Enzymatic Degradation of Plastic

Recent Methods, Applications, and Solutions for the Recycling of Polymers

Plastic is everywhere: researchers have been able to detect it on Mount Everest [1] as well as in the Mariana Trench [2]. Particularly alarming was the recently published study that found microplastics even in human blood [3]. In order to solve this global problem, research is being conducted into ways of degrading polymer materials efficiently. In addition to chemical and physical methods, the enzymatic degradation of plastics in particular has become the focus of interest for many research groups. In the following, we present current research articles that provide promising approaches for possible applications. In addition, you will learn about the potential of the enzymatic plastic degradation in short interviews with thought leaders in the field.
You can also learn more about the solutions provided by Sartorius that are helping to enable this promising research on page 18.

References
[1] I. E. Napper et al., One Earth (2020) 3, 621–630.
[2] X. Peng et al., Geochem. Persp. Let. (2018) 9, 1–5.
[3] H. A. Leslie et al., Environment International (2022) 163, 107199.

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Wiley Analytical Science

Plastic Pollution: A Global Challenge

Current Microplastics Research at a Glance

Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia

While microplastics have been detected in numerous biotic and abiotic environments, less is known about their presence in groundwater. Recently, Australian researchers reported on the analysis of the eight most commonly found polymers in samples from capped groundwater monitoring bores. Microplastics were detected in all samples, with PE, PP, PS and PVC detected in all seven bores.

Full article: S. Samandra *et al.:* Science of The Total Environment, 802, 2022; DOI: 10.1016/j.scitotenv.2021.149727.

First documentation of plastic ingestion in the arctic glaucous gull *(Larus hyperboreus)*

Researchers from Norway and Finland investigated the occurrence of plastics in glaucous gulls, which is a sentinel species for the health of the arctic marine ecosystem. They found a frequency of occurrence of 14.3% (n = 21) of microplastic particles, with all plastics being identified as user plastics and consisted of polypropylene (PP) and polystyrene (PS).

Full open access article: S. C. Benjaminsen *et al.:* First documentation of plastic ingestion in the arctic glaucous gull (Larus hyperboreus), Science of The Total Environment, 834, 2022; DOI: 10.1016/j.scitotenv.2022.155340.

Microplastic contamination of the drilling bivalve *Hiatella arctica* in Arctic rhodolith beds

More and more studies are reporting the detection of microplastics in the Arctic. Researchers from Germany were able to confirm these findings: in their current research article, they report on the analysis of samples from hollow rhodoliths gouged by the bivalve Hiatella arctica. They showed that 100% of the examined specimens were contaminated with microplastics.

Full open access article: S. Teichert *et al.:* Microplastic contamination of the drilling bivalve Hiatella arctica in Arctic rhodolith beds, Scientific Reports, 11:14574, 2022; DOI: 10.1038/s41598-021-93668-w.

A comprehensive biotechnological and molecular insight into plastic degradation by microbial community

Introduction

Plastics, one of the most extensively consumed materials in human history, have polluted this world more after the COVID-19 pandemic owing to the vast amount of rejected PPEs. Both PE and PP jointly hold ~54% of different plastic usage, whereas PVC (~14%) and PET (~8%) hold the second and third places respectively. Around 90% of these plastics contribute to the environmental pollution at least 500 years after disposal, which leads to barren lands, the release of toxic and hazardous fumes into the atmosphere, choking and death of aquatic animals, transportation of microplastic particles via air and water circulation throughout the world, and ecotoxicity due to the leaching of various toxic and carcinogenic chemicals from rejected plastics.

People, becoming more aware of these adverse effects, made several attempts toward plastic waste treatment, such as the promotion of plastic recycling, the manufacturing of bitumen modifiers from plastics in road construction, liquid fuel production from plastic waste using thermochemical biomass conversion technologies, and many more as illustrated in **Figure 1**. Biodegradation is an eco-friendly solution to this plastic waste management. Hydrophobicity, high molecular weight, and long-chain polymer structure make the plastic typically unfavorable for biodegradation. But some specialized marine microbes (bacteria such as *Arthrobacter, Bacillus, Micrococcus, Pseudomonas, Corynebacterium, Streptomyces, and Nocardia, and fungi such as Fusarium spp., Aspergillus spp., and Penicillium spp.*) have the po-

tential to disrupt the structure and plastic's polymeric chain via physicochemical reactions and thus to use them as a carbon source. Biodegradation is also facilitated by enzymes (such as oxygenases, dehydrogenases, lipases, and esterases) promoting the oxidation and fragmentation of polymers. This review paper documents different biotechnological and molecular pathways in microbial plastic degradation along with the identification of current knowledge gaps, future challenges, technologies, and key research areas.

Microbial degradation of petrochemical plastic variants

The effective polymerization of elements like C, H, O, N, Cl, and S present in the plastic provides high strength, durability, and stability. Various microorganisms
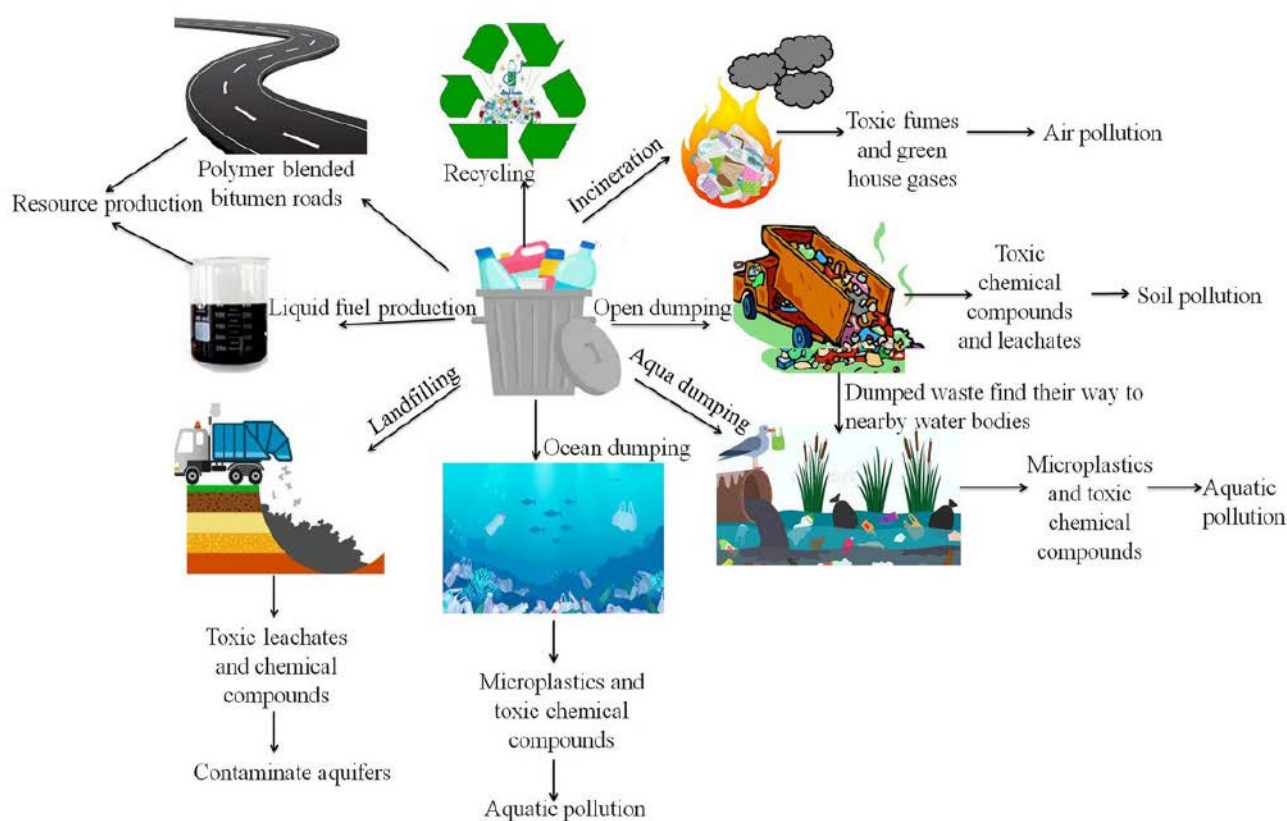

