

Review

Engineering whole-cell catalysts to use plastic waste as a feedstock

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The extensive production, durability, and waste mismanagement of plastic polymers have led to a highly concerning environmental problem. Recycling methods aim to reduce the amount of plastic pollution, and among them, biological processes have emerged as an interesting alternative for the management of plastic waste that is difficult to collect or can not be recycled by other methods. While there has been significant progress in the field, in particular related to the enzymatic hydrolysis of polyesters, most biological methods rely on the use of enzymes *in vitro*, using collected plastics. In this review, we explore the status of technologies using whole-cell catalysts that could be used for *in vivo* upcycling of plastic waste — with plastic becoming a microbial feedstock — and for the development of biodegradation strategies in relevant environments. We have identified a number of barriers related to polymer bioavailability, enzyme activity and secretion, and the use of strains and microbial communities that need to be overcome to materialize a much-needed solution to plastic pollution.

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Introduction

Plastics are arguably one of the most successful and versatile materials ever developed. In 2023, global production reached 413.8 million metric tons (Mt), generating 360.2 Mt of waste, a figure expected to more than

double by 2040 as annual plastic waste generation is forecast to surpass 615 Mt [1]. Plastics have permeated every aspect of our daily lives, finding applications not only as containers but also in insulation, construction, fabric production, and many other fields [2].

Alongside their versatility is their durability. Plastics are particularly stable polymers, persisting far longer than their intended lifespan, making them highly recalcitrant materials [3]. This property, coupled with chronic waste mismanagement, has resulted in their unwanted accumulation in the environment [4]. Plastics pollute every niche on the planet, harming flora and fauna both as macro- and as microplastics, the latter formed from the breakdown of larger plastic fragments [5]. A wide number of methods are used to manage plastic waste [6], including mechanical [7] and chemical recycling [8], and recently, using biology for polymer breakdown [9]. These bio-based approaches rely on enzymes used *in vitro* to cleave plastic polymers [10], sometimes releasing constituent monomers that can be purified and re-polymerized to produce virgin-like plastics [11] or transformed into value-added compounds in a process known as upcycling [12]. Alternatively, another upcycling approach is to exploit plastic waste as a carbon source for microorganisms, where the degradation products are assimilated by microbial metabolism to produce other molecules [13]. While enzymes act faster, whole-cell catalysts offer a cheaper, self-sustaining route [9,14].

In this review, we focus on the development of whole-cell microbial catalysts capable of directly feeding on plastic waste (Figure 1). This approach is promising not only for treating collected plastics, as with current enzymatic treatments, but also for bioremediating plastics, including microplastics, potentially offering a much-needed solution to a major environmental challenge [15]. The following sections discuss the current status and challenges involved in engineering microorganisms for the removal and assimilation of plastic waste, with a focus on polyethylene terephthalate (PET) as a case study, given that most of the advances in the field have been carried out using this polymer as a target for biological management.

Can microbes eat all the plastic?

A question we often receive is ‘Are you not worried that microbes might escape into the environment and eat all

