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Letter

# Enzymatic Degradation of Polyethylene Terephthalate Plastics by Bacterial Curli Display PETase

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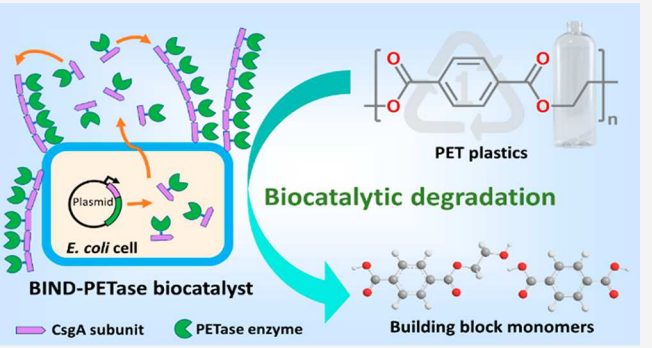
**ABSTRACT:** The extensive production and use of polyethylene terephthalate (PET) have generated an enormous amount of plastic waste, which potentially threatens the environment and humans. Enzyme biocatalysis is a promising green chemistry alternative, relative to the conventional fossil-derived production process, to achieve plastic waste treatment and recycling. In this work, we created a biocatalyst, BIND-PETase, by genetically engineering the curli of an *Escherichia coli* cell with a functional PETase enzyme for biocatalytic degradation of PET plastics. BIND-PETase could degrade PET to generate degradation products at the concentration level of greater than 3000 $\mu$M under various reaction conditions. The effects of key reaction parameters, including pH, temperature, plastic substrate mass load, and surfactant addition were characterized. BIND-PETase was reusable for PET degradation and remained stable with no significant enzyme activity loss when stored at both 4 °C and room temperature for 30 days (Student's *t* test, $p > 0.05$). Notably, BIND-PETase could enable the degradation of PET microplastics in wastewater effluent matrix. Moreover, BIND-PETase could depolymerize highly crystalline postconsumer PET waste materials under ambient conditions with degradation efficiency of 9.1% in 7 days. This study provides a new horizon for developing environmentally friendly biocatalytic approaches to solve the plastic degradation and recycling challenge.




**KEYWORDS:** *Biocatalysis, enzymatic depolymerization, plastic waste, biofilm-integrated biocatalyst, polyethylene terephthalate (PET), curli*

## INTRODUCTION

With an annual production exceeding 26 million tons, polyethylene terephthalate (PET) is the most widely manufactured and used polyester plastic because of its excellent mechanical and thermal stability.[1,2] However, massive postconsumer PET waste has been generated, which is prevalent in diverse environmental matrices,[3−5] potentially threatening the health of different organisms.[6,7] Such adverse impacts of PET plastic pollution would be compounded by its extreme environmental degradation resistance.[8] Additionally, continuing production of new PET consumes nonrenewable petroleum resources.[9] Therefore, it is critically important to address the plastic waste crisis for environmental sustainability.

There is an urgent need to develop innovative and efficient technologies for plastic waste treatment and recycling. Traditional solid waste treatment methods such as landfill and incineration have severe drawbacks including secondary pollution and incapability in valorization of plastic waste.[10−12] Current mechanical and chemical recycling either deteriorate PET plastic properties or require a large amount of energy and costly chemical input.[13−15] In contrast, enzyme-catalyzed PET recycling, which has high specificity and efficiency under mild reaction conditions with minimal energy and chemical use,[16−18] has potential to work as an environmentally friendly alternative to conventional fossil-derived production process.[19] The enzyme PETase from the bacterium *Ideonella sakaiensis* is a prime candidate for biocatalytic PET degradation because of its activity in hydrolyzing highly crystalline PET (crystallinity > 20%) at mesophilic temperatures.[20−23] It is still challenging to use the free PETase for practical applications due to its costly and time-consuming purification processes, short working lifespan, and nonreusability, although the improvement in the depolymerization capability of PET hydrolases by protein engineering has enabled the use of free enzymes to step closer to economical large-scale plastic recycling.[18,24−26] Engineering biocatalytic materials with immobilization of the target enzyme is a promising approach to overcome the above-mentioned