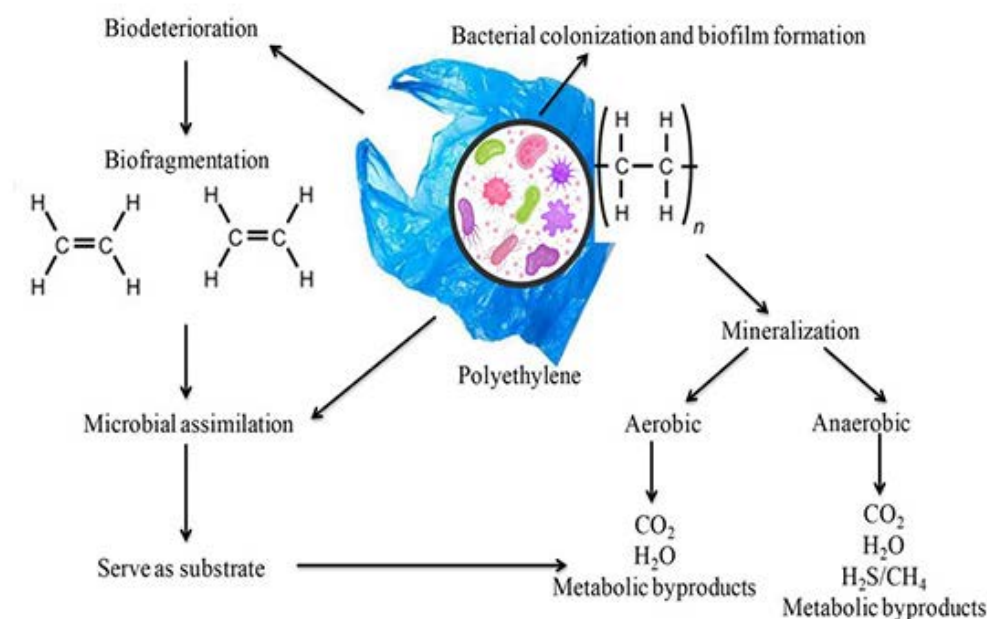**Fig. 1**: Fate of plastic waste in environment.

Fig. 2: Schematic representation of mechanism involved in biodegradation of plastic.

and their enzymes can slow down this polymerization process, for example, PE can be degraded by various bacteria and fungi such as *Pseudomonas, Staphylococcus, Streptomyces, Rhodococcus, Aspergillus, Penicillium, Cladosporium, Rhodococcus ruber, Brevibacillus borstelensis, Streptomyces spp., Bacillus cereus, Penicillium simplicissimum,* and *Aspergillus niger.* The PE degradation rate can be further increased via several photo-oxidation and chemical treatments often observed in different bacterial and microbial consortiums. The PET degradation rate depends on the presence of actinomycetes and enzymes, whereas numerous bacterial strains promote the PA, PS, and PVC degradation rate through biofilm formation on the polymer surfaces.

Mechanism of biodegradation

Several studies have documented the role of microorganisms, enzymes, and metabolites from different bacteria and fungi in plastic degradation, but they still deserve further attention for the identification of novel microbes and enzymes and the clarification of metabolic pathways for efficient and fast degradation. Either these biocatalysts use the plastics directly as a source of nutrients or they efficiently catalyze the

polymers into carbon dioxide and water. As the microbes attach and colonize on the polymer surfaces, the microbial biodegradation mechanism starts and then degrades the polymer into low molecular weight oligomers, dimmers, and monomers, which are eventually transformed into CO_2, and H_2O. **Figure 2** shows the aerobic and anaerobic mechanisms in plastic biodegradation, where oxygen molecules serve as electron acceptors during aerobic biodegradation, and inorganic materials such as sulfate, nitrate, and manganese act as electron acceptors during anaerobic biodegradation. The final degradation products are CO_2, H_2O, and microbial mass in aerobic biodegradation, but CH_4, CO_2, and H_2O in anaerobic biodegradation.

As biodegradation is an inherently slow process, hence optimization of the governing factors is necessary to enhance the degradation rate. The key process parameters are microbes, pH level, temperature, characteristics of polymer substrate, and surfactants. Plastic biodegradation is heavily influenced by the microbial consortium (including community, composition, and colonization), biofilm formation, enzymatic makeup, adaptability, and tolerance to the substrate. Also, the degradation

byproducts such as dissolved organic carbon and chemical leachates affect the growth and rate of biodegradation. The microbial growth is directly related to the pH of the substrate, which influences hydrolysis reaction rates by modifying the acidic or basic conditions, thus regulating biodegradation. At the higher pH of a substrate, biodegradation is increased and is further influenced by the temperature. Most mesophiles operate at an optimum temperature range of 25–30°C, whereas the polymer's softening temperature inversely influences enzymatic activity. Typically, polymers with high melting points are less susceptible to biodegradation and vice-versa.

Molecular weight, shape, size, and composition of the polymer substrate further influence biodegradation. Molecular weight is inversely related to degradability, whereas polymer composition (structural complexity, number of carbon atoms, and degree of polymerization) is coherently linked to biodegradability. Biodegradation is higher for a large surface area than for a smaller counterpart. Amphiphilic compounds, such as chemical surfactants or biosurfactants, promote biodegradation by increasing the surface area of hydrophobic water-soluble substances. The

amphiphilic nature of biosurfactants endorses the microorganism attachment on the hydrophobic polymer surfaces and thereby using them as a nutrient source. This has been reported for incubation of LDPE in 0.5% non-ionic surfactant and different degradation rates (3–6%) of HDPE and LDPE in freshwater, brackish water, and ocean water. Particularly, cell surface hydrophobicity is increased by the non-ionic surfactants, which in turn enhances biofilm formation and initiates biodegradation.

Influence of genes, enzymes, and metabolic pathways on biodegradation

Plastic oxidation increases the polymer hydrophobicity and thereby promotes microbial colonization, thus playing a major role in biodegradation. For example, PE biodegradation is influenced by copper-binding enzyme laccase produced by actinomycete Rhodococcus ruber and Aspergillus flavus. The PE metabolism involves acetyl-CoA and succinyl-CoA in the TCA cycle, followed by the production of NADH compounds. This chemical energy is used in ATP production via the respiratory chain, along with CO_2 and H_2O, marking the complete mineralization of PE. Specialized Alkane hydroxylase (alkB) genes in Pseudomonas spp. E4 strain can achieve 28.6% organic carbon degradation of PE in 80 days and show 19.3% mineralization potential of PE organic carbon in *Escherichia coli* BL21 strain. Similar to enzymes, hydrolases also facilitate plastic biodegradation. Bacteria I. sakaiensis 201-F6 produce PETase and MHETase when adhering to the PET substrate and degrade the PET into simple compounds such as TPA, MHET, and BHET. Further hydrolases catabolize MHET into TPA and ethylene glycol, and then TPA initially into PCA, and eventually 2-pyrone-4,6-dicarboxylic acid. This transforms into pyruvate and oxaloacetate in a TCA cycle and further gets assimilated as CO_2, and H_2O as schematically shown in **Figure 3**. Other potential PET degraders with enzymes (such as polyesterase cutinase and hydrolase) are Humicola insolens, Humicola cutinases, Penicillium citrinum, and Fusarium oxysporum fungi. PET esterases (such as metagenome-derived esterases MGS0156, and GEN0105) also mediate the hydrolysis of compounds like bis(benzoyloxyethyl)-terephthalate and polycaprolactone.

