently feasible.

How Do We Translate Experimental Results to What Occurs in the Natural Environment?

As discussed previously, there are currently several limitations to the ability to use what has been observed experimentally as an indication or reflection of what is occurring naturally (i.e., on the surface of plastic objects in landfills, the ocean, etc.). This discrepancy between experimental conditions and what may be occurring in the natural environment can prevent the eventual development of successful bioremediation technology and any larger conclusions about the impact of these prokaryotes on existing plastic pollution in the environment.

Improvements to existing capabilities of how plastic degradation can be observed experimentally in combination with advancements in how microbial communities can be studied at a larger scale (with regards to large, untreated plastic substrate) are essential for the eventual resolution of this unanswered question. Bridging this gap is necessary for further study of the large-scale implications of this microbial trait.

Conclusion

While much progress has been made in our understanding of plastic degradation by prokaryotes, there remains a significant amount of knowledge to be discovered. The gaps in knowledge in this field have remained a significant obstacle to the utilization of plastic degraders as effective and sustainable tools, and inconsistency in the quantification and identification of microbial plastic degraders has only further contributed to the negative effects of this gap in knowledge. In particular, the environmental conditions that influence the degradation of plastic *in vivo*, and the molecular mechanisms through which this degradation occurs naturally – both by individual strains and synergistically by microbial consortia – are of great importance, especially given that understanding both of these factors are a necessary step in fully realizing the potential for these organisms to operate in the process of bioremediation and effectively mitigate the impact of plastic pollution on the natural environment.

Future research should prioritize the identification and elucidation of molecular mechanisms for plastic degradation to ensure the advancement of biotechnological and bioremediatory approaches to plastic-degrading microbes and their enzymes. Such advancement can hopefully lead to the full-scale implementation of bioremediation practices and biotechnology-led sustainability improvements to traditional methods of plastic waste management. However, given the novelty of our understanding of this microbial trait and the current state of molecular analysis, such implementation is likely a distant future.

While full-scale implementation of these enzymes – either for the purpose of mitigating plastic waste directly or improving existing waste management – has not and likely will not be accomplished given the current state of knowledge and technology, prokaryotic plastic degradation is a critical area of research and the continuation of current research/the development of future research is vital for environmental sustainability and the procurement of a future free from the burden of the millions of tons of plastic waste that currently pollute the Earth.

References

1. Giant molecules: from nylon to nanotubes. (2010). *Choice Reviews Online*, *47*(11), 47–6257. https://doi.org/10.5860/choice.47-6257

2. *Global plastic production 2021 | Statista*. (2023, September 20). Statista. https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/to

3. *Single-Use Plastics Ban | Nantucket, MA - Official website*. (n.d.). https://www.nantucket-ma.gov/1475/Single-Use-Plastics-Ban

4. Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Phil. Trans. R. Soc. B* 364 1985–1998. 10.1098/rstb.2008.0205

5. Rüthi, J., Cerri, M., Brunner, I., Stierli, B., Sander, M., & Frey, B. (2023). Discovery of plastic- degrading microbial strains isolated from the alpine and Arctic terrestrial plastisphere. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1178474

6. Jamieson AJ, Brooks L, Reid W, Piertney S, Narayanaswamy B, Linley T. 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *R Soc Open Sci* 6:180667. doi: 10.1098/rsos.180667.

7. Romera-Castillo C, Pinto M, Langer TM, Álvarez-Salgado XA, Herndl GJ. 2018. Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. *Nat Commun*9:1430. doi: 10.1038/s41467-018-03798-5.

8. Li, P.; Wang, X.; Su, M.; Zou, X.; Duan, L.; Zhang, H. Characteristics of Plastic Pollution in the Environment: A Review. *Bull. Environ. Contam. Toxicol.* **2021**, *107*, 577–584.

9. Gewert, B., Plassmann, M., & MacLeod, M. (2015). Pathways for degradation of plastic polymers floating in the marine environment. *Environmental Science: Processes & Impacts*, *17*(9), 1513–1521. https://doi.org/10.1039/c5em00207a

10. A. L. Andrady, Mar. Pollut. Bull., 2011, 62, 1596–1605.

11. Zimmermann, L.; Bartosova, Z.; Braun, K.; Oehlmann, J.; Völker, C.; Wagner, M. Plastic Products Leach Chemicals That Induce In Vitro Toxicity under Realistic Use Conditions. *Environ. Sci. Technol.* **2021**, *55*, 11814–11823

12. Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances*, *26*(3), 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005

13. Mohanan, N., Montazer, Z., Sharma, P., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.580709

14. Cai, Z., Li, M., Zhu, Z., Wang, X., Huang, Y., Li, T. J., Gong, H., & Yan, M. (2023). Biological degradation of plastics and microplastics: A recent perspective on associated mechanisms and influencing factors. *Microorganisms*, *11*(7), 1661. https://doi.org/10.3390/microorganisms11071661

15. Chandra, S., Sharma, R., Singh, K. *et al.* Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Ann Microbiol* **63**, 417–431 (2013). https://doi.org/10.1007/s13213-012-0543-3

16. Ru, J., Huo, Y., & Yang, Y. (2020). Microbial degradation and valorization of plastic wastes. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00442

17. Carpenter, S. (2021, March 10). The Race To Develop Plastic-Eating Bacteria. *Forbes*. https://www.forbes.com/sites/scottcarpenter/2021/03/10/the-race-to-develop-plastic-eating-bacteria/?sh=4846c9097406

18. Tanasupawat, S., Takehana, T., Yoshida, S., Hiraga, K., & Oda, K. (2016). Ideonella sakaiensis sp. nov., isolated from a microbial consortium that degrades poly(ethylene terephthalate). *International Journal of Systematic and Evolutionary Microbiology*, *66*(8), 2813–2818. https://doi.org/10.1099/ijsem.0.001058

19. Geyer, R. (2020). A brief history of plastics. In *Springer eBooks* (pp. 31–47). https://doi.org/10.1007/978-3-030-38945-1_2