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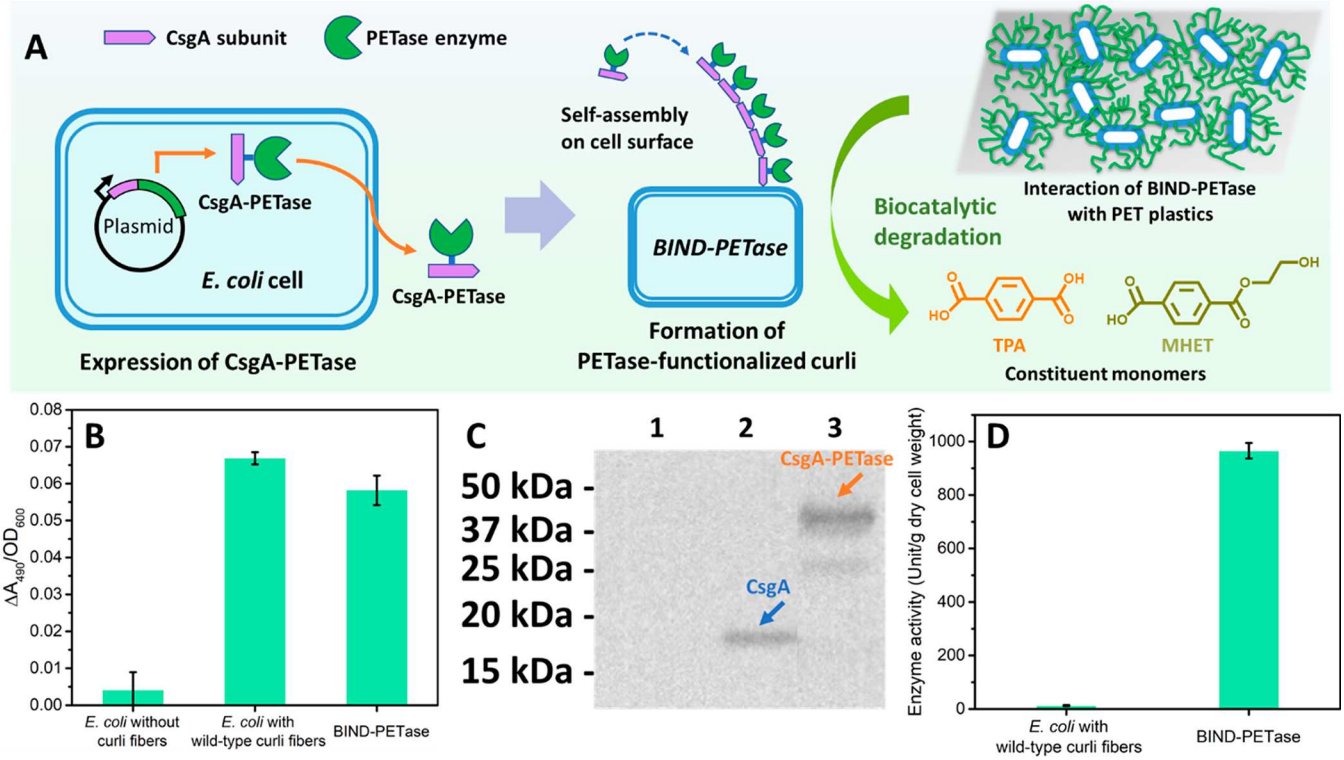


**Figure 1.** Construction and characterization of the BIND-PETase biocatalyst. (A) Schematic diagram for the design of BIND-PETase for biocatalytic degradation of PET. (B) Congo red binding assay. The experiments were conducted in triplicate, and values represent the mean ± standard error. (C) Western blot analysis. The blue and orange arrows indicate the wild-type CsgA subunit and CsgA-PETase fusion, respectively. (D) Enzyme activity of the BIND-PETase biocatalyst. One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1 $\mu$mol of *p*NPB substrate per minute under the conditions specified in Section S5. The experiments were conducted in triplicate, and values represent the mean ± standard error.