Figure 1

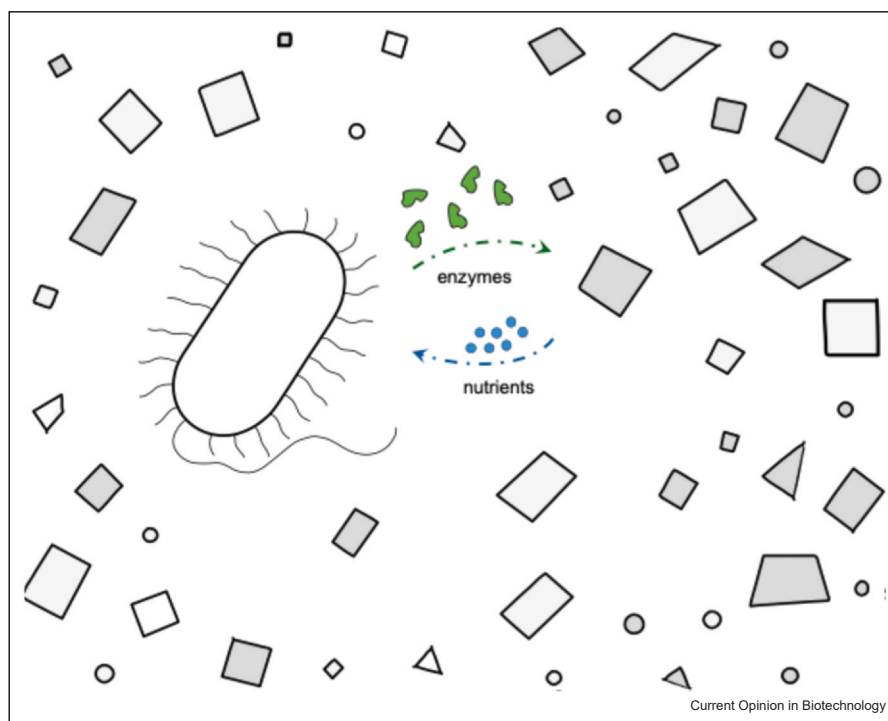


Diagram of a whole-cell catalyst for plastic waste. Natural or engineered microorganisms secrete enzymes (represented in green) that contribute to the depolymerization of plastic waste releasing nutrients (represented in blue) that can be assimilated by the same cells. The illustration exemplifies their use for the removal of microplastics represented as geometrical shapes.

the plastics?'. The short answer is no, and it is highly unlikely to happen anytime soon. As much as biotechnologists would love to use microbes that break down plastic as easily as other polymers, the reality is that microbial biodegradation of plastics is very slow [16].

For context, the EU standard EN13432 defines industrial biodegradability for bioplastics as losing 90% of dry weight within 6 months at temperatures between 50 °C and 60 °C. The requirements for home compostable bioplastics are similar, although at lower temperatures (20–30 °C), which slows down plastic decomposition. Moreover, nonbiodegradable plastics constitute the vast majority of those used in our daily lives and are extremely recalcitrant. For example, standard polyethylene films may persist for centuries in natural environments, undergoing mostly fragmentation rather than true biodegradation [17]. In comparison, PET containers such as clamshells and bottles can take hundreds of years to degrade, primarily by fragmentation; under optimal conditions, complete depolymerization takes approximately 72 years at 35 °C, while 50% conversion into monomers may require up to 162 years in marine environments [16,18]. These figures highlight

the wide gap between natural biodegradation rates and desirable environmental standards.

Therefore, the idea that engineered microbes could accidentally escape and rapidly degrade plastics before their intended use ends, while appealing in science fiction, is not grounded in reality. In fact, engineering microorganisms able to accelerate plastic degradation to prevent century-long environmental accumulation would be highly desirable.

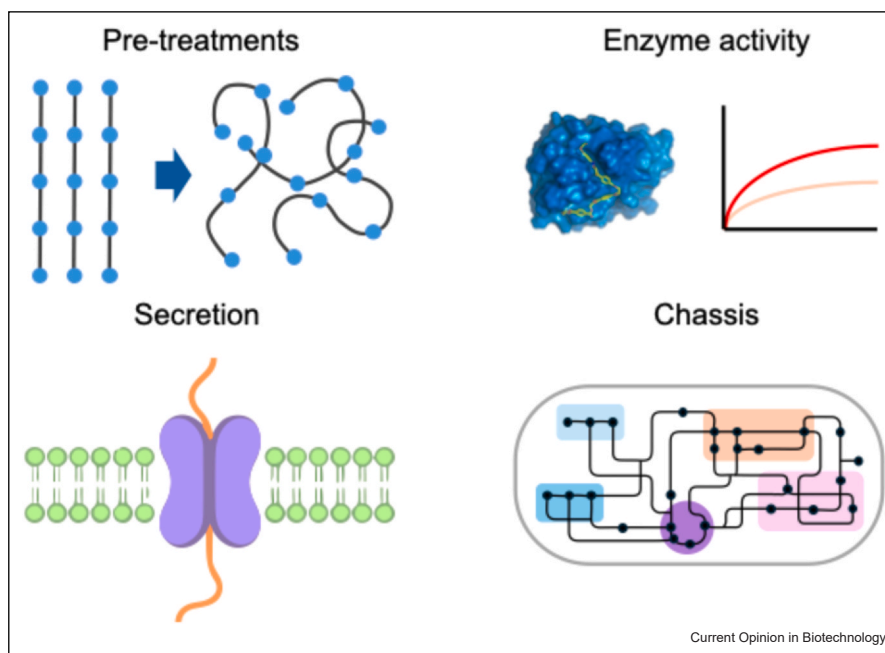
The challenge of engineering whole-cell catalysts

Using whole-cell catalysts to hydrolyze plastics has various limitations. In this section, we will explore the state of the art and technical barriers to the development of relevant applications. These are summarized in Figure 2.