20. Geyer, R., Jambeck, J., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, *3*(7). https://doi.org/10.1126/sciadv.1700782

21. Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M. M., Scott, S. L., & Suh, S. (2020). Degradation rates of plastics in the environment. *ACS Sustainable Chemistry & Engineering*, *8*(9), 3494 3511. https://doi.org/10.1021/acssuschemeng.9b06635

22. Jang, M., Yang, H., Park, S., Sung, H. K., Koo, J. M., Hwang, S. Y., Jeon, H., Oh, D. X., & Park, J. (2022). Analysis of volatile organic compounds produced during incineration of non-degradable and biodegradable plastics. *Chemosphere*, *303*, 134946. https://doi.org/10.1016/j.chemosphere.2022.134946

23. Lin, X., Zhang, S., Shu, Y., Zhang, R., Shi, X., & Song, L. (2023). A landfill serves as a critical source of microplastic pollution and harbors diverse plastic biodegradation microbial species and enzymes: Study in large-scale landfills, China. *Journal of Hazardous Materials*, *457*, 131676. https://doi.org/10.1016/j.jhazmat.2023.131676

24. Rose, R., Richardson, K. H., Latvanen, E. J., Hanson, C. A., Resmini, M., & Sanders, I. A. (2020). Microbial degradation of plastic in aqueous solutions demonstrated by CO2 evolution and quantification. *International Journal of Molecular Sciences*, *21*(4), 1176. https://doi.org/10.3390/ijms21041176

25. Sielicki, M., Focht, D. D., & Martín, J. P. (1978). Microbial degradation of [14C]polystyrene and 1,3-diphenylbutane. *Canadian Journal of Microbiology*, *24*(7), 798–803. https://doi.org/10.1139/m78-134

26. Kotova, I., Taktarova, Y., Цавкелова, E. A., Егорова, Bubnov, I. A., Malakhova, D. V., Shirinkina, L., Sokolova, T. G., & Bonch-Osmolovskaya, E. A. (2021). Microbial Degradation of Plastics and to Make it More Efficient. *Microbiology*, *90*(6), 671–701. https://doi.org/10.1134/s0026261721060084

27. Rajandas, H., Parimannan, S., Sathasivam, K. V., Ravichandran, M., & Yin, L. S. (2012). A novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation. *Polymer Testing*, *31*(8), 1094–1099. https://doi.org/10.1016/j.polymertesting.2012.07.015

28. Montazer, Z., Najafi, M. B. H., & Levin, D. B. (2020). Challenges with Verifying Microbial Degradation of Polyethylene. *Polymers*, *12*(1), 123. https://doi.org/10.3390/polym12010123

29. Darby, R. T., & Kaplan, A. M. (1968). Fungal susceptibility of polyurethanes. *Applied Microbiology*, *16*(6), 900–905. https://doi.org/10.1128/aem.16.6.900-905.1968

30. Yang, S., Wu, W., Brandon, A. M., Fan, H., Receveur, J. P., Li, Y., Wang, Z., Fan, R., McClellan, R. L., Gao, S., Ning, D., Phillips, D., Peng, B., Wang, H., Cai, S., Li, P., Cai, W., Lee, D., Yang, J., . . . Criddle, C. S. (2018). Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae). *Chemosphere*, *212*, 262–271. https://doi.org/10.1016/j.chemosphere.2018.08.078

31. Peng, B., Li, Y., Fan, R., Chen, Z., Chen, J., Brandon, A. M., Criddle, C. S., Zhang, Y., & Wu, W. (2020). Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of Zophobas atratus (Coleoptera: Tenebrionidae): Broad and limited extent depolymerization. *Environmental Pollution*, *266*, 115206. https://doi.org/10.1016/j.envpol.2020.115206

32. Peng, B., Chen, Z., Chen, J., Yu, H., Zhou, X., Criddle, C. S., Wu, W., & Zhang, Y. (2020). Biodegradation of Polyvinyl Chloride (PVC) in Tenebrio molitor (Coleoptera: Tenebrionidae) larvae. *Environment International*, *145*, 106106. https://doi.org/10.1016/j.envint.2020.106106

33. Peng, B., Li, Y., Fan, R., Chen, Z., Chen, J., Brandon, A. M., Criddle, C. S., Zhang, Y., & Wu, W. (2020b). Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of Zophobas atratus (Coleoptera: Tenebrionidae): Broad and limited extent depolymerization. *Environmental Pollution*, *266*, 115206. https://doi.org/10.1016/j.envpol.2020.115206

34. Song, Y., Qiu, R., Hu, J., Li, X., Zhang, X., Chen, Y., Wu, W., & He, D. (2020). Biodegradation and disintegration of expanded polystyrene by land snails Achatina fulica. *Science of the Total Environment*, *746*, 141289. https://doi.org/10./j.scitotenv.2020.141289

35. Giacomucci, L., Raddadi, N., Soccio, M., & Fava, F. (2019). Polyvinyl chloride biodegradation by Pseudomonas citronellolis and Bacillus flexus. *New Biotechnology*, *52*, 35–41. https://doi.org/10.1016/j.nbt.2019.04.005

36. Kinoshita, S., Kageyama, S., Iba, K., Yamada, Y., & Okada, H. (1975). Utilization of a cyclic dimer and linear oligomers of .EPSILON.-aminocaproic acid by Achromobacter guttatus KI72. *Agricultural and Biological Chemistry*, *39*(6), 1219–1223. https://doi.org/10.1271/bbb1961.39.1219

37. Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., Barbe, V., Meistertzheim, A., & Ghiglione, J. (2019). Microbial Ecotoxicology of Marine Plastic Debris: A review on colonization and biodegradation by the "Plastisphere." *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00865

38. Pérez-García, P., Chow, J., Costanzi, E., Gurschke, M., Dittrich, J., Dierkes, R. F., Molitor, R., Applegate, V., Feuerriegel, G., Tete, P., Danso, D., Thies, S., Schumacher, J., Pfleger, C., Jaeger, K., Gohlke, H., Smits, S. H. J., Schmitz, R. A., & Streit, W. R. (2023). An archaeal lid-containing feruloyl esterase degrades polyethylene terephthalate. *Communications Chemistry*, *6*(1). https://doi.org/10.1038/s42004-023-00998-z