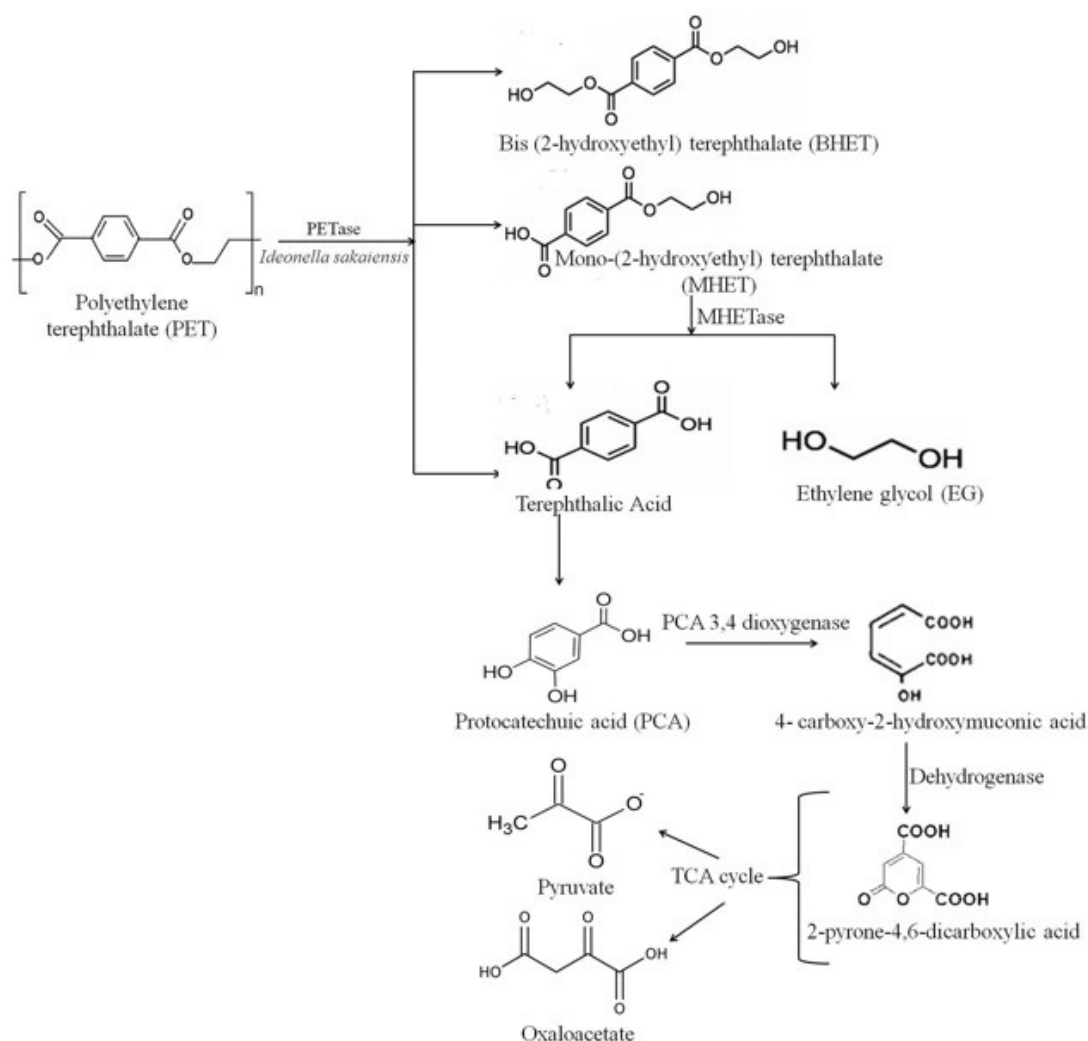The oxidation of *P. putida F1, PS,* composed of styrene monomers, has several steps, which initiates with

Fig. 3: Enzymatic reactions involved in biodegradation of PET.

styrene epoxide with enzymes styrene monooxygenase, followed by further oxidation of styrene epoxide to phenylacetaldehyde, which is then catabolized into PAA. In the lower pathway of styrene metabolism, PAA is transformed to phenylacetyl-CoA, which enters TCA cycle in the form of acetyl-CoA and succinyl-CoA after a series of enzymatic reactions. *P. putida CA-3* demonstrates a specialized pathway Phenylacetyl-CoA catabolon, which utilizes the activity of catabolic operon and assists its growth on styrene, facilitating the PS biodegradation to medium-chain length polyhydroxyalkanoates. Synthetic polymer PUR, another petrochemical variant, is also degraded by the activity of bacteria like *P. chlororaphis, P. protegens, P. putida,* and *Comamonas acidovorans TB-35* and fungi like *Fusarium solani, Candida rugosa, Aspergillus fumigates, Candida ethanolica,* and *Penicillium chrysogenum* with the help of enzymes like lipase, hydrolases, and esterases. Microbes like *Bacillus cereus, Vibrio furnisii, Bacillus sphaericus, Anoxybacillus rupiensis, Bacil-*

lus subtilis, and *Brevundimonas vesicularis* degrade the synthetic PA, widely used in textiles, carpets, and sportswear. **Figure 4** schematically represents these vital microbes and enzymes involved in plastic degradation.

Biotechnological developments in plastic biodegradation and future perspective

The metabolic versatility of the microorganisms capable of degrading synthetic polymers is an alternative to the chemical and physical depolymerization methods. Recent advancements in synthetic biology and metabolic engineering have promoted the development of engineered microbial strains for safe, eco-friendly plastic waste degradation and recycling. For example, a native marine bacterium can be transformed into a hydrocarbon degrader via gene transformation or recalcitrant plastics can be degraded via independent or combined engineered microbial degraders. Similar genetic approaches can be strategically used to merge the functions of genes

and enzymes facilitating plastic degradation, which includes genome manipulation, recombinant gene expression, and protein engineering. Using systems metabolic engineering (SysME), microbes with optimized cellular performance can be developed to achieve plastic degradation and this can be enhanced by different gene-editing tools and approaches (such as Zinc finger proteins, TALENs, CRISPR/Cas9) along with genes encoding enzymes (such as esterase, PETase, depolymerase, and laccase) for plastic degradation in a non-degrading microbe. These engineered strains enhance the degradation as compared to the natural microbes, which is evident in an engineered enzyme cutinase reducing PUR degradation time from 41.8 to 6.2 hours, as compared to wild-type cutinase. However, biodegradation is extensively dependent on the polymer's chemical structure and the degrading microbe's metabolic and enzymatic system, thus most microbes have shown disappointing field results as compared to the laboratory conditions. Biodegra-
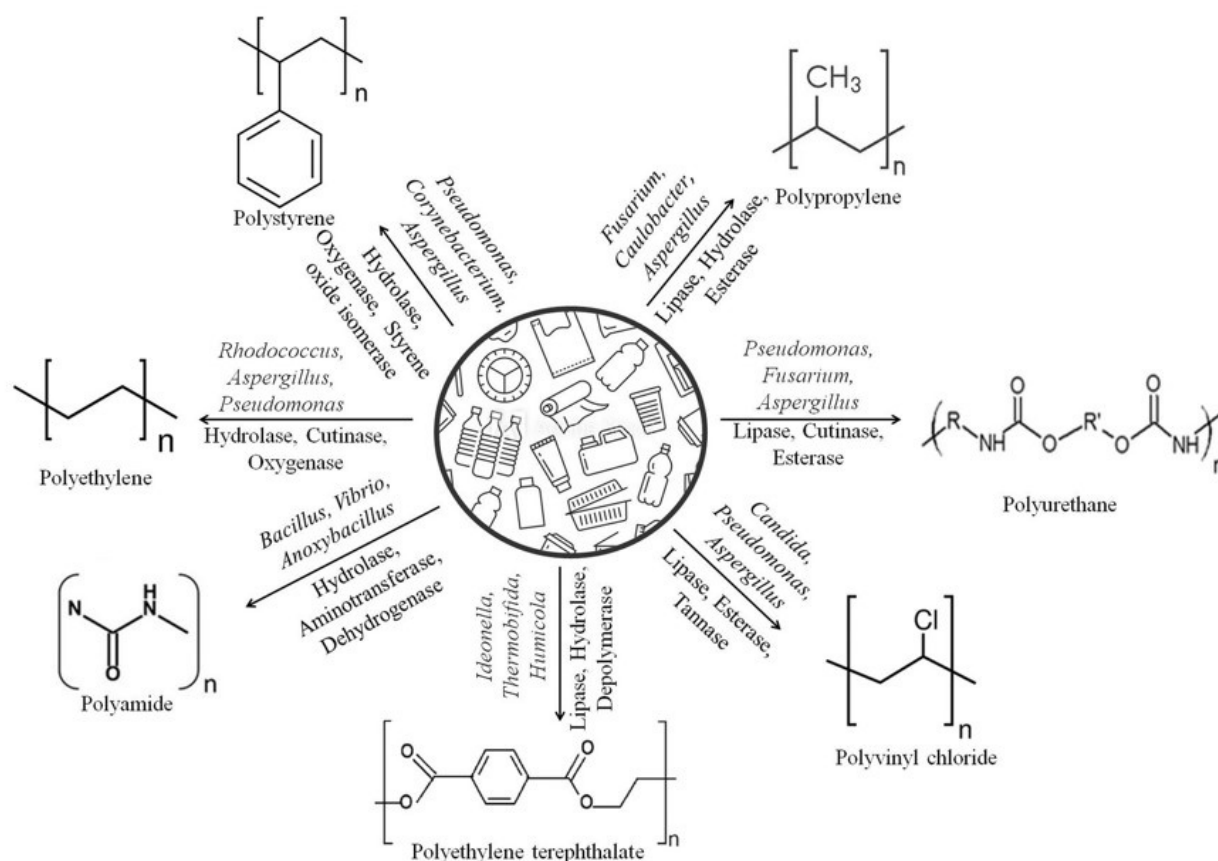

