

Applications

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Biocatalytic approaches for plastic recycling

Biokatalytische Konzepte für das Plastikrecycling

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Abstract: This article presents an overview of the evolution, impact, and challenges of plastics, focusing on the difficulties in recycling processes and introducing biocatalytic technologies as a promising solution for efficient plastic degradation. While there are many obstacles in accessibility of plastics for enzymatic degradation, recent achievements in industrial application and discoveries of new enzyme classes for polymer degradation highly encourage further investment in this technology paving the way towards broad industrial application of biocatalysis in plastic recycling.

Keywords: biocatalysis; enzyme engineering; plastic recycling; synthetic polymer degradation

Zusammenfassung: Dieser Artikel bietet einen Überblick über die Entwicklung, die Auswirkungen und die Herausforderungen von Kunststoffen, wobei der Fokus auf den Schwierigkeiten in den Recyclingprozessen liegt und biokatalytische Technologien als vielversprechende Lösung für einen effizienten Abbau von Kunststoffen vorgestellt werden. Obwohl es viele Hürden bei der Zugänglichkeit von Kunststoffen für den enzymatischen Abbau gibt, ermutigen jüngste Errungenschaften in der industriellen Anwendung und die Entdeckung neuer Enzymklassen für den Polymerabbau zu weiteren Investitionen in diese Technologie, die den Weg für eine breite industrielle Anwendung der Biokatalyse im Kunststoffrecycling ebnet.

Schlagwörter: Biokatalyse; Enzym-engineering; Plastikrecycling; Abbau synthetischer Polymere

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1 The story of plastics

Plastics have become a defining feature of modern life within the contemporary era which thus often is referred to as the “plastic age”. Indeed, the exponential increase in plastic contamination since the 1950’s closely mirrors the trajectory of global plastic production, marking plastics as a significant geological indicator of human impact on the planet. Plastics, as defined by the International Union of Pure and Applied Chemistry (IUPAC), are polymeric materials that may contain additives (e.g., inorganic fillers, softeners, thermal stabilizers, fire retardants, UV stabilizers and colorants) [1] to enhance performance or reduce costs [2] and their ascent to becoming the material of choice for numerous applications is well-founded: Due to their polymeric structure, they exhibit high molecular weight and plasticity. Plastics outclass other materials due to the ease of manufacture, high resistance to water, chemicals or light, and a good performance in a wide range of temperature. Furthermore, they provide a high strength/weight ratio and most importantly, low production costs [1]. While first approaches of humans to use polymers like natural rubber lead back to the ancient Mesoamericans in 1600 BC, the first fully synthetic polymer, Bakelite, was invented in 1909 [3], [4] and marked the birth of a new class of heavily used materials with global production soaring from 1.5 million tons in 1950 to an astounding 390 million tons in 2021 [5], [6]. From packaging fresh foods to insulating electrical instruments, as well as usage in lightweight construction materials and essential healthcare products, plastics have played a pivotal role in advancing health, safety, and convenience.

Despite these benefits, the vast production and inadequate management of plastic waste have led to environmental contamination on a global scale ranging from the depths of the Mariana Trench to remote Antarctic food chains. Approximately 60 % of plastic debris, due to its lower density than water, floats in surface waters, contributing to large-scale accumulations like the Great Pacific Garbage Patch (Figure 1) [7]–[9].

The categorization of plastic debris based on size, including micro- and nanoplastics, highlights another dimension of the problem. These small particles infiltrate

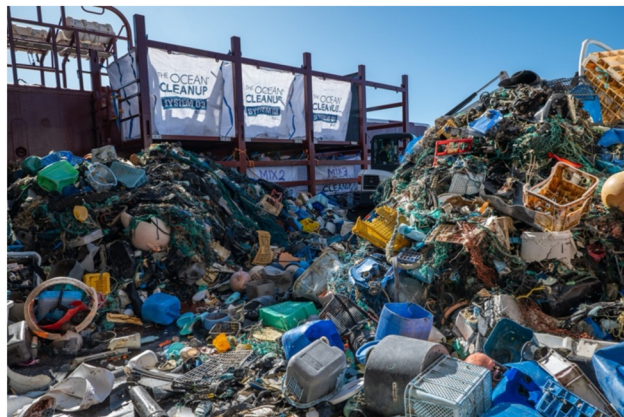


Figure 1: Plastic catch in the pacific garbage patch by “the ocean cleanup” in 2023 [10].

marine ecosystems, which impacts wildlife and potentially human health. The ability of plastic surfaces to adsorb harmful pollutants ranging from pathogenic microorganisms to intrinsic chemical additives in plastics such as phthalates raises further concerns about the environmental and health consequences of plastic waste [11]–[13].