limitations,[27] but developing effective PETase-immobilized biocatalysts remains underexplored. Notably, conventional enzyme immobilization methods require laborious protein purification and carrier material production, and the immobilization processing may impair enzyme functionality due to physical or chemical bonding.[27] Recently, an innovative platform called biofilm-integrated nanofiber display (BIND) has been developed, enabling efficient and autonomous protein immobilization on the curli of *Escherichia coli*.[28] Curli are proteinaceous amyloid nanofibers on the surface of *E. coli* as a major constituent of biofilm.[29] The BIND platform allows a functional display of the desirable enzyme through fusion with the building block monomer of curli, CsgA subunit, via a synthetic biology approach (Figure 1A). The BIND platform is advantageous over conventional immobilization methods because it circumvents the complicated steps of protein purification, immobilization material preparation, and processing, while maximally retaining the enzyme activity.[28] In addition, the BIND strategy would benefit the interaction between the enzyme and solid substrates given the critical role of curli in mediating the contact between bacterial biofilm and external surfaces.[28] Inspired by successful application of BIND to display various peptides and proteins,[28,30,31] we aim to exploit this platform to develop a new renewable PET-degrading biocatalyst.

Specifically, this study created a whole-cell biocatalyst, named BIND-PETase, by functionally immobilizing PETase on the self-assembled *E. coli* curli nanofibers for PET degradation (Figure 1A). The study characterized the effectiveness of the BIND-PETase biocatalyst in degrading PET under various reaction conditions, stability during storage, and reusability in PET degradation. Moreover, in relevance to application scenarios, we demonstrated the capability of BIND-PETase in (i) degrading PET microplastics in complex wastewater effluent matrix and (ii) depolymerizing highly crystalline postconsumer PET waste materials. This study provides a new dimension for developing environmentally friendly biocatalytic approaches to solve the plastic waste challenge.

## MATERIALS AND METHODS

**Chemicals, Reagents, and Bacterial Strains.** The detailed information is provided in Section S1.

**Construction of BIND-PETase.** The *csgA* gene was amplified from the genomic DNA of *E. coli* K-12 with 5′ *Nde*I and 3′ *Bam*HI sites. The *PETase* gene codon-optimized for *E. coli* expression was amplified from the plasmid pET21b(+)-Is-PETase[20] with 5′ *Bam*HI and 3′ *Xho*I sites. These two genes were inserted into the pBbE1a backbone digested with *Nde*I and *Xho*I via a three-way ligation to construct pBbE1a-CsgA-PETase (Table S1). The CsgA gene was ligated with the pBbE1a backbone to obtain pBbE1a-CsgA (Table S1). The correctness of the resultant plasmids was confirmed by Sanger sequencing.

The *E. coli* PHL628 cells were transformed with pBbE1a-CsgA-PETase. Cultivation and induction conditions to obtain BIND-PETase biocatalyst were detailed in Section S2. *E. coli* PHL628 transformed with pBbE1a-CsgA was included in parallel to prepare the cells with wild-type curli. The obtained


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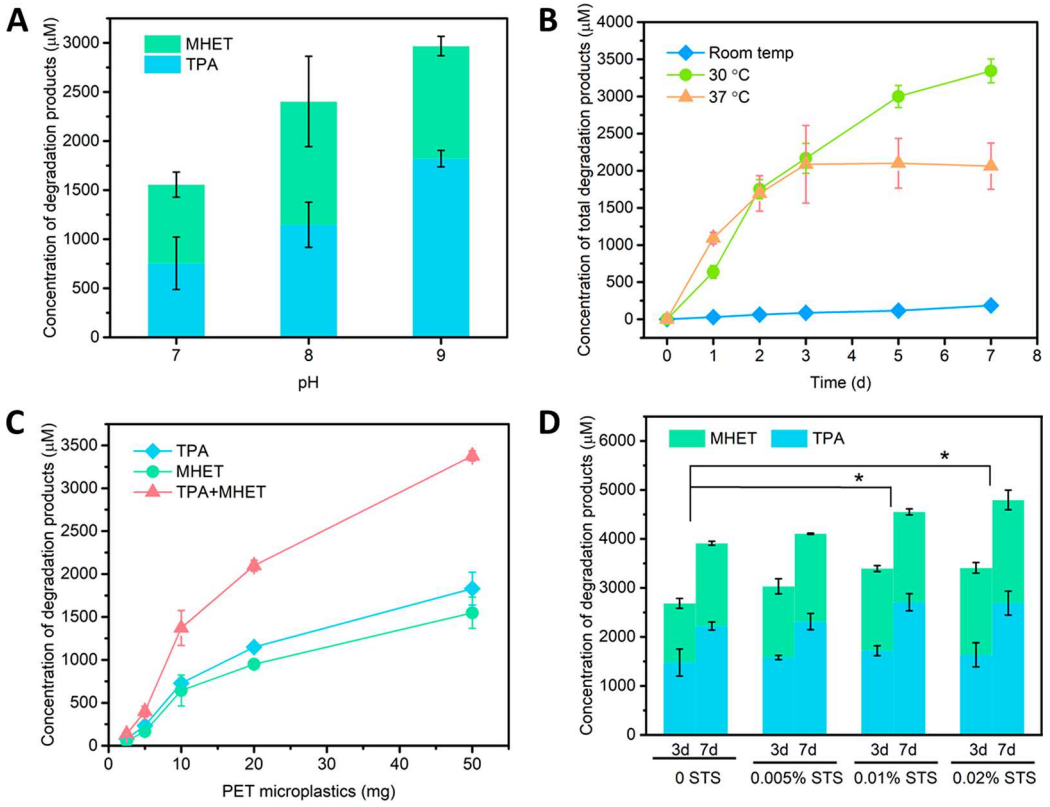