Fig. 4: Major microbes and enzymes involved in degradation of petrochemical plastic variants.

dation pathway prediction systems and chemical toxicity prediction systems have been developed to assess biodegradation, and much useful information related to metabolic pathways, genes, microbes, enzymes, and multistep enzymatic reactions promoting biodegradation have been included in the UM-BBD, MetaCyc, and BioCyc databases. These databases are useful in analyzing and identifying the degrading enzymes and predicting the degradation pathways of little-known toxicants. However, the unavailability of experimental validation is the main limitation of the pathway prediction systems. In addition, current knowledge gaps include the diversity of synthetic polymer degrading microbes and enzymes, tailoring of the metabolic pathway of the synthetic polymers, and characterization and identification of novel polymer degraders with high degradation potential. These topics need more research attention in the future, where a combined approach of bioinformatics, metabolic engineering, system biology, and genetic and molecular techniques may provide innovative insight into plastic biodegradation.

Conclusion

The toxicity and tenacity of the petrochemical plastics to remain stable after disposal causes a significant threat to the environment, and is further damaged by the conventional waste disposal methods. Thermal and catalytic pyrolysis are some potential waste management techniques that convert plastics to oil and green fuel, but the high energy demand, high production cost, reuse, and limitations of catalyst regeneration reduce the process sustainability. Under appropriate conditions, several microorganisms from the bacterial, fungal, and actinomycetes domains act as potential plastic degraders. Microbes with catalyzing capability for plastic degradation with the help of enzymes (such as hydrolases, esterases, lipases, and tannases) are an eco-friendly alternative to physicochemical depolymerization methods but suffer from the inherently slow biodegradation rate. Genetic manipulation, synthetic biology, metabolic engineering, and bioinformatics are the potential approaches for increased biodegradation rates. The biotransformation and reuse of plastic wastes promote a circular economy owing to reduced environmental pollution and economic development. Available literature indicates a knowledge gap regarding the microbes and the plastic-substrate interactions, the diversity of synthetic polymer degrading microbes and enzymes, and the experimental validation requirement of the dynamics of polymer biodegradation as confirmed by the bioinformatics prediction systems to determine the exact fate of plastic pollutants in nature. Future research is essential to fill these knowledge gaps toward efficient and effective plastic waste biodegradation via different biotechnological tools and approaches.

Abbreviations

BHET Bis-(2-hydroxyethyl)-terephthalate

HDPE High-density polyethylene

LDPE Low-density polyethylene

MHET Mono-(2-hydroxyethyl)-terephthalate

PA Polyamides

PAA Phenylacetic acid

PCA Protocatechuic acid

PE Polyethylene

PET Polyethylene terephthalate

PP Polypropylene

PPE Personal protective equipment

PS Polystyrene

PUR Polyurethane

PVC Polyvinyl chloride

TCA Tricarboxylic acid

TPA Terephthalic acid

Understanding consequences and tradeoffs of melt processing as a pretreatment for enzymatic depolymerization of poly(ethylene terephthalate)

Introduction

The enzymatic recycling of polymers, which was initially studied on naturally degradable ones such as poly(ethylene glycol), poly(vinyl alcohol), and poly(lactic acid), has received more attention nowadays because of their lower energy cost and eco-friendly recycling methods as compared to the current chemical recycling processes. For example, mechanical recycling of poly(ethylene terephthalate) (PET) involves grinding, washing, melting, molecular weight, and colour modification of PET wastes for a maximum of six times eventually leading to ~25% of relatively uncontaminated post-consumer and post-industrial scraps from rigid packaging (i.e. bottles and thermoformed containers). On the other hand, enzymatic recycling is more tolerant of mixed wastes and contaminants and produces chemically identical feedstocks for repolymerization. This is very similar to chemical recycling showing a higher yield (>90%) at ~70ºC, but an order of magnitude lower depolymerization rate (~10 hours at best). Despite that, advancements in enzyme engineering and substrate modification have led to open new windows to explore enzymatic recycling.

Enzymatic recycling of PET has been successfully achieved via enzymes like PETase, TfCut2, and leaf branch compost cutinase (LCC), and among them, LCC can readily hydrolyze amorphous PET to produce monomer products including terephthalic acid (TPA). *Ideonella sakaiensis* is a hydrolyzing enzyme secreting PETase and MHETase enzymes, that work together to depolymerize PET into its monomeric components. The PETase and MHETase enzyme structures are similar in their α/β-hydrolase fold to cutinases and lipases, and subtle variations in these enzymes' folding structures impact their ability to bind and depolymerize PET. A molecular dynamics simulation of the folding structure and binding capabilities of PETase indicates a preference for PETase to bind to labile carbonyls of substrates at room temperature. In addition, improved activity and thermostability of engineered LCC enzymes (at 72–75ºC with 2–3 $mg_{LCC}g_{PET}^{-1}$ concentrations) can improve enzymatic depolymerization of PET at a minimum of 90% conversion over 10 hours. Comparatively less effort has been put into the optimal preparation of substrates for enzymatic deconstruction, rather it has been mainly hypothesized and shown in some studies that the crystalline polymers degrade much slower than amorphous ones when subjected to enzymatic depolymerization. Besides this, particles with higher specific surface area (SSA) in the enzymatic depolymerization of biomass experience faster depolymerization in heterogeneous systems. In most experimental systems, amorphous PET powders, PET fibers, or PET bottle flakes have been used as target substrates but lack details on how substrates' form factors can affect depolymerization rates.

The processing techniques, such as twin-screw extrusion and grinding/milling, allow controllable variation in polymer properties including molecular weight, crystallinity, viscosity, and SSA prior to enzymatic recycling and eventually accelerating the depolymerization rates. In this study, consequences and energy tradeoffs of pretreating postconsumer recycled bottle-grade PET (RPET) before enzymatic depolymerization with an unpurified LCC solution to obtain TPA has been reported. The as-received RPET flakes contain <1 wt% of plastic and paper-label scraps as well as coloured plastic-bit impurities. The use of an unpurified enzyme cocktail reveals the potential for removing costly enzyme purification steps in scaled-up activities. Thermal property measurement via differential scanning calorimeter indicates a reduced crystallinity and increased SSA of extruded RPET (ex-RPET) compared to as-received flakes for current processing techniques. A simple energy balance is used to determine the required energy for the heating of extruded and pre-treated samples in a reaction medium within 25–65°C for specific energy consumption measurement. The energy and material property tradeoffs are then compared between mechanically pre-exposed RPET and enzymatic natural bindable surface areas for depolymerization.