39. Ellis, L. D., Rorrer, N. A., Sullivan, K. P., Otto, M., McGeehan, J., Román-Leshkov, Y., Wierckx, N., & Beckham, G. T. (2021). Chemical and biological catalysis for plastics recycling and upcycling. *Nature Catalysis*, *4*(7), 539–556. https://doi.org/10.1038/s41929-021-00648-4

40. Albertsson, A., & Karlsson, S. (1990). The influence of biotic and abiotic environments on the degradation of polyethylene. *Progress in Polymer Science*, *15*(2), 177–192. https://doi.org/10.1016/0079-6700(90)90027-x

41. Parikh, M., Gross, R. A., & McCarthy, S. P. (1993). The effect of crystalline morphology on enzymatic degradation kinetics. *Biodegradable Polymers and Packaging*, 407–415.

42. Tokiwa, Y., Calabia, B. P., Ugwu, C. U., & Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences*, *10*(9), 3722–3742. https://doi.org/10.3390/ijms10093722

43. Zheng, Y., Yanful, E. K., & Bassi, A. (2005). A review of plastic waste biodegradation. *Critical Reviews in Biotechnology*, *25*(4), 243–250. https://doi.org/10.1080/07388550500346359

44. Zadjelovic, V., Erni-Cassola, G., Obrador-Viel, T., Lester, D. W., Eley, Y., Gibson, M. I., Dorador, C., Golyshin, P. N., Black, S., Wellington, E. M. H., & Christie-Oleza, J. A. (2022). A mechanistic understanding of polyethylene biodegradation by the marine bacterium Alcanivorax. *Journal of Hazardous Materials*, *436*, 129278. https://doi.org/10.1016/j.jhazmat.2022.129278

45. Azeko, S. T., Etuk-Udo, G. A., Odusanya, O. S., Malatesta, K., Anuku, N., and Soboyejo, W. O. (2015). Biodegradation of linear low density polyethylene by *Serratia marcescens* subsp. *marcescens* and its cell free extracts. *Waste Biomass Valor.* 6, 1047–1057. doi: 10.1007/s12649-015-9421-0

46. Sarmah, P., and Rout, J. (2018). Efficient biodegradation of low-density polyethylene by cyanobacteria isolated from submerged polyethylene surface in domestic sewage water. *Environ. Sci. Pollut. Res. Int.* 25, 33508–33520. doi: 10.1007/s11356- 018- 3079- 7

47. Albertsson, A. C. (1980). Microbial and oxidative effects in degradation of poly- ethene. *J. Appl. Polym. Sci.* 25, 1655–1671. doi: 10.1002/app.1980.070250813

48. Sen, S. K., & Raut, S. (2015). Microbial degradation of low density polyethylene (LDPE): A review. *Journal of Environmental Chemical Engineering*, *3*(1), 462–473. https://doi.org/10.1016/j.jece.2015.01.003

49. Zhang, Y., Pedersen, J. N., Eser, B. E., & Guo, Z. (2022). Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery. *Biotechnology Advances*, *60*, 107991. https://doi.org/10.1016/j.biotechadv.2022.107991

50. Pometto, A. L., Lee, B. T., & Johnson, K. E. (1992). Production of an extracellular polyethylene-degrading enzyme(s) by Streptomyces species. *Applied and Environmental Microbiology*, *58*(2), 731–733. https://doi.org/10.1128/aem.58.2.731-733.1992

51. Chigor, V. N., Nwankwo, C. E. I., Ogbodo, U., & Ugwu, J. N. (2020). Polyethylene Biodegradation Potentials of Pseudomonas aeruginosa and Micrococcus sp. Isolated from Waste Dumps and Farmlands in Nsukka, Enugu State, Nigeria. *Microbiology Research Journal International*, 10–18. https://doi.org/10.9734/mrji/2020/v30i430208

52. Liu, R., Men, L., Zhang, Z., Guan, F., Tian, J., Wang, B., Wang, J., Zhang, Y., & Zhang, W. (2019). Biodegradation of Polyethylene by Enterobacter sp. D1 from the Guts of Wax Moth Galleria mellonella. *International Journal of Environmental Research and Public Health*, *16*(11), 1941. https://doi.org/10.3390/ijerph16111941

53. Ruslan, R., Iqbal, M. N., Pekey, A. Y., Dewi, A. P., & Djamaan, A. (2018). ISOLATION AND CHARACTERIZATION OF POLYSTYRENE-DEGRADING BACTERIA BACILLUS SP. ITP 10.1.1 FROM SOIL SAMPLE OF JAYAWIJAYA MOUNTAINS, PAPUA, INDONESIA. *International Research Journal of Pharmacy*, *9*(10), 85–88. https://doi.org/10.7897/2230-8407.0910231

54. Chen, L., & Lin, Z. (2021). Polyethylene: Properties, production and applications. *2021 3rd International Academic Exchange Conference on Science and Technology Innovation (IAECST)*. https://doi.org/10.1109/iaecst54258.2021.9695646

55. Sudhakar, M., Doble, M., Murthy, P. S., & Venkatesan, R. (2008). Marine microbe-mediated biodegradation of low- and high-density polyethylenes. *International Biodeterioration & Biodegradation*, *61*(3), 203–213. https://doi.org/10.1016/j.ibiod.2007.07.011

56. Awasthi, S. K., Srivastava, P., Singh, P., Tiwary, D., & Mishra, P. K. (2017). Biodegradation of thermally treated high-density polyethylene (HDPE) by Klebsiella pneumoniae CH001. *3 Biotech*, *7*(5). https://doi.org/10.1007/s13205-017-0959-3

57. Yang, J., Yang, Y., Wu, W. M., Zhao, J., and Jiang, L. (2014). Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ. Sci. Technol.* 48, 13776–13784. doi: 10.1021/es504038a

58. Bombelli, P., Howe, C. J., and Bertocchini, F. (2017). Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*. *Curr. Biol.* 27, R292–R293. doi: 10.1016/j.cub.2017.02.060

59. Brandon, A. M., Garcia, A., Khlystov, N. A., Wu, W., & Criddle, C. S. (2021). Enhanced Bioavailability and Microbial Biodegradation of Polystyrene in an Enrichment Derived from the Gut Microbiome of Tenebrio molitor (Mealworm Larvae). *Environmental Science & Technology*, *55*(3), 2027–2036. https://doi.org/10.1021/acs.est.0c04952

60. Gahleitner, M., & Paulik, C. (2017). Polypropylene and other polyolefins. In *Brydson's plastics materials* (pp. 279-309). Butterworth-Heinemann.