As the world grapples with this issue, the need for effective plastic waste management and innovative recycling technologies, including biocatalysis, becomes ever more pressing.

2 The challenging task of plastic recycling

The exponential growth in plastic production has not been matched by equivalent increases in recycling. Historically, before 1980, nearly all plastic waste was discarded. Since then, incineration and recycling rates have gradually increased. Despite the growth in recycling, a significant portion of the produced plastics remains unrecycled. As of 2015, an estimated 12 % of global plastic waste was incinerated, only 9 % recycled, and 79 % accumulated in landfills or the natural environment [14]. In addition to the inherent toughness of plastics, a major challenge in achieving economically viable plastic recycling is the dependence of recycling effectiveness on the purity of the polymers. Contaminants can drastically hinder the recycling potential as only pure degradation products can be reused for high quality polymer production, necessitating advanced sorting techniques. Near Infrared (NIR) detection, for example, is commonly used to differentiate between polymers, such as distinguishing polyamides from PET waste [15]. However, the vast diversity in plastic compositions poses a substantial challenge. Two plastics of the same type, like packaging

consisting of PE, can vary significantly in their composition of colorants, modifiers, etc. complicating the recycling process. Multilayer products further complicate this issue, as their complex layers of different materials make efficient recycling more difficult. Chemical recycling, which breaks down plastics to their molecular components, is affected by such impurities, but also faces further challenges. High reaction temperatures, aggressive solvents and catalysts, or extreme pH values drive up costs, creating a highly questionable ecological benefit. Mechanical recycling processes, such as grinding, melting, and reshaping, are cheaper and less complex. For instance, many thermoplastics, like PET, PE, or PP, can be melted and reshaped with little loss of quality; however, these processes are more strongly affected by impurities and additionally are limited in their scope and effectiveness. Usually, mechanical recycling generates products of lower quality, thus failing to significantly reduce the need for producing new, virgin polymers [16].

Energy recovery methods like pyrolysis and incineration offer an increasingly considered alternative that does not rely on polymer purity. Yet, they come with their own set of environmental concerns, like greenhouse gas emissions and air pollution. Most importantly, these approaches only gain energy, but no material is recycled and hence new polymers must be produced from petrol-based resources again with negative impacts on the climate.

In light of these challenges, biocatalysis emerged as a promising approach, leveraging the specificity and efficiency of enzymes and microorganisms to break down and recycle plastics. This innovative method offers a potential solution to the limitations of traditional recycling techniques, paving the way for more sustainable and efficient plastic waste management [17].

3 Biocatalytic technologies for plastic degradation

Biodegradation, the breakdown of organic matter by living organisms, is a vital process in nature and a cornerstone of biocatalytic plastic degradation. Organisms use enzymes as biocatalysts, or molecular tools, for biochemical reactions in their metabolism. These processes typically require the cooperation of multiple organisms or enzymes and can occur in both aerobic and anaerobic environments, such as different layers of landfills or composts. Over the past two decades, biocatalysis, has emerged as a practical and environmentally friendly alternative to traditional catalysis. The field has evolved through waves of technological research and innovations, particularly in protein engineering. Early applications of biocatalysis utilized

enzyme-containing components of living cells for chemical transformations. With the advent of gene technology in the 1980s and 1990s, it became possible to clone and express enzymes in microbial hosts, opening new possibilities for optimizing biocatalysts for non-natural substrates, like plastics [18]–[20]. Just like chemical recycling of plastics, biodegradation results in the full decomposition of the polymers into their monomeric building blocks (this is possible in principle for polymers with hydrolyzable bonds, like PET, polyurethane and polyamides like nylon), but with the significant advantage of mild reaction conditions. Enzymes, optimized for natural environments, negate the need for extreme temperatures, high pressure, or toxic reagents, making biodegradation potentially both cost-effective and environmentally friendly. The success of enzymatic plastic degradation largely depends on the accessibility of chemical bonds within the polymers. Additionally, factors such as crystallinity, molecular orientation, and cross-linking play a crucial role, with amorphous regions generally more susceptible to degradation [21]. Therefore, the variability in polymer structures poses a significant challenge in identifying and developing effective biocatalysts. For instance, some fully synthetic polymers, like polyether-polyurethanes, which are used for mattresses, insulation, coatings, or shoe soles contain bonds that are usually not found in nature and thus are less commonly targeted by naturally occurring enzymes. Other polymers, like PE or PP, do not contain any reactive chemical bonds in their main chains and thus may only be targeted by slow and unspecific oxidative bioreactions with a non-uniform and complex product scope. Furthermore, here the original building blocks (ethylene or propylene) can not be accessed. Thus, biodegradation holds particular promise for certain types of plastics. Ester bonds, commonly found in natural polymers and lipids, are cleaved by esterases or lipases, making them key targets in the setup of biodegradation of polyester-based plastics, like PET, a plastic widely used in bottles and clothing.

A primary challenge in the application of biocatalytic technologies for plastic degradation is the level of enzymatic activity. While several enzymes, such as specific cutinases or polyesterases, have shown promise in degrading polymers like PET, their activity levels often fall short of what is economically viable and hence enzyme engineering has been used to make the enzyme suitable for large-scale processing as described in the next chapter. Beyond that, process engineering plays a crucial role in enhancing the efficiency of the process at industrial scale. One significant aspect is high conversion within a short time and high

substrate loading. In addition, it must be ensured that accumulation of the product must not have a negative impact on the enzymes' activity [22]. Acidification from PET hydrolysis may inactivate the enzymes. Techniques such as (continuous) extraction or crystallization of the product can improve the overall efficiency of the process and facilitate product isolation. Another aspect is proper pre-treatment of the polymer substrate to increase accessibility of the chemical bonds to be cleaved by the enzyme. Techniques like grinding and extrusion-based amorphization enhance the interaction between the enzymes and the plastic substrates and thus can lead to more efficient degradation.

4 Enzyme engineering

To overcome many of the above-mentioned hurdles, enzyme engineering plays a pivotal role in advancing biocatalysis, also in regards to plastic recycling. Enzyme engineering involves the modification of enzyme sequences to enhance their efficiency, stability, and specificity for the desired reaction. This process, generally achieved through techniques like directed evolution and rational protein design [23]–[25], enables the tailoring of enzymes for specific industrial applications by making them more active towards their substrates or more robust at high temperatures, extreme pH, or towards organic solvents. In the late 1990s, advanced protein engineering, combined with high-throughput screening, led to 'directed evolution'. Since then, biocatalysis has significantly benefited from molecular biology advancements, affordable genome sequencing, bioinformatics, and computer modeling [26].

Rational design involves specific modifications to amino acids within the enzyme via site-directed mutagenesis, relying on structural and mechanistic understanding of enzymes. Recent developments, like the enzyme-structure prediction tool "Alpha Fold", significantly empowers rational design approaches. Directed evolution, in contrast, employs iterative cycles of random mutagenesis along the whole enzyme to generate vast libraries of enzyme variants. This approach allows for the discovery of beneficial mutations that might not be achievable through rational design alone and does not need much information about the enzymes' structure or mechanism. However, vast library creation requires efficient screening and selection assays requiring potent high-throughput screening methods to identify improved versions out of thousands or millions of variants.

A groundbreaking showcase of successful enzyme engineering and process engineering for plastic recycling is the achievement made by the French company Carbios which