**Figure 2.** Degradation of PET by BIND-PETase under different reaction conditions. (A) Degradation of PET microplastics at different pH values. The experiments were performed in 50 mM $Na_2HPO_4$−HCl buffer (pH 7 and 8) and 50 mM glycine-NaOH (pH 9) with 1.2 U/mL of BIND-PETase and 20 mg of PET at 30 °C for 3 days. (B) Degradation of PET at different temperatures. The experiments were performed in 50 mM glycine-NaOH buffer (pH 9) with 1.2 U/mL of BIND-PETase and 20 mg of PET. (C) Degradation of various mass loads of PET. The experiments were performed in 50 mM glycine-NaOH buffer (pH 9) with 1.2 U/mL of BIND-PETase at 30 °C for 3 days. (D) Effects of sodium tetradecyl sulfate (STS) surfactant on degradation of PET. The asterisk denotes the statistically significant difference ($p < 0.05$, one-way ANOVA test). The experiments were performed in 50 mM glycine-NaOH buffer (pH 9) with 1.2 U/mL of BIND-PETase and 20 mg of PET at 30 °C. All experiments were conducted in triplicate, and values represent the mean ± standard error.

biocatalyst was stored in phosphate buffer (50 mM $Na_2HPO_4$−HCl, 100 mM NaCl, pH = 7) at 4 °C prior to use. BIND-PETase was characterized using Congo red (CR) binding assay, Western blot analysis, and enzyme activity assay. The details are provided in SI (Sections S3−S5).

**PET Degradation by BIND-PETase.** The degradation experiments were performed as follows: 1.2 U/mL of BIND-PETase was added into 1.5 mL of 50 mM glycine-NaOH buffer (pH 9) containing 20 mg of commercial semicrystalline PET (particle size < 300 μm) and mixed gently by pipetting up and down with the PET plastic, followed by static incubation at 30 °C. These experimental conditions were also used to investigate the effects of different reaction factors, including pH, temperature, microplastic substrate mass load, and surfactant addition, unless otherwise specified. The secondary effluent used in PET microplastic degradation was collected from the South Bend Wastewater Treatment Plant, and its characteristics are shown in Table S2. The effluent was 0.2 μm membrane filtered and adjusted to pH 9 before use. The degradation experiments were performed in 1.5 mL of wastewater effluent containing 1.2 U/mL of BIND-PETase and 20 mg of commercial PET microplastics with static incubation at 30 °C. At the predetermined time, the reaction solutions were centrifuged at 16,000*g* for 5 min, and the supernatants were filtered through a 0.2 μm nylon syringe filter for degradation product quantification using high-performance liquid chromatography (Section S6). Controls without biocatalyst addition were also included. All experiments were conducted in triplicate.

The degradation efficiency was calculated as follows

$$\text{Degradation efficiency (\%)} = \frac{(c_{TPA} + c_{MHET}) \times V \times (M_{MHET} - M_{H_2O})}{m_{PET}} \times 100 \quad (1)$$

where $c_{TPA}$ and $c_{MHET}$ are the molar concentration of terephthalic acid (TPA) and mono(2-hydroxyethyl) terephthalate (MHET), respectively, and $V$ is the reaction volume. $M_{MHET}$ and $M_{H_2O}$ are the molecular weight of MHET and $H_2O$, respectively, and $M_{MHET} - M_{H_2O}$ represents the molecular weight of the monomer subunit of PET. $m_{PET}$ is the PET mass added into the reaction.