61. Cacciari, I., Quatrini, P., Zirletta, G., Mincione, E., Vinciguerra, V., Lupattelli, P., et al. (1993). Isotactic polypropylene biodegradation by a microbial community: physicochemical characterization of metabolites produced. *Appl. Environ. Microbiol.* 59, 3695–3700. doi: 10.1128/aem.59.11.3695-3700.1993

62. Jeon, H. J., and Kim, M. N. (2016). Isolation of mesophilic bacterium for biodegradation of polypropylene. *Int. Biodeter. Biodegr.* 115, 244–249. doi: 10.1016/j.ibiod.2016.08.025

63. Sielicki, M., Focht, D. D., & Martín, J. P. (1978). Microbial degradation of [14C]polystyrene and 1,3-diphenylbutane. *Canadian Journal of Microbiology*, *24*(7), 798–803. https://doi.org/10.1139/m78-134

64. Yang, S., Wu, W., Brandon, A. M., Fan, H., Receveur, J. P., Li, Y., Wang, Z., Fan, R., McClellan, R. L., Gao, S., Ning, D., Phillips, D., Peng, B., Wang, H., Cai, S., Li, P., Cai, W., Lee, D., Yang, J., . . . Criddle, C. S. (2018). Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae). *Chemosphere*, *212*, 262–271. https://doi.org/10.1016/j.chemosphere.2018.08.078

65. Yang, Y., Wang, J., and Xia, M. (2020). Biodegradation and mineralization of polystyrene by plastic-eating superworms *Zophobas atratus*. *Sci. Total Environ.* 708:135233. doi: 10.1016/j.scitotenv.2019.135233

66. Lou, Y., Ekaterina, P., Yang, S., Lu, B., Liu, B., Ren, N., Corvini, P. F., & Xing, D. (2020). Biodegradation of Polyethylene and Polystyrene by Greater Wax Moth Larvae (Galleria mellonella L.) and the Effect of Co-diet Supplementation on the Core Gut Microbiome. *Environmental Science & Technology*, *54*(5), 2821–2831. https://doi.org/10.1021/acs.est.9b07044

67. Nakamiya, K., Hashimoto, S., Ito, H., Edmonds, J. S., Yasuhara, A., and Morita, M. (2005). Microbial treatment of bis (2-ethylhexyl) phthalate in polyvinyl chloride with isolated bacteria. *J. Biosci. Bioeng.* 99, 115–119. doi: 10.1263/jbb.99.115

68. Latorre, I., Hwang, S., and Montalvo-Rodriguez, R. (2012). Isolation and molecular identification of landfill bacteria capable of growing on di-(2-ethylhexyl) phthalate and deteriorating PVC materials. *J. Environ. Health Sci. Part A* 47, 2254–2262. doi: 10.1080/10934529.2012.707549

69. Anwar, M. S., Kapri, A., Chaudhry, V., Mishra, A., Ansari, M. W., Souche, Y., et al. (2016). Response of indigenously developed bacterial consortia in progressive degradation of polyvinyl chloride. *Protoplasma* 253, 1023–1032. doi: 10.1007/ s00709- 015- 0855- 9

70. Zhang, Z., Peng, H., Yang, D., Zhang, G., Zhang, J., & Ju, F. (2022). Polyvinyl chloride degradation by a bacterium isolated from the gut of insect larvae. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-32903-y

71. Müller, R., Kleeberg, I., & Deckwer, W. (2001). Biodegradation of polyesters containing aromatic constituents. *Journal of Biotechnology*, *86*(2), 87–95. https://doi.org/10.1016/s0168-1656(00)00407-7

72. Mohanan, N., Montazer, Z., Sharma, P., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.580709

73. Benyathiar, P., Kumar, P., Carpenter, G. S., Brace, J. G., & Mishra, D. K. (2022). Polyethylene Terephthalate (PET) Bottle-to-Bottle Recycling for the Beverage industry: A review. *Polymers*, *14*(12), 2366. https://doi.org/10.3390/polym14122366

74. Marten, E., Müller, R., & Deckwer, W. (2003). Studies on the enzymatic hydrolysis of polyesters I. Low molecular mass model esters and aliphatic polyesters. *Polymer Degradation and Stability*, *80*(3), 485–501. https://doi.org/10.1016/s0141-3910(03)00032-6

75. Marten, E., Müller, R., & Deckwer, W. (2005). Studies on the enzymatic hydrolysis of polyesters. II. Aliphatic–aromatic copolyesters. *Polymer Degradation and Stability*, *88*(3), 371–381. https://doi.org/10.1016/j.polymdegradstab.2004.12.001

76. Müller, R. J., Schrader, H., Profe, J., Dresler, K., and Deckwer, W. D. (2005). Enzymatic degradation of poly (ethylene terephthalate): rapid hydrolyse using a hydrolase from *T. fusca*. *Macromol. Rapid Commun.* 26, 1400–1405. doi: 10.1002/marc.200500410

77. Kawai, F., Oda, M., Tamashiro, T., Waku, T., Tanaka, N., Yamamoto, M., et al. (2014). A novel Ca^{2+}-activated, thermostabilized polyesterase capable of hydrolyzing polyethylene terephthalate from *Saccharomonospora viridis* AHK190. *Appl. Microbiol. Biotechnol.* 98, 10053–10064. doi: 10.1007/s00253- 014- 5860- y