Discussion on SSA

RPET flakes have been melt-processed at 9 g.min⁻¹ feed rate, 200 RPM screw speed twin-screw extruder through a single strand die head into a chilled water bath, producing cylindrical strands of ex-RPET (~0.5 mm diameter) with a modified thermal history, chain structure, and molecular weight. Exposure of variable length ex-RPET strands to enzymatic depolymerization and analysis of depolymerization behavior show the influence of modifying substrate SSA. Different SSA's of 4.3±0.74, 4.4±0.73, and 5.6±0.19 mm² mg⁻¹ are achieved by preparing ex-RPET strands of 200 mm (1 piece), 20 mm (10 pieces), and 3 mm (67 pieces) respectively. For a constant mass, sectioning of additional strands creates new strand-ends and thus increasing SSA while keeping the crystallinity and other morphological features almost unchanged. **Figure 1** summarizes the time-dependent enzymatic depolymerization of these ex-RPETs at a substrate concentration of 2 gL⁻¹ and enzyme concentrations of 30 nm. The product yield shows significant variability within the lowest SSA substrates

(1×200 mm and 10×20 mm ex-RPETs) between 3–5 days as product release began to rapidly rise. Unlike that, the higher SSA substrates (67×3 mm ex-RPETand 30 mesh ground) produce a better-defined average and less variability, which is due to the higher number of test pieces involved. A 30% increase in SSA is observed from low to high SSA substrates and this change is revealed in the disappearance of the induction phase during the start of depolymerization. Due to the low depolymerization rate, the induction phase in low SSA strands is ~24–36 hours indicating an inadequate surface area for all available enzymes to bind and actively break down the ex-RPETs. The ground particulate samples have ~75% larger SSA than the strands resulting in a significantly short (<8 hour) induction phase, increased depolymerization rate, and reduced final product concentration. Researches indicate that the steady enzymatic depolymerization rate for a low SSA insoluble substrate can be delayed due to the formation of an enzyme-substrate complex for further depolymerization. The use of purified enzymes, higher enzyme concentration, and higher SSA to promote fast access to substrate-binding sites can reduce the induction phase to form such enzyme-substrate complexes. When all material properties related to depolymerization rate remain constant, varying SSA only impacts the length of the induction phase. Even though the induction phase takes considerable time (>12 hours) for degrading, the depolymerization rate after the induction phase remains constant regardless of its duration. When the induction phase is eliminated, the depolymerization rate approaches an asymptotic limit defined by the speed at which enzymes bind
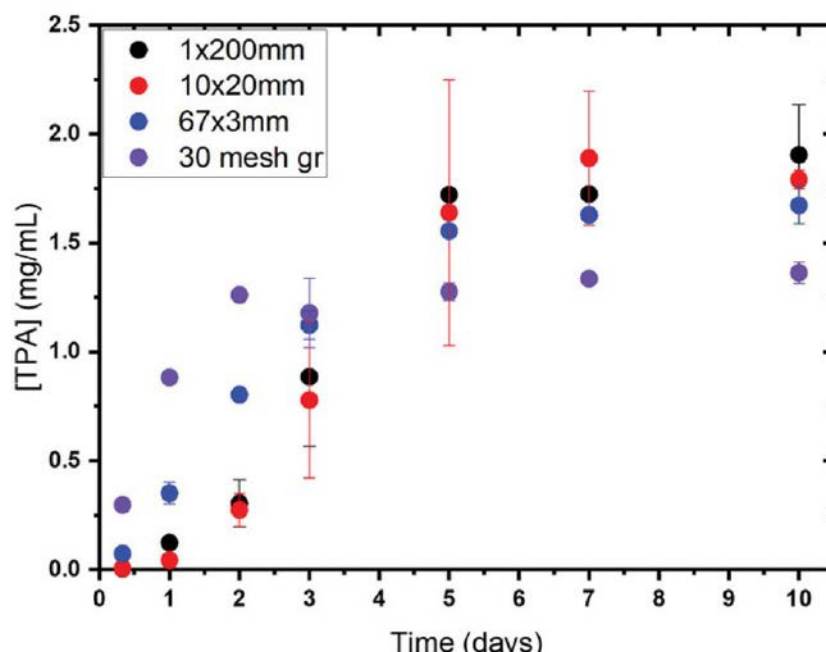

Fig. 1: TPA produced from the enzymatic depolymerization of ex-PRET strands of different lengths where S 2 g L^{-1}, E = 30 nм, pH ≈ 8 in potassium phosphate buffer. Expected maximum conversion for 2 g L^{-1} substrate concentration is ≈ 1.72 g$_{TPA}$ L^{-1}. Error bars are a single standard deviation, n = 3.

and depolymerize free polymers. **Table 1** shows higher crystallinity but lower MAF values for high SSA ground samples leading to a reduced final product concentration. Both crystallinity and pH lower the TPA yield leading to uncontrolled acidification of the reaction system, which is another reason for lower product concentration.

Depolymerization mechanisms and behavior

Polymerization and depolymerization of PET occur through the polycondensation of TPA and the hydrolysis of ester bonds (i.e. esterase enzymes) respectively. The proposed enzymatic mechanism is the aromatic ring in TPA entering a binding site within the enzyme, wherein an α/β-hydrolase

domain works to hydrolyze the ester bonds connecting TPA to its neighboring EG monomers. **Figure 2** shows the SEM images of the degraded substrates demonstrating depolymerization behaviors of ex-RPETs. The ungraded strand has a relatively pristine, smooth surface with no visible surface defects, air pockets, or obvious favorable areas for depolymerization **(Figure 2a,b)**. During the 3-days course, pitting appears randomly with no distinct pattern and large continuous smooth, and untouched surface areas (Figure 2c-n), suggesting that the enzymes deconstruct one full polymer chain in a local binding site before hopping to the next available site. **Figure 2k** shows one fully degraded channel, where two pits meet from opposite sides of the strand indicating

	T_g [°C]	T_m [°C]	Crystallinity [%]	RAF [%]	MAF [%]
1 x 200 mm/10 x 200 mm	66.5 ±0.42	253.8±0.25	7.8±0.37	5.2±2.6	87±2.3
67 x 3 mm	70.4±2.0	251.6±0.08	6.5±0.05	8.5±5.2	85.1±5.15
30 mesh gr	64.2±0.74	252.4±0.17	12.6±2.21	15.3±6.05	72.1±8.25

Table 1: Thermal properties of ex-RPET strands and ground ex-RPET including glass transition temperature (T_g), melt temperature (T_m), percent crystallinity, percent rigid amorphous fraction (RAF), and percent mobile amorphous fraction (MAF). Error is one standard deviation, n = 3.

a focused direction of depolymerization for LCC enzymes and thereby an anisotropic substrate degradation. Researches show relatively similar pit formation across the substrates during isotropic etching, except when the multiple pits are impinged and separating edges are broken. A slightly different result is observed for enzymatic depolymerization of 250 µm PET films, where minimal pitting is noticed even after 5 days possibly influenced by a number of factors such as substrate form and thickness, crystallinity, and enzyme-specific properties. For longer depolymerization reactions (>5 days) on substrates, the entire strand disappears leaving behind fragments difficult to recover, wash and analyze. **Figure 3** shows one such recovered strand after a 7-days course with the presence of both amorphous and crystalline regions. Pristine, smooth, and sharp fibrils are observed within the crystalline spherulites, whereas the amorphous region is found percolated, rough, and preferentially attacked by the enzymes. This suggested a degraded crystalline region around amorphous material and the crystals do not readily undergo enzymatic depolymerization, rather simply falling out of the substrate into the reaction solution as micro or nanoscopic waste byproducts. Unless crystallinity is fully removed from the substrate, 100% recovery of the TPA and EG component monomers may not be achievable.