## RESULTS AND DISCUSSION

**Functional Display of PETase on *E. coli* Curli.** The BIND-PETase biocatalyst was successfully constructed by engineering the *E. coli* curli system through synthetic biology techniques. The *csgA* gene encoding CsgA protein, the monomeric component of curli nanofibers, was transcriptionally linked to the *PETase* gene regulated by a *trc* promoter in an expression plasmid (Figure S1). *E. coli* PHL628 was used as


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chassis for BIND-PETase construction because of its genomic *csgA* deletion and enhanced heterologous curli expression.[32] When induced by isopropyl β-D-1-thiogalactopyranoside (IPTG), the CsgA-PETase fusion was initially expressed intracellularly and then translocated extracellularly via the curli biogenesis machinery (Figure 1A). Subsequently, the CsgA subunit spontaneously self-assembled to form PETase-functionalized curli nanofibers and covalently anchored on the cell surface, yielding the whole-cell BIND-PETase biocatalyst.

We demonstrated successful immobilization of functional PETase on *E. coli* curli nanofibers through comprehensive characterization of the BIND-PETase biocatalyst. First, to confirm the curli nanofiber formation, we conducted a CR binding assay as described in Section S3, a standard method for curli identification.[30,31] BIND-PETase showed a red color after CR binding, verifying the curli nanofiber formation (Figure S2). Meanwhile, BIND-PETase and cells with wild-type curli had comparable CR binding, suggesting minimal interference of fused PETase with the five repeat units, R1−R5, of the CsgA involved in forming β-strand-loop-β-strand motifs of the core of the curli fiber (Figure 1B). Western blot analysis probing the CsgA showed the presence of bands corresponding to the wild-type CsgA (17 kDa, for *E. coli* with wild-type curli) and CsgA-PETase fusion (45 kDa, for BIND-PETase) (Figure 1C). BIND-PETase had an enzyme activity of 966 ± 29 U/g dry cell weight as determined using a typical soluble substrate *p*-nitrophenyl butyrate (*p*NPB)[21,33] (Figure 1D). Furthermore, BIND-PETase was active in degrading PET into TPA and MHET (Figure S3), conforming to the hydrolytic degradation mechanisms of PETase.[20,21] Collectively, these results demonstrated successful construction of functional BIND-PETase biocatalyst.

**Effects of Key Reaction Parameters on PET Degradation by BIND-PETase.** Batch experiments were performed to determine how key reaction parameters influence the catalytic capability of BIND-PETase. The pH value and temperature are crucial factors for enzymatic PET degradation. The degradation product concentration increased from 1556.8 μM at pH 7 to 2967.8 μM at pH 9 for PET degradation by BIND-PETase (Figure 2A). At 30 °C, the BIND-PETase biocatalyst could degrade 4.79% of PET and remained active for 7 days, while the degradation efficiency was 0.27% at room temperature (Figure 2B and Figure S4). As temperature increased to 37 °C, BIND-PETase remained active for 3 days with the degradation efficiency of 3.08%. For a comparison between BIND-PETase and free PETase on thermostability, we also produced free PETase enzyme (Section S7) and used it in PET degradation at 30 and 37 °C (Section S8). The production of PET degradation products leveled off for free PETase after 1 day at 30 and 37 °C, indicating the deactivation of free PETase (Figure S5). Our results showed that BIND-PETase had improved thermostability in plastic degradation activity relative to free PETase. With the identified pH and temperature conditions, we further investigated PET degradation with varied substrate mass. As the PET microplastics mass addition increased from 2.5 to 50 mg, the degradation product concentration increased from 137.0 to 3378.1 μM accordingly (Figure 2C), while the degradation efficiency (mass ratio of the product over the substrate) initially increased from 1.57% to 3.93% and then decreased to 1.94% (Figure S6), which suggested that the biocatalyst amount could be the limiting factor for interacting with and catalyzing the degradation of the excessive plastic substrate. The optimal BIND-PETase concentration used was 1.2 U/mL as our preliminary experiments showed decreased PET degradation activity when the concentration of BIND-PETase was higher than 1.2 U/mL. This phenomenon might be due to the macromolecular crowding effect of immobilized PETase, which could reduce the enzymatic PET degradation by negatively influencing the enzyme−substrate interactions, as also observed previously.[21,34] Furthermore, as surfactants were found to promote enzyme−plastic interactions,[23,35] we characterized how sodium tetradecyl sulfate (STS), an anionic surfactant, would affect PET degradation by BIND-PETase. Addition of STS ranging from 0.005% to 0.02% could enhance PET degradation in a dose-dependent manner (Figure 2D and Figure S7). For example, the PET degradation efficiency in the presence of 0.02% STS increased by 26.9% when compared to the STS-free condition. Such enhancement could be attributed to the amphiphilic nature of STS which facilitated the interaction between BIND-PETase and PET. In summary, pH and temperature are two dominant influential factors for the PET degradation activity of BIND-PETase.