78. Taniguchi, I., Yoshida, S., Hiraga, K., Miyamoto, K., Kimura, Y., & Oda, K. (2019). Biodegradation of PET: current status and application aspects. *ACS Catalysis*, *9*(5), 4089–4105. https://doi.org/10.1021/acscatal.8b05171

79. Maity, W., Maity, S., Bera, S., & Roy, A. (2021). Emerging roles of PETASE and MHETASE in the biodegradation of plastic wastes. *Applied Biochemistry and Biotechnology*, *193*(8), 2699–2716. https://doi.org/10.1007/s12010-021-03562-4

80. Jansen, B., Schumacher-Perdreau, F., Peters, G., and Pulverer, G. (1991). Evidence for degradation of synthetic polyurethanes by *Staphylococcus epidermidis*. *Zentralbl. Bakteriol.* 276, 36–45. doi: 10.1016/s0934-8840(11)80216-1

81. Stepien, A. E., Zebrowski, J., Piszczyk, Ł., Boyko, V. V., Riabov, S. V., Dmitrieva, T., et al. (2017). Assessment of the impact of bacteria *Pseudomonas denitrificans*, *Pseudomonas fluorescens*, *Bacillus subtilis* and yeast *Yarrowia lipolytica* on commercial poly (ether urethanes). *Polym. Test.* 63, 484–493. doi: 10.1016/j. polymertesting.2017.08.038

82. Schmidt, J., Wei, R., Oeser, T., Dedavid, E., Silva, L. A., Breite, D., et al. (2017). Degradation of polyester polyurethane by bacterial polyester hydrolases. *Polymers* 9:65. doi: 10.3390/polym9020065

83. Gambarini, V., Pantos, O., Kingsbury, J. M., Weaver, L., Handley, K. M., & Lear, G. (2021). Phylogenetic distribution of Plastic-Degrading microorganisms. *MSystems*, *6*(1). https://doi.org/10.1128/msystems.01112-20

84. Priya, A., Dutta, K., & Daverey, A. (2021). A comprehensive biotechnological and molecular insight into plastic degradation by microbial community. *Journal of Chemical Technology and Biotechnology*, *97*(2), 381–390. https://doi.org/10.1002/jctb.6675

85. Gravouil, K., Ferru-Clément, R., Colas, S., Hélye, R., Kadri, L., Bourdeau, L., Moumen, B., Mercier, A., & Ferreira, T. (2017). Transcriptomics and Lipidomics of the Environmental Strain Rhodococcus ruber Point out Consumption Pathways and Potential Metabolic Bottlenecks for Polyethylene Degradation. *Environmental Science & Technology*, *51*(9), 5172–5181. https://doi.org/10.1021/acs.est.7b00846

86. Restrepo-Flórez, J., Bassi, A., & Thompson, M. R. (2014). Microbial degradation and deterioration of polyethylene – A review. *International Biodeterioration & Biodegradation*, *88*, 83–90. https://doi.org/10.1016/j.ibiod.2013.12.014

87. Lucas, N., Bienaimé, C., Belloy, C., Quéneudec, M., Silvestre, F., & Nava-Saucedo, J. (2008). Polymer biodegradation: Mechanisms and estimation techniques – A review. *Chemosphere*, *73*(4), 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064

88. Santo, M., Weitsman, R., and Sivan, A. (2013). The role of the copper-binding enzyme–laccase–in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*. *Int. Biodeter. Biodegr.* 84, 204–210. doi: 10.1016/j.ibiod. 2012.03.001

89. Sowmya, H. V., Ramalingappa, Krishnappa, M., & Thippeswamy, B. (2014). Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district. *Environment, Development and Sustainability*, *17*(4), 731–745. https://doi.org/10.1007/s10668-014-9571-4

90. Sowmya, H. V., Ramalingappa, Krishnappa, M., & Thippeswamy, B. (2014). Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district. *Environment, Development and Sustainability*, *17*(4), 731–745. https://doi.org/10.1007/s10668-014-9571-4

91. Lv, S., Li, Y., Zhao, S., & Shao, Z. (2024). Biodegradation of typical plastics: from microbial diversity to metabolic mechanisms. *International Journal of Molecular Sciences*, *25*(1), 593. https://doi.org/10.3390/ijms25010593

92. Zhang, Y., Pedersen, J. N., Eser, B. E., & Guo, Z. (2022b). Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery. *Biotechnology Advances*, *60*, 107991. https://doi.org/10.1016/j.biotechadv.2022.107991

93. Jeon, H. J., & Kim, M. N. (2015). Functional analysis of alkane hydroxylase system derived from Pseudomonas aeruginosa E7 for low molecular weight polyethylene biodegradation. *International Biodeterioration & Biodegradation*, *103*, 141–146. https://doi.org/10.1016/j.ibiod.2015.04.024

94. M. G., Jeon, H. J., & Kim, M. N. (2012). Biodegradation of polyethylene by a soil bacterium and ALKB cloned recombinant cell. *Journal of Bioremediation and Biodegradation*, *03*(04). https://doi.org/10.4172/2155-6199.1000145

95. Zadjelovic, V., Erni-Cassola, G., Obrador-Viel, T., Lester, D. W., Eley, Y., Gibson, M. I., Dorador, C., Golyshin, P. N., Black, S., Wellington, E. M. H., & Christie-Oleza, J. A. (2022b). A mechanistic understanding of polyethylene biodegradation by the marine bacterium Alcanivorax. *Journal of Hazardous Materials*, *436*, 129278. https://doi.org/10.1016/j.jhazmat.2022.129278

96. Hou, L., & Majumder, E. L. (2021). Potential for and distribution of enzymatic biodegradation of polystyrene by environmental microorganisms. *Materials*, *14*(3), 503. https://doi.org/10.3390/ma14030503

97. Mooney, A., Ward, P. G., & O'Connor, K. E. (2006). Microbial degradation of styrene: biochemistry, molecular genetics, and perspectives for biotechnological applications. *Applied Microbiology and Biotechnology*, *72*(1), 1–10. https://doi.org/10.1007/s00253-006-0443-1