Energy tradeoffs

The findings related to SSA and reaction kinetics highlight the importance of tradeoffs in PET recycling. As the induction phase decreases with increasing SSA, the substrates undergoing the induction phase may save energy at the expense of time, but suffers from increased crystallinity depending on the reaction temperature. A manual pre-grinding of the substrate can increase energy costs and decrease final product yield by saving time. **Table 2** summarises these methods to increase SSA. Excluding the Wiley Mill grinder's fixed capital cost, the cost of increasing SSA is manually estimated considering (i) material loss during grinding (~87%
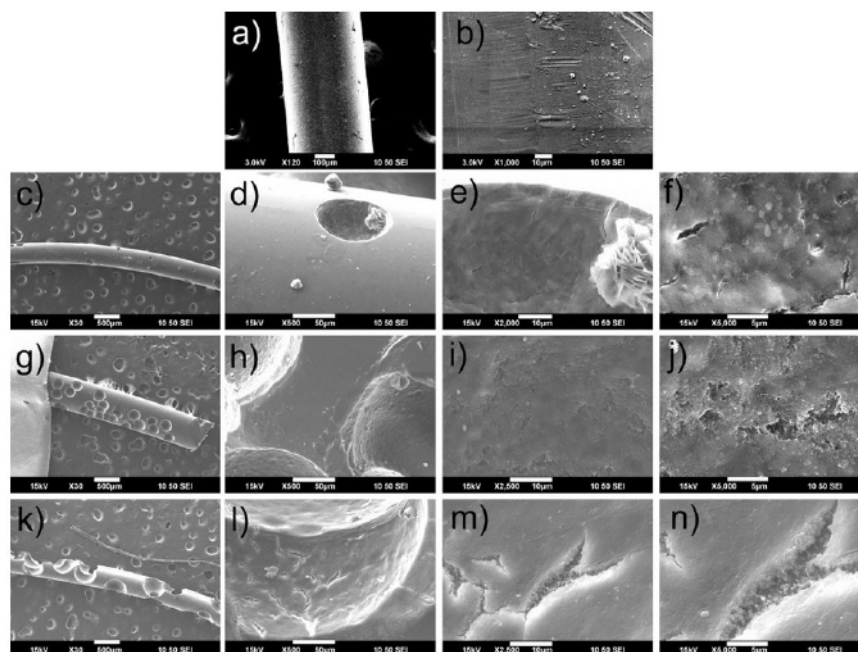

Fig. 2: SEM images of a,b) virgin ex-RPET strands and degraded ex-RPET strands over c–f) 1 day, g–j) 2 days, and k–n) 3 days.
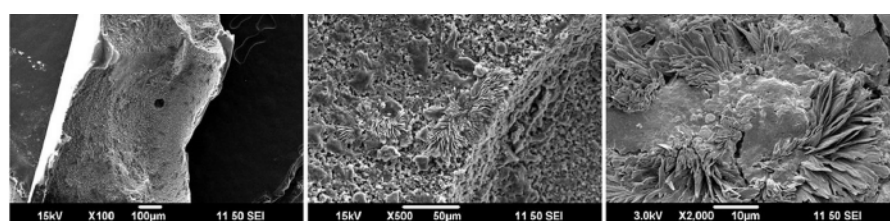

Fig. 3: SEM images of a,b) virgin ex-RPET strands and degraded ex-RPET strands over c–f) 1 day, g–j) 2 days, and k–n) 3 days.

product yield from 100g ex-RPETs leading to ~13% material loss via fine powder-coating and micro-particulates trapped inside the grinder), (ii) grinder power consumption (0.015 kWh for ~87% yield), and (iii) increased crystallinity due to shearing via grinder blades and slower cooling of the extrudate.

The piled-up ground ex-RPETs outside the extruder die hole cool slowly, but ex-RPETs with a high aspect ratio cool rapidly via a chilled water bath when exiting the extruder die head. These high aspect ratio strands are difficult to grind uniformly, so only globular chunks of ex-RPETs are used in this work. Among

	Incubation	Manual pregrind
Specific surface area	$\approx 4.3–5.6 \ mm^2 \ mg^{-1}$	$\approx 7.5 \ mm^2 \ mg^{-1}$
Operation time	$\approx 24 – 36$ h induction phase	
+ depolymerization time	≈ 4 min	
+ depolymerization time		
Specific energy cost	0 (assuming no heat loss in an adiabatic system)	$0.175 \pm 0.017 \ kWh \ kg^{-1}$
Substrate loss	0%	$\approx 13\%$
End product concentration	1.7 g L^{-1} (max 1.72 g L^{-1})	1.3 g L^{-1} (max 1.72 g L^{-1})

Table 2: Summary of incubation versus manual pregrind processes to increase SSA.

them, 3 mm strands show higher SSA without a large increase in crystallinity but lead to higher energy consumption (5.3 kWh kg^{-1} PET). The large blades of the grinder cause abrasive heating and shearing of the material leading to a doubling of the crystallinity and RAF values in ground samples as compared to the ex-RPET strands **(Table 1).** As these two properties do not show significant depolymerization behaviour, thus increasing their amount reduces the amount of degradable SSA. The ground ex-RPETs reach maximum depolymerization in 2 days but achieve a product concentration via high-performance liquid chromatography (HPLC) of TPA ~1.3 gL^{-1} indicating ~25% less product released by the enzyme solution. The non-degradable portions of the substrate remain in the solution as waste byproducts to be appropriately collected and discarded from the reactor later. Cryomilling, a particle size reduction method, can maintain low crystallinity while increasing SSA, but suffers from a very high substrate cooling cost as compared to the Wiley Mill grinder. Goodfel-

low can produce fine-powder industrial PET with higher SSA, but shows >30% crystallinity. Hence being an energetically expensive process (3.8 kWh kg^{-1} PET), extrusion of PET to amorphous substrates is necessary to achieve better grindability and significantly lower crystallinity. Heating of reaction medium and substrate grinding cost 0.233 and 0.0175 kWh kg^{-1} PET respectively under realistic conditions. This can increase SSA up to 75% at expense of substrate crystallinity.

Conclusion

SSA is a key factor in the enzymatic depolymerization of PET. At lower SSA, a long induction phase of 24–36 hours is inevitable with low product yield and after this phase, the depolymerization rate is independent of SSA. At higher SSA, more active sites on the target substrates are open for enzymes to bind to and thereby increasing the overall depolymerization rate. The induction phase is reduced by an order of a few hours and the depolymerization rate increases rapidly. The depolymerization is initiated

in select areas leading to methodical deconstruction substrate rather than isotropic etching. Finally, the mechanical grinding of ex-RPETs drastically increases SSA and reduces induction time. Despite that, the increased crystallinity after grinding poses a big challenge by forming increased waste byproducts after depolymerization. Thus, further research is needed to maintain low crystallinity but high SSA for optimal enzymatic recycling technologies.

Finding extracellular hydrolytic activity in microorganisms

Interview with Dr. Hermann J. Heipieper

Please briefly present your research approach in the field of plastic degradation with microbial enzymes.

Within the EU-project "From Plastic waste to Plastic value using Pseudomonas putida Synthetic Biology (P4SB) we could isolate a bacterium, Pseudomonas capeferrum TDA1, from a plastic dump site in Leipzig, Germany, that is able to degrade polyurethane (PU) oligo- and monomers [1,2]. This bacterium is already part of a defined microbial mixed culture for utilization of polyurethane monomers (Utomo et al. 2020. ACS Sustain. Chem. Eng. 8:17466-17474).

What was the most surprising finding for you in the course of your research on this topic?

Different cell fractions of Pseudomonas capeferrum TDA1 grown on a PU oligomer were tested for extracellular hydrolytic activity. Strikingly, purified outer membrane vesicles (OMV) of P. capeferrum TDA1 grown on PU showed higher esterase activity than cell pellets. Hydrolases in the OMV fraction possibly involved in extracellular PU degrada-

Colonies of Pseudomonas careerism TDA1 growing on agar plates with a PU oligomer as sole carbon and energy source

tion were identified by mass spectrometry [3,4].