**Storage Stability and Reusability of BIND-PETase.** BIND-PETase could remain stable in storage and was reusable. When stored at 4 °C or room temperature for 30 days, BIND-PETase showed no significant change in enzyme activity (Figure S8A) (Student's *t* test, $p > 0.05$). The storage stability of BIND-PETase surpassed the PETase enzyme immobilized on magnetic iron oxide nanoparticles, which had 40%−70% loss of its enzyme activity during 2 week storage at 4 °C.[36] In addition, the whole-cell BIND-PETase biocatalyst could be recovered from the reaction solution by centrifugation and reused. The enzyme activity of BIND-PETase in *p*NPB hydrolysis retained 87.3% of the initial value after six repeated reaction cycles (Figure S8B). In PET degradation, BIND-PETase was recovered by low-speed centrifugation (200*g*) to separate from plastic particles for repeated use. The BIND-PETase biocatalyst was reused for three reaction cycles with no loss of catalytic activity after the second reaction cycle and 55% loss of activity after the third cycle (Figure S8C), which could be due to the prolonged reaction time at a relatively high pH condition compared with that for the hydrolysis of *p*NPB. In contrast, the degradation activity of *E. coli* cell surface-displayed PETase was reduced to ∼50% and less than 10% after the second and third cycles of reuse, respectively,[34] while the yeast cell surface-displayed PETase could still retain ∼50% of initial activity after seven cycles[37] probably due to the different host cell properties.

It is noted that the use of centrifugation to recover BIND-PETase is not suitable for large-scale applications. Instead, as a whole-cell biocatalyst, BIND-PETase would be possibly recovered by filtration, sedimentation, and decantation methods for repeated use in practical scenarios.[38] The stability during storage and reusability of BIND-PETase would benefit its real-world applications for PET degradation.

**BIND-PETase Was Capable of PET Microplastic Degradation in Wastewater and Postconsumer PET Waste Depolymerization.** Wastewater treatment plants (WWTPs) contribute substantially to the plastic pollution in aquatic environments.[39] Although the current WWTP processes could remove microplastics, given the tremendous volume of effluent, a significant amount of microplastics (∼$5.00 \times 10^5$ to $1.39 \times 10^{10}$ particles/day) could still be released with effluent into the receiving water systems.[39−42] PET accounts for ∼4%−35% of the microplastics discharged.[39]



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Therefore, developing microplastic-targeted treatment technology is critically needed, but such research is still limited. The biocatalyst described in this study could be potentially used in polishing processes targeting at microplastic removal from wastewater effluents. We performed a proof-of-concept experiment to evaluate the efficacy of BIND-PETase for degrading PET microplastics in wastewater effluent. To provide an optimal pH condition for the biocatalytic reaction, the pH of wastewater effluent was adjusted to 9.0 from 6.9. The PET microplastic degradation proceeded steadily during the experimental time frame with the generation of degradation products at a concentration of 4334.5 $\mu$M after 15 days (Figure 3A), indicating the maintenance of catalytic activity of BIND-PETase in wastewater effluent. Meanwhile, the degradation product concentration in wastewater effluent (3316.4 ± 347.5 $\mu$M) was not significantly different from that in a pure buffer (3343.9 ± 160.5 $\mu$M) after 7 day incubation (Student's $t$ test, $p > 0.05$) (Figure 3A), suggesting that the complex wastewater effluent matrix did not significantly influence the BIND-PETase activity. PET microplastics could potentially pose toxic effects to aquatic organisms,[43,44] while the enzymatic PET degradation products, including TPA, MHET, and ethylene glycol, have no or low aquatic ecotoxicity.[45