98. Awasthi, S. K., Kumar, M., Kumar, V., Sarsaiya, S., Anerao, P., Ghosh, P., Singh, L., Liu, H., Zhang, Z., & Awasthi, M. K. (2022). A comprehensive review on recent advancements in biodegradation and sustainable management of biopolymers. *Environmental Pollution*, *307*, 119600. https://doi.org/10.1016/j.envpol.2022.119600

99. Arutchelvi, J., Sudhakar, M., Arkatkar, A. S., & Uppara, P. V. (2008). Biodegradation of polyethylene and polypropylene. *ResearchGate*. https://www.researchgate.net/publication/228090462

100. Müller, R., Schrader, H., Profe, J., Dresler, K., & Deckwer, W. (2005). Enzymatic Degradation of Poly(ethylene terephthalate): Rapid Hydrolyse using a Hydrolase from T. fusca. *Macromolecular Rapid Communications*, *26*(17), 1400–1405. https://doi.org/10.1002/marc.200500410

101. Barth, M., Oeser, T., Wei, R., Then, J., Schmidt, J., & Zimmermann, W. (2015). Effect of hydrolysis products on the enzymatic degradation of polyethylene terephthalate nanoparticles by a polyester hydrolase from Thermobifida fusca. *Biochemical Engineering Journal*, *93*, 222–228. https://doi.org/10.1016/j.bej.2014.10.012

102. Wei, R., Oeser, T., Then, J., Kühn, N., Barth, M., Schmidt, J., & Zimmermann, W. (2014). Functional characterization and structural modeling of synthetic polyester-degrading hydrolases from Thermomonospora curvata. *AMB Express*, *4*(1). https://doi.org/10.1186/s13568-014-0044-9

103. Yan, F., Wei, R., Cui, Q., Bornscheuer, U. T., & Liu, Y. (2020). Thermophilic whole-cell degradation of polyethylene terephthalate using engineered Clostridium thermocellum. *Microbial Biotechnology*, *14*(2), 374–385. https://doi.org/10.1111/1751-7915.13580

104. Li, W.-J., Jayakody, L. N., Franden, M. A., Wehrmann, M., Daun, T., Hauer, B., et al. (2019). Laboratory evolution reveals the metabolic and regulatory basis of ethylene glycol metabolism by *Pseudomonas putida* KT2440. *Environ. Microbiol.* 21, 3669–3682. doi: 10.1111/1462-2920.14703

105. Kim, N. K., Lee, S., & Park, H. (2022). Current biotechnologies on depolymerization of polyethylene terephthalate (PET) and repolymerization of reclaimed monomers from PET for bio-upcycling: A critical review. *Bioresource Technology*, *363*, 127931. https://doi.org/10.1016/j.biortech.2022.127931

106. Hosaka, M., Kamimura, N., Toribami, S., Mori, K., Kasai, D., Fukuda, M., & Masai, E. (2013). Novel Tripartite Aromatic Acid Transporter Essential for Terephthalate Uptake in Comamonas sp. Strain E6. *Applied and Environmental Microbiology*, *79*(19), 6148–6155. https://doi.org/10.1128/aem.01600-13

107. Wei, R., & Zimmermann, W. (2017). Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? *Microbial Biotechnology*, *10*(6), 1308–1322. https://doi.org/10.1111/1751-7915.12710

108. Ali, S. S., Elsamahy, T., Al-Tohamy, R., Zhu, D., Mahmoud, Y. A., Koutra, E., Metwally, M. A., Kornaros, M., & Sun, J. (2021). Plastic wastes biodegradation: Mechanisms, challenges and future prospects. *Science of the Total Environment*, *780*, 146590. https://doi.org/10.1016/j.scitotenv.2021.146590

109. Tamargo, A., Molinero, N., Reinosa, J., Alcolea-Rodriguez, V., Portela, R., Bañares, M. Á., Fernández, J., & Moreno-Arribas, M. V. (2022). PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-021-04489-w

110. Atanasova, N., Stoitsova, S., Paunova-Krasteva, T., & Kambourova, M. (2021). Plastic degradation by extremophilic bacteria. *International Journal of Molecular Sciences*, *22*(11), 5610. https://doi.org/10.3390/ijms22115610

111. Almeida, C. M. R., Reis, I., Couto, N., Bordalo, A. A., & Mucha, A. P. (2012). Potential of the microbial community present in an unimpacted beach sediment to remediate petroleum hydrocarbons. *Environmental Science and Pollution Research*, *20*(5), 3176–3184. https://doi.org/10.1007/s11356-012-1240-2

112. Priya, A., Sarma, P. M., & Lal, B. (2016). Isolation and characterization of Candida vishwanathii strain TERI MS1 for degradation of petroleum hydrocarbons in marine environment. *Desalination and Water Treatment*, *57*(46), 22099–22106. https://doi.org/10.1080/19443994.2015.1134351

113. Sánchez, C. (2021). Microbial capability for the degradation of chemical additives present in petroleum-based plastic products: A review on current status and perspectives. *Journal of Hazardous Materials*, *402*, 123534. https://doi.org/10.1016/j.jhazmat.2020.123534

114. Mohan, L., Cherian, E., & Joshi, T. J. (2022). Emerging issues and challenges for plastic bioremediation. In *Elsevier eBooks* (pp. 589–600). https://doi.org/10.1016/b978-0-323-90452-0.00010-4

115. Shahnawaz, M., Sangale, M. K., & Ade, A. B. (2019). Ex situ bioremediation technology for plastic degradation. In *Springer eBooks* (pp. 77–83). https://doi.org/10.1007/978-981-13-7492-0_7

116. Syranidou, E., Karkanorachaki, K., Amorotti, F., Avgeropoulos, A., Kolvenbach, B. A., Zhou, N., Fava, F., Corvini, P. F., & Kalogerakis, N. (2019). Biodegradation of mixture of plastic films by tailored marine consortia. *Journal of Hazardous Materials*, *375*, 33–42. https://doi.org/10.1016/j.jhazmat.2019.04.078