What are the advantages of using enzymes for the degradation of polymers compared to other methods?

Microbes must take up chemicals to degrade them, which is not possible for high molecular weight polymers such as plastics. These polymers are degraded to oligomers or monomers by extracellular enzymes. Therefore, it is more promising to work directly with these extracellular enzymes. They can be produced in large quantities using genetically modified cell factories in containments, a technology that is already state of the art for enzymes used in detergents or in the food industry, for example.

Which microorganisms are most important for the decomposition of plastics, and which enzymes play a decisive role here?

Enzymes that can degrade polyester plastics are usually esterases or cutinases, which are able to hydrolyze the ester bonds. In order to be attacked by enzymes, however, the plastics must be converted from their crystalline to an amorphous structure. In the case of PET, this is generally done by thermal treatment at 70°C. Enzymes from thermophilic bacteria such as Thermobifida fusca are therefore particularly promising for plastics applications, as they can also be used at these high temperatures.

Which classes of plastics are best suited for enzymatic degradation? And which are the most challenging?

This depends heavily on the chemical compound that makes up the plastic. Polyesters such as polyethylene terephthalate (PET) and polyester polyurethane (PU) can be hydrolyzed by enzymes that also degrade naturally occurring plant and animal polymers. In contrast, plastics such as polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC), which consist of C-C bonds, and polyether polyurethane (PU) are very difficult to degrade. Here, PS and PVC in particular are more or less inert to be attacked enzymatically. Therefore, most research successes so far have been described for PET and polyester-PU. The most important research question for environmental microbiologists working on this topic is which plastics can be attacked by microorganisms at all. This information can be helpful both for industry with regard to the chemical structure of their future plastic products and for legislation (e.g. local governments or the EU Commission) with regard to the future banning of certain types of plastics, especially for single use.

From your point of view, which research approaches and methods are the most advanced and promising ones for an industrial application?

For future industrial application, the most advanced process is the use of enzymes or enzyme mixtures, respectively, to convert PET into its monomers terephthalic acid and ethylene glycol for their reuse to make virgin plastics [5,6].

How do you estimate the possibility of using microbial decomposition to degrade plastic on a large scale in the near future? When do you think enzymatic degradation of plastic will be ready for application? What problems will have to be solved, and which prerequisites have to be established first?

Tournier et al. [6] promised to start an industrial-scale recycling process in the next few years, converting PET into its monomers terephthalic acid

and ethylene glycol. Such a process requires pure PET (bottles). Therefore, an EU-wide introduction of an effective collection system for used PET bottles would be necessary, e.g. through a deposit system as already exists in several countries.

Could enzymatic degradation of plastic also be used to remove plastic particles from the environment?

The problem of billions of tons of plastic waste already in landfills or even in the sea will certainly not be solved by bioremediation using microorganisms or enzymes. Therefore, it is now important to evaluate which plastic compounds are biodegradable. For a sustainable future of plastics, more biodegradable plastics would have to be introduced and better recycling would have to be organized, e.g. through a deposit system for PET bottles, as already exists in several EU countries. It is conceivable that industry will use more degradable precursors in the manufacture of plastics in the future, taking into account scientific findings on degradability by microorganisms. However, a fundamental shift to new plastic compounds in the future will only be possible through changes in policy and legislation, e.g. by the EU. What is needed is a transition from a linear, oil-based "below ground" to a circular, bio-based "above ground" plastics economy.
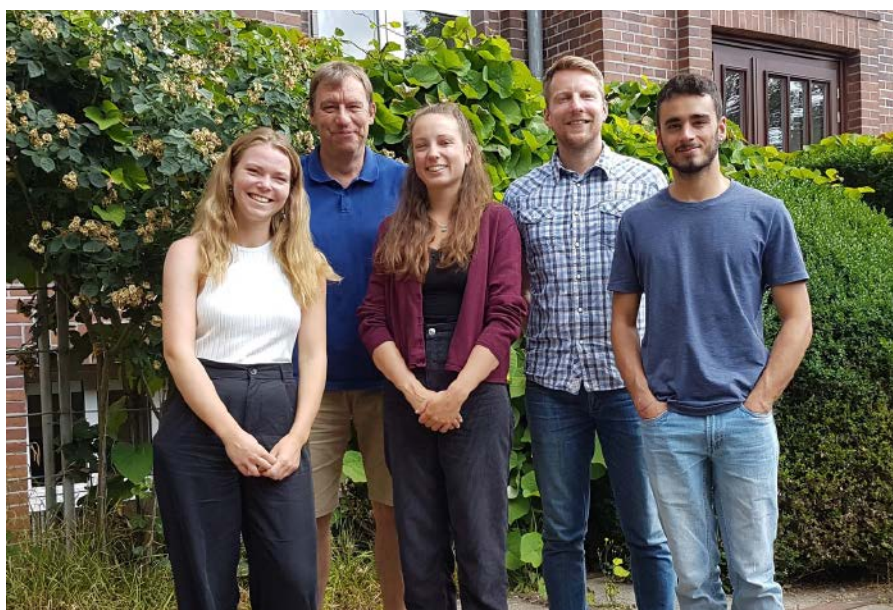
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BIOGRAPHY

Dr. Hermann J. Heipieper. Was awarded his PhD in Biology/Microbiology in 1992 from the University of Münster, Germany. From 1993 to 1999 he was Postdoc at the University of Wageningen, Netherlands, at the Sapienza University of Rome, Italy; and at Consorzio Mario Negri Sud, Italy. Since July 1999 he is head of the group Microbial Adaptation and Contaminant Degradation in the Helmholtz Centre for Environmental Research - UFZ. Main subjects of his work is the microbial degradation of organic pollutants and microbial adaptation to environmental stresses on the level of membrane composition and surface properties.
Dr. Heipieper was coordinator of two and participant in six projects of the EU-commission, including the RTD project entitled: "From Plastic waste to Plastic value using Pseudomonas putida Synthetic Biology (P4SB).

The UFZ group of Hermann J. Heipieper

Plastic degradation in the environment using biodegrading microbes

Interview with Dr. Anshu Priya

What are the advantages of using enzymes for the degradation of polymers compared to other methods?

Compared to the physical, chemical methods of plastic degradation, enzymatic degradation is natural, green, cost-effective, biologically safe and environmentally friendly. Unlike the conventional methods, enzymatic degradation is not energy intensive and does not generate toxic by-products. Further, compared to microbial cell-based polymer degradation, enzymatic degradation is more specific in its action and does not have complications related to requirement of growth media and optimum growth conditions for their action.

Which microorganisms are most important for the decomposition of plastics, and which enzymes play a decisive role here?

Several microorganisms belonging to fungi, yeast, bacteria, algae, actinomycetes, as pure cultures or as consortiums have been known to decompose plastic. Some of the potential degraders are Ideonella, Pseudomonas, Aspergillus, Streptomyces, Thermobifida, Penicillium, Rhodococcus, Xanthobacter, Cladosporium, Brevibacillus, Bacillus.

Microbial enzymes belonging to the hydrolase family such as esterases, lipases, depolymerases, and PETases efficiently degrade the carbon backbone and the chemical bonds of plastics breaking them down to simpler monomeric subunits which are further assimilated and accumulated by the microorganisms and are broken down into by-products such as H_2O, CO_2, CH_4, N_2 etc.

Which classes of plastics are best suited for enzymatic degradation? And which are the most challenging?

Extensively branched low-density polyethylene (LDPE) has a tendency to degrade more easily by enzymatic action as compared to linear low-density polyethylene (LLDPE) or high-density polyethylene (HDPE). Polymers such as polyethylene, nylon are some of the polymers which are very difficult to biodegrade.

From your point of view, which research approaches and methods are the most advanced and promising ones for an industrial application?