has developed an efficient and scalable enzymatic process for PET recycling. After an amorphization and micronization pre-treatment of the PET post-consumer waste, they apply an engineered variant of the leaf- and branch compost cutinase (LCC), an enzyme which degrades the natural polyester cutine in plant cell walls and which was found in a compost metagenome in 2012 by Sulaiman et al. in Japan (Figure 2) [27]. In 2020, the company could demonstrate that after 10 h of incubation of pre-treated waste PET (200 g/L) with the engineered cutinase mutant LCC-ICCG, more than 90 % of the polymer was degraded into its monomers terephthalic acid and ethylene glycol. These monomers were subsequently used to produce virgin and high-quality PET bottles [28]. This approach not only offers an effective recycling solution but also contributes to the creation of a bio-based circular economy for plastics. To reach these goals, Carbios has established long-term strategic partnerships, including with Novozymes, a specialist in the development and production of industrial enzymes and microorganisms, to ensure a steady supply of enzymes necessary for their PET recycling process. Currently, the world's first biological PET-recycling plant located in Longjumeau, France, is under construction. This plant will process an estimated 50,000 tons of PET waste per year starting in 2025 [29]. The Carbios method presents a significantly more environmentally friendly alternative to traditional plastic recycling methods, avoiding the need for high temperatures and pressure, or toxic reagents. The economic viability of this process, despite the challenges in maintaining pH levels and managing byproducts, offers a promising outlook for the recycling industry. The ability of the process to convert waste PET into high-quality raw materials for new plastics production positions Carbios as a key player in the future of sustainable plastic management.

5 Future perspectives and innovations

To expand industrial-scale plastic recycling beyond PET and polyesters in general, scientists explore novel enzymes that can degrade nylons (polyamides) [30]–[32] as well as polyurethane (PU) [22] as we have recently demonstrated by the discovery of three urethanase-bond hydrolyzing enzymes in a metagenomic library. These enzymes now represent a valuable starting point for enzyme engineering – similar to improving LCC as described above – and the mining of further enzymes to achieve large-scale processing of polyurethane waste materials.

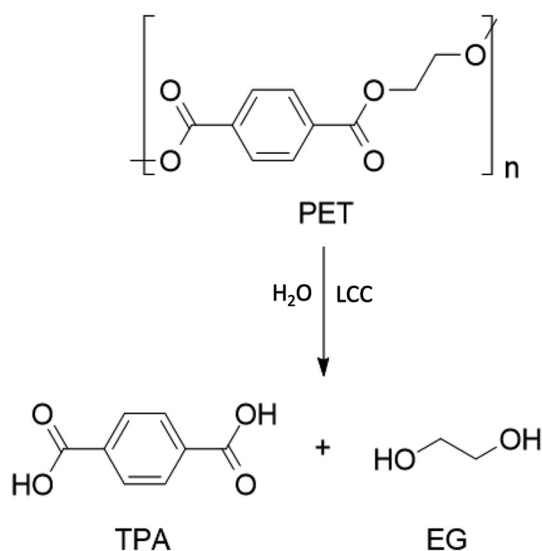


Figure 2: Biocatalytic hydrolysis of polyethylene terephthalate (PET). Under aqueous conditions, the ester bonds within the backbone of the polymer are hydrolyzed by an esterase, such as LCC. The monomeric products, terephthalic acid (TPA) and ethylene glycol (EG) can then be reused for the production of high quality virgin PET.

Another active research area is the upcycling of plastic waste to higher value products. For example, our recent study proposed an upcycling from waste PET by introducing highly concentrated calcium ions into the enzymatic PET degradation reaction. This not only reduces the need for pH control during enzymatic PET degradation, but simultaneously generates calcium terephthalate, which has the potential to be utilized as a lithium battery anode [33]. Further, given the growing scientific and political interest in biodegradable plastics, like PLA or PHA, for their potential sustainability, it is crucial to prioritize research and technology development on efficient bio-based recycling methods that focus on monomer recovery rather than biodegradation. Only monomer recovery can close a circular recycling system, whereas supposedly natural biodegradation will waste valuable resources and may leave behind invisible and non-degradable microplastics.

To conclude, the future of plastic recycling is poised at the intersection of biotechnology and engineering. Biocatalytic plastic recycling already represents a significant leap forward in the transformation of the plastic industry to a circular economy. Techniques like enzymatic degradation, spearheaded by advancements in enzyme technology and process engineering, offer a more sustainable and efficient alternative to traditional recycling methods. The innovations in bioplastic production align with these sustainable goals and pave the way to a circular economy in

harmony with nature. Recycling of plastic waste is mandatory and prevention of avoidable use of plastics and their reuse should always be preferred over recycling where it is possible.

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Bionotes



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