117. Son, H. F., Cho, I. J., Joo, S., Seo, H., Sagong, H., Choi, S. Y., Lee, S. Y., & Kim, K. (2019). Rational Protein Engineering of Thermo-Stable PETase from Ideonella sakaiensis for Highly Efficient PET Degradation. *ACS Catalysis*, *9*(4), 3519–3526. https://doi.org/10.1021/acscatal.9b00568

118. Wei, R., Oeser, T., Schmidt, J., Meier, R., Barth, M., Then, J., & Zimmermann, W. (2016). Engineered bacterial polyester hydrolases efficiently degrade polyethylene terephthalate due to relieved product inhibition. *Biotechnology and Bioengineering*, *113*(8), 1658–1665. https://doi.org/10.1002/bit.25941

119. Ma, Y., Yao, M., Li, B., Ding, M., He, B., Chen, S., Zhou, X. D., & Yuan, Y. (2018). Enhanced Poly(ethylene terephthalate) Hydrolase Activity by Protein Engineering. *Engineering*, *4*(6), 888–893. https://doi.org/10.1016/j.eng.2018.09.007

120. Muriel-Millán, L. F., Millán-López, S., & Pardo-López, L. (2021). Biotechnological applications of marine bacteria in bioremediation of environments polluted with hydrocarbons and plastics. *Applied Microbiology and Biotechnology*, *105*(19), 7171–7185. https://doi.org/10.1007/s00253-021-11569-4

121. Verschoor, J., Kusumawardhani, H., Ram, A. F. J., & De Winde, J. H. (2022). Toward Microbial Recycling and upcycling of Plastics: Prospects and challenges. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.821629

122. Berini, F., Casciello, C., Marcone, G. L., & Marinelli, F. (2017). Metagenomics: novel enzymes from non-culturable microbes. *FEMS Microbiology Letters*, *364*(21). https://doi.org/10.1093/femsle/fnx211

123. Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Sil- vestre, F., and Nava-Saucedo, J.-E. (2008) Polymer biodegradation: mechanisms and estimation techniques - a review. Chemosphere 73: 429–442.

124. Gambarini, V., Pantos, O., Kingsbury, J. M., Weaver, L., Handley, K. M., & Lear, G. (2022b). PlasticDB: a database of microorganisms and proteins linked to plastic biodegradation. *Database*, *2022*. https://doi.org/10.1093/database/baac008

125. Montazer, Z., Habibi Najafi, M. B., and Levin, D. B. (2019). Microbial degradation of low-density polyethylene and synthesis of polyhydroxyalkanoate polymers. *Can. J. Microbiol.* 65, 1–11. doi: 10.1139/cjm-2018-0335

126. Yoon, M. G., Jeon, H. J., and Kim, M. N. (2012). Biodegradation of polyethylene by a soil bacterium and *AlkB* cloned recombinant cell. *J. Bioremediat. Biodegrad.* 3:145.

127. Sowmya, H. V., Ramalingappa, Krishnappa, M., & Thippeswamy, B. (2014b). Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district. *Environment, Development and Sustainability*, *17*(4), 731–745. https://doi.org/10.1007/s10668-014-9571-4

128. Nakamiya, K., Sakasita, G., Ooi, T., & Kinoshita, S. (1997). Enzymatic degradation of polystyrene by hydroquinone peroxidase of Azotobacter beijerinckii HM121. *Journal of Fermentation and Bioengineering*, *84*(5), 480–482. https://doi.org/10.1016/s0922-338x(97)82013-2

129. Yang, Y., Yang, J., Wu, W. M., Zhao, J., Song, Y., Gao, L., et al. (2015c). Biodegradation and mineralization of polystyrene by plastic-eating mealworms. 2. role of gut microorganisms. *Environ. Sci. Technol.* 49, 12087–12093. doi: 10.1021/acs.est.5b02663

130. Atiq, N., Garba, A., Ali, M. I., Andleeb, S., Khan, N. A., and Robson, G. D. (2010). Isolation and identification of polystyrene biodegrading bacteria from soil. *Afr. J. Microbiol. Res.* 4, 1537–1541.

131. Kumari, A., Chaudhary, D. R., and Jha, B. (2019). Destabilization of polyethylene and polyvinylchloride structure by marine bacterial strain. *Environ. Sci. Pollut. Res. Int.* 26, 1507–1516. doi: 10.1007/s11356-018-3465-1

132. Nakamiya, K., Hashimoto, S., Ito, H., Edmonds, J. S., Yasuhara, A., and Morita, M. (2005). Microbial treatment of bis (2-ethylhexyl) phthalate in polyvinyl chloride with isolated bacteria. *J. Biosci. Bioeng.* 99, 115–119. doi: 10.1263/jbb.99.115

133. Ronkvist, Å. M., Xie, W., Lu, W., and Gross, R. A. (2009). Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate). *Macromolecules* 42, 5128–5138. doi: 10.1021/ma9005318

134. Nakajima-Kambe, T., Onuma, F., Kimpara, N., and Nakahara, T. (1995). Isolation and characterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source. *FEMS Microbiol. Lett.* 129, 39–42. doi: 10.1111/j.1574-6968.1995.tb07554.x

135. Shah, Z. A., Gulzar, M., Hasan, F., & Shah, A. A. (2016). Degradation of polyester polyurethane by an indigenously developed consortium of Pseudomonas and Bacillus species isolated from soil. *Polymer Degradation and Stability*, *134*, 349–356. https://doi.org/10.1016/j.polymdegradstab.2016.11.003

136. Bryant, J. A., Clemente, T. M., Viviani, D. A., Fong, A. A., Thomas, K. A., Kemp, P. F., Karl, D. M., White, A., & DeLong, E. F. (2016). Diversity and activity of communities inhabiting plastic debris in the North Pacific gyre. *MSystems*, *1*(3). https://doi.org/10.1128/msystems.00024-16