All the plastic management methods currently being used by the industries have their own pros and cons. The conventional, physical and chemical methods are energy intensive, costly and generate secondary environmental pollution while the biodegradation process is inherently slow in its action. A hybrid method using a safe, green and economic approach, utilizing all the three, physical, chemical and biological processes can prove to be the most promising for degradation of plastics. Further, emphasis should also be laid on reduction and reuse of plastic to minimize their generation.

Could enzymatic degradation of plastic also be used to remove plastic particles from the environment?

The enzymatic degradation of plastic can be used to remove plastic particles from the environment, however, this type of applications of enzymes are difficult to conduct. The enzymes have poor shelf life, high degree of instability and need specific conditions for their catalytic activity; change in parameters such as pH, temperature may lead to their denaturation. Thus, it is very difficult to conduct real time enzymatic degradation of polymers in an environment at variable pH, temperature and adverse conditions. Further, high cost of isolation and purification of enzymes makes their environmental application economically unfeasible. However, with biodegrading microbes, plastic degradation in the environment can be well achieved.

BIOGRAPHY

Dr. Anshu Priya is a biotechnologist. She has completed her PhD from Indian Institute of Technology Patna, India and is currently engaged in research and development related to microbial biotechnology, environmental management, resource recovery, bio-product synthesis at City University of Hong Kong. She has vast experience in research in the field of environmental biotechnology, bioprocessing, biometallurgy, waste valorization and resource recovery. Dr. Priya's research work is focused on conversion of waste to wealth for sustainable development. She is recipient of various scientific awards, grants and fellowship including Young Researcher Award. Dr. Priya has several scientific publications to her credit. She is also Editor and Reviewer of various journals of international repute.

Enzymatic functionalization and degradation of synthetic polymers

Interview with Prof. Dr. Wolfgang Zimmermann and Dr. Christian Sonnendecker

Please briefly present your research approach in the field of plastic degradation with microbial enzymes.

In the area of polymer biotechnology, the enzymatic functionalization and degradation of synthetic polymers has been a focus of our research group for more than 20 years. For the development of enzyme-related technologies we benefit from natural biodiversity to obtain novel biocatalysts using metagenomic and bioinformatic approaches. Previous work has demonstrated the biocatalytic functionalization of synthetic polyesters with applications in the textile and laundry industry. Access to a portfolio of powerful polyester hydrolases and their further optimization by genetic engineering resulted in the development of novel environmentally benign processes for the enzymatic degradation and recycling of post-consumer plastic waste streams.

What was the most surprising finding for you in the course of your research on this topic?

Microbial polyester hydrolases described so far are similar in structure and function. By exploiting their relaxed substrate specificity, we expected to find hydrolases which could also use synthetic polyesters as substrate. Still, we were surprised to find biocatalysts in natural environments with such a high activity against polyethylene terephthalate (PET) like the recently discovered PHL7 enzyme, indicating a high diversity of polyester hydrolases in nature.

What are the advantages of using enzymes for the degradation of polymers compared to other methods?

The use of enzymes for the degradation and recycling of polyesters allows their ecofriendly conversion to the

Fig. 1: Enzymatic depolymerization of PET thermoform packaging.

monomers at mild reaction conditions in contrast to energy-intensive conventional chemical or mechanical methods. An enzymatic hydrolysis can also be of advantage for the processing of waste composed of different types of plastics which are difficult to recycle by other methods.

Which microorganisms are most important for the decomposition of plastics, and which enzymes play a decisive role here?

Actinomycete bacteria, for example Thermomonospora species and fungi, for example Humicola insolens are producing important polyester hydrolases.

Fig. 2: Isolation of thermophilic actinomycetes from a compost site.
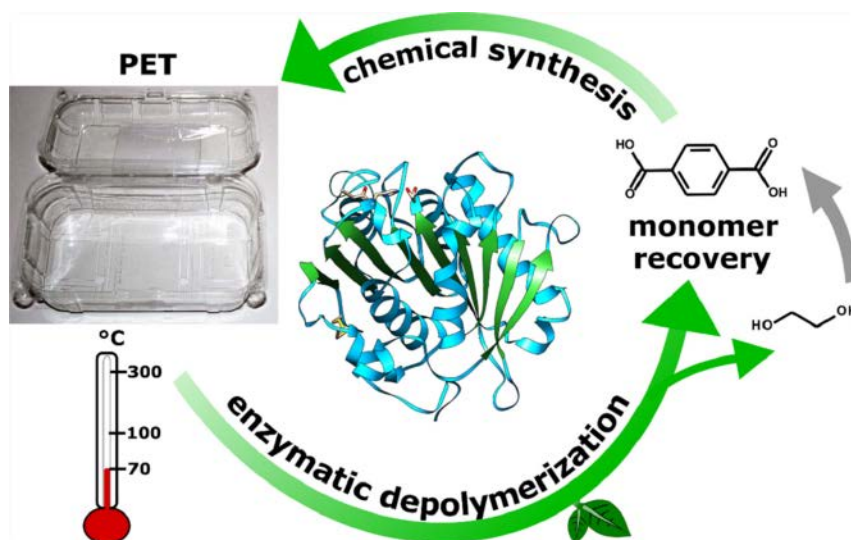
Fig. 3: Enzymatic closed-loop recycling of PET.

to be avoided. The recently discovered polyester hydrolases are able to rapidly degrade food packaging containers without any pretreatment. However, suitable collection schemes for this important post-consumer plastic waste stream have not been established yet.

Could enzymatic degradation of plastic also be used to remove plastic particles from the environment?

This is rather unlikely. Polyester hydrolases for the degradation of PET waste cannot work efficiently outside a contained environment with temperatures around 60–70°C.

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Which classes of plastics are best suited for enzymatic degradation? And which are the most challenging?

It is now possible to fully degrade amorphous PET using enzymes. Enzymes able to degrade other types of plastics such as polyethylene, polypropylene, and polyamides are presently under investigation but are still far from an application in plastic degradation processes.

How do you estimate the possibility of using microbial decomposition to degrade plastic on a large scale in the near future? When do you think

enzymatic degradation of plastic will be ready for application? What problems will have to be solved, and which prerequisites have to be established first?

We presume that the use of microorganisms in a degradation process for plastic waste at any large scale is unlikely in the near future. In contrast, enzymatic degradation of pre-treated bottle PET waste has already been demonstrated at pilot scale by a French company. To become commercially competitive, energy-intensive and costly pretreatments to convert crystalline PET to amorphous PET which can be hydrolyzed by polyester hydrolases have

BIOGRAPHY

Prof. Dr. Wolfgang Zimmermann
studied biochemistry, microbiology, and chemistry at the University of Ulm and Heidelberg, receiving his Diplom degree in 1981 and doctorate in 1984 from the University of Heidelberg. From 1985 to 1987 post-doctoral fellow at the University of Manchester, Institute of Science and Technology, UK. From 1988 to 1993 Assistant Professor at the Department of Biotechnology, ETH Zürich, Switzerland. From 1993 to 1999 Professor in Biotechnology at Aalborg University, Denmark and from 1999 to 2004 Professor and Chair in Bioprocess Technology at Chemnitz University of Technology, Germany. From 2005 to 2019 Professor and Chair in Microbiology and Bioprocess Technology and since 2019 Professor Emeritus at the Institute of Analytical Chemistry, Leipzig University, Germany.

BIOGRAPHY

Dr. Christian Sonnendecker
From 2008 to 2013, he studied biochemistry at Leipzig University. Following, he worked as research fellow in the team of Prof. Wolfgang Zimmermann. From 2015 to 2019 he performed his PhD in biochemistry with a scholarship funded by ESF. During that period, he worked in the field of protein engineering of cyclodextrin glucanotransferases. Since 2018, he performs research on enzymatic PET degradation, including the screening for new enzyme candidates from metagenomes and protein-engineering. Since 2020 he works in the group of Prof. Jörg Matysik as a contributor for the EU projects ENZYCLE and MIPLACE.

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