Plastic bioavailability and pretreatments

The biodegradability of plastic polymers is largely determined by the availability of hydrolysable bonds, which are closely linked to polymer crystallinity. In highly crystalline regions, polymer chains are tightly packed, reducing bond mobility and limiting enzymatic activity [19]. In the case of PET, crystallinity above 30%

Figure 2



Technical challenges for the development of effective whole-cell catalysts for the degradation and upcycling of plastic waste. Faster and effective plastic removal would be achieved by overcoming each of these barriers: increasing the bioavailability of amorphous plastic particles, generating enzymes with optimized activity against plastic substrates, improving the secretory capabilities of organisms expressing these enzymes, and engineering microorganisms (or consortia) with efficient metabolic pathways for plastic breakdown products (PETase structure credit Rodrigo Leandro Silveira).

significantly inhibits enzymatic hydrolysis, a threshold commonly exceeded in post-consumer waste [20].

To overcome this barrier, both industrial processes and laboratory-scale research have adopted pretreatment strategies aimed at reducing crystallinity and increasing surface area [21]. In industrial settings, extrusion followed by mechanical shear is frequently employed [11,21], while in laboratory-scale studies, cryomilling remains the standard method for particle size reduction [22]. Many other physical and chemical methods [23,24], such as solvent exposure [25], are also used to disrupt the ordered polymer structure, especially in high-crystallinity plastics.

These pretreatments are effective for collected plastic waste processed under controlled conditions; however, they are far less applicable to uncollected microplastics, small plastic particles typically less than 5 mm in size, dispersed in the environment [5]. Microplastics derived from textiles, in particular, are often highly crystalline and embedded in complex matrices such as soil, sludge, or aquatic systems. This severely limits the accessibility of microbial catalysts and presents a major bottleneck for *in situ* degradation [26]. There is therefore a need for pretreatment strategies suitable for environmental

deployment, ideally low-energy and non-toxic, with advances including engineered microbes that secrete softening agents or enzymes able to function on crystalline substrates.

Plastic-degrading enzymes

Recent years have seen dramatic advances in enzyme optimization, largely thanks to protein engineering and directed evolution supported by machine learning and artificial intelligence strategies [25,27,28]. These approaches have resulted in optimized esterases and amidases with improved activities against PET [29], nylon [30], and polyurethane [31], along with enhancements in durability and thermostability, required for industrial relevance [21,32]. Despite these advancements, the developed enzymes still have limited activity unless the polymer substrate has undergone some form of pretreatment to amorphise its structure or increase its surface area.

One important point to consider is that many enhanced enzymatic activities have been validated mainly *in vitro*, under optimal conditions and in a non-standardized way [33–35], making direct comparisons difficult. Moreover, whole-cell catalysis also requires conditions compatible with cell viability, requiring specific pH, salinity, and

especially temperature, which may limit the use of certain enzymes. Additionally, properties of proteins optimal for *in vivo* activity differ from those required *in vitro*; for example, it is generally thought that thermophilic PET hydrolases such as PHL7 can only perform well at high temperatures; however, recent data suggest they may outperform mesophilic enzymes at lower temperatures during whole-cell catalysis [36,37]. In whole-cell systems, diffusion, substrate availability, cell membrane permeability, and local microenvironment drastically affect enzyme kinetics; therefore, translating *in vitro* kinetics to *in vivo* whole-cell systems remains a bottleneck and calls for better predictive models.

Extracellular protein secretion

A key aspect of whole-cell plastic degradation depends on enzymes being active outside of the cells, requiring secretion to the extracellular medium by the producing microbes. However, most species engineered for these purposes are gram-negative bacteria, which have notoriously low secretion capacities [38]. Nonetheless, model organisms like *Escherichia coli*, which are easy to genetically manipulate, and species from the *Pseudomonas* genus [39], known for their ability to metabolize aromatic compounds, are widely used. Most of these approaches have exploited the Sec and Tat secretory machineries [40], which show limited enzyme activity in culture supernatants [37], highlighting the opportunity to optimize secretion efficiency by engineering the signal peptides and transport machinery involved in these pathways.

To address the limitations of gram-negative hosts, other microorganisms with greater secretory capacity, such as the gram-positive *Bacillus subtilis* [41] and yeasts like *Pichia pastoris* and *Saccharomyces cerevisiae* [42] have been explored. These organisms secrete larger amounts of protein and offer a more efficient platform for extracellular plastic-degrading enzyme production [43,44]. However, they also require adaptation for effective plastic degradation, particularly regarding substrate uptake and monomer metabolism. Nonetheless, the overexpression of enzymes can impose metabolic stress [45], so for all hosts, consideration must be given to the dynamic regulation of protein expression, where constitutive expression of hydrolases may not be efficient at scale. However, using responsive systems with inducible promoters (e.g. responding to terephthalate) can help tune expression depending on substrate availability [46].

Beyond secretion, there is growing interest in other strategies to achieve extracellular enzymatic activity. Recent studies have explored surface display, where enzymes are anchored to the cell surface *via* fusion to membrane proteins, allowing direct contact with plastic substrates [47–49]. Another approach involves extracellular vesicles, which are naturally released by some bacteria for

functions such as cellular communication, and can be engineered to carry enzymes into the environment for plastic degradation outside of the cell [50,51].

Consolidated chassis and artificial consortia

Another important aspect is the selection of the right microbial chassis for use as a whole-cell catalyst. If the resulting monomers posthydrolysis are to be assimilated, microorganisms with native metabolic pathways for those monomers are typically selected [52]. Examples include bacterial species naturally endowed with metabolic pathways for the assimilation of the PET monomers, such as *Comamonas* [53] and *Pseudomonas umsongensis* [54] for terephthalate, or *Pseudomonas putida* for ethylene glycol [52,55].

However, these organisms can be difficult to engineer, prompting efforts to modify strains more amenable for genetic manipulation such as *P. putida* KT2440, whose protocatechuate degradation pathway has been expanded to also metabolize terephthalate [55] and even *E. coli* for the upcycling of terephthalate into valuable products such as paracetamol, vanillin, and adipic acid [12,56,57]. Additionally, PET degradation proceeds via the oligomeric intermediates bis(2-hydroxyethyl) terephthalate and mono(2-hydroxyethyl) terephthalate, and whole-cell systems must also handle oligomers, which are known to inhibit further degradation if not metabolized or removed [58]. These systems might benefit from the co-expression of MHETase's to improve conversion efficiency [59].

Another important aspect is the evolutionary stability of any engineered strains. It is well known that recombinant functions are typically selected against and outcompeted when strains are deployed in relevant environments [60]. This also affects the ability to secrete relevant enzymes. For example, a negative correlation has been shown between recombinant protein secretion and the growth rate of *Lactiplantibacillus plantarum* [61]. Overcoming limitations like these is key to a successful deployment and could be aided by the use of plastics as feedstocks. In this scenario, the engineered organisms could be endowed with a selective advantage. In addition, evolutionary processes would lead to the fine-tuning of engineered functions as illustrated by the adaptive laboratory evolution of *P. putida* KT2440, resulting in a strain with improved growth on terephthalate [62].

Besides consolidated strains, artificial microbial communities present an attractive alternative whereby combining organisms that specialize in different tasks in a consortia would reduce the metabolic burden and expression challenges observed in a single organism [63,64]. In nature, polymer degradation occurs in consortia, so future efforts should learn from natural microbial consortia to guide more efficient synthetic

designs, or by mining functional consortia *via* metagenomics or enrichment [65]. Such engineered division of labor has been shown using a two-strain *E. coli*–*P. putida* system for PET hydrolysis and PHA production [66], and a consortium involving two *P. putida* strains achieved faster and more complete conversion of PET hydrolysate compared to monocultures [67].

Concluding remarks: the road ahead

Developing robust whole-cell microbial catalysts for plastic degradation is an important step toward addressing the environmental accumulation of hard-to-collect plastics, particularly microplastics for which no alternative technologies exist. Significant progress has been made in key areas, such as enzyme optimization and substrate upcycling, but it is important to acknowledge that the field is PET-dominated due to its chemical accessibility, and that progress on polyolefins is emerging but poses significant distinct challenges. Therefore, much remains to be done before whole-cell microbial systems can be deployed outside the lab [9].

Primarily, most efforts to date have focused on engineered microorganisms that can function well under controlled laboratory conditions. These strategies are far from relevant operational settings, such as wastewater treatment plants, where the removal of microplastics from sludge is urgently needed [68,69], and variables like pH, temperature, salinity, and nutrient availability (e.g. phosphate, carbon, and nitrogen) are difficult to control. Yet, while microorganisms capable of partial plastic degradation under these complex conditions have been reported [70], they remain underexplored as chassis for further strain optimization regardless of whether they can assimilate plastic degradation products or not, which in any case would lead to much-needed microplastics biodegradation. Engineering strains that can not only survive but also colonize these environments and express enzymes *in situ* would represent a major milestone for the field.

With continued research into biosafety, future applications could extend to more dynamic ecosystems. While it is unlikely that engineered microbes could escape and degrade all plastic in the environment, it remains essential to design them with built-in safeguards to eliminate that possibility. Finally, bridging the gap between lab-based proof of concept and real-world deployment will require not only innovative microbial engineering but also careful integration with environmental, regulatory, and societal considerations.

CRedit authorship contribution statement

All authors contributed to the writing and editing of this manuscript.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they do not have a conflict of interests.

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