137. Ogonowski, M., Motiei, A., Ininbergs, K., Hell, É., Gerdes, Z., Udekwu, K. I., Bacsik, Z., & Gorokhova, E. (2018). Evidence for selective bacterial community structuring on microplastics. *Environmental Microbiology*, *20*(8), 2796–2808. https://doi.org/10.1111/1462-2920.14120

138. Jeon, H. J., and Kim, M. N. (2016a). Comparison of the functional characterization between alkane monooxygenases for low-molecular-weight polyethylene biodegradation. *Int. Biodeter. Biodegr.* 114, 202–208. doi: 10.1016/j.ibiod.2016. 06.012

139. Wei, R., Breite, D., Song, C., Gräsing, D., Ploss, T., Hille, P., et al. (2019a). Biocatalytic degradation efficiency of postconsumer polyethylene terephthalate packaging determined by their polymer microstructures. *Adv. Sci.* 6:1900491. doi: 10.1002/advs.201900491

140. Akutsu, Y., Nakajima-Kambe, T., Nomura, N., & Nakahara, T. (1998). Purification and Properties of a Polyester Polyurethane-Degrading Enzyme from Comamonas acidovorans TB-35. *Applied and Environmental Microbiology*, *64*(1), 62–67. https://doi.org/10.1128/aem.64.1.62-67.1998

141. Ruiz, C., Main, T., Hilliard, N. P., and Howard, G. T. (1999). Purification and characterization of two polyurethanase enzymes from *Pseudomonas chlororaphis*. *Int. Biodeter. Biodegr.* 43, 43–47. doi: 10.1016/s0964-8305(98) 00067- 5

142. Stern, R. V., and Howard, G. T. (2000). The polyester polyurethanase gene (pueA) from *Pseudomonas chlororaphis* encodes a lipase. *FEMS Microbiol. Lett.* 185, 163–168. doi: 10.1111/j.1574-6968.2000.tb09056.x

143. Howard, G. T., Crother, B., and Vicknair, J. (2001b). Cloning, nucleotide sequencing and characterization of a polyurethanase gene (pueB) from *Pseudomonas chlororaphis*. *Int. Biodeterior. Biodegrad.* 47, 141–149. doi: 10.1016/s0964- 8305(01)00042- 7

144. Zhai, X., Zhang, X., & Yu, M. (2023). Microbial colonization and degradation of marine microplastics in the plastisphere: A review. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1127308

145. Urbanek, A. K., Rymowicz, W., & Mirończuk, A. M. (2018). Degradation of plastics and plastic-degrading bacteria in cold marine habitats. *Applied Microbiology and Biotechnology*, *102*(18), 7669–7678. https://doi.org/10.1007/s00253-018-9195-y

146. Vaksmaa, A., Egger, M., Lüke, C., Martins, P. D., Rosselli, R., Asbun, A. A., & Niemann, H. (2022). Microbial communities on plastic particles in surface waters differ from subsurface waters of the North Pacific Subtropical Gyre. *Marine Pollution Bulletin*, *182*, 113949. https://doi.org/10.1016/j.marpolbul.2022.113949

147. Amobonye A., Bhagwat P., Singh S., Pillai S. (2021). Plastic biodegradation: frontline microbes and their enzymes. Sci. Total Environ. 759:143536. doi: 10.1016/j.scitotenv.2020.143536

148. Ruiz, C., and Howard, G. T. (1999). Nucleotide sequencing of a polyurethanase gene (pulA) from *Pseudomonas fluorescens*. *Int. Biodeter. Biodegr.* 44, 127–131. doi: 10.1016/s0964-8305(99)00074-8

149. Bollinger, A., Thies, S., Knieps-Grünhagen, E., Gertzen, C., Kobus, S., Höppner, A., Ferrer, M., Gohlke, H., Smits, S. H. J., & Jaeger, K. (2020). A Novel Polyester Hydrolase From the Marine Bacterium Pseudomonas aestusnigri – Structural and Functional Insights. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00114

150. Carr, C. M., Clarke, D. J., & Dobson, A. D. W. (2020). Microbial Polyethylene Terephthalate Hydrolases: Current and future Perspectives. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.571265

151. Acero, E. H., Ribitsch, D., Steinkellner, G., Gruber, K., Greimel, K., Eiteljoerg, I., Trotscha, E., Wei, R., Zimmermann, W., Zinn, M., Cavaco-Paulo, A., Freddi, G., Schwab, H., & Guebitz, G. (2011). Enzymatic Surface Hydrolysis of PET: Effect of Structural Diversity on Kinetic Properties of Cutinases from Thermobifida. *Macromolecules*, *44*(12), 4632–4640. https://doi.org/10.1021/ma200949p

152. Danso, D., Schmeisser, C., Chow, J., Zimmermann, W., Wei, R., Leggewie, C., Li, X., Hazen, T., & Streit, W. R. (2018). New Insights into the Function and Global Distribution of Polyethylene Terephthalate (PET)-Degrading Bacteria and Enzymes in Marine and Terrestrial Metagenomes. *Applied and Environmental Microbiology*, *84*(8). https://doi.org/10.1128/aem.02773-17

153. *Information on EC 3.1.1.101 - poly(ethylene terephthalate) hydrolase and Organism(s) Ideonella sakaiensis and UniProt Accession A0A0K8P6T7 - BRENDA Enzyme Database*. (n.d.). https://www.brenda-enzymes.org/enzyme.php?ecno=3.1.1.101&organism%5b%5d=Ideonella+sakaiensis&showtm=0&UniProtAcc=A0A0K8P6T7&onlyTable=Reference

154. Zadjelovic, V., Gibson, M., Dorador, C., & Christie-Oleza, J. (2020). Genome of Alcanivorax sp. 24: A hydrocarbon degrading bacterium isolated from marine plastic debris. *Marine Genomics*, *49*, 100686. https://doi.org/10.1016/j.margen.2019.05.001

155. Bardají, D. K. R., Furlan, J. P. R., & Stehling, E. G. (2019). Isolation of a polyethylene degrading Paenibacillus sp. from a landfill in Brazil. *Archives of Microbiology*, *201*(5), 699–704. https://doi.org/10.1007/s00203-019-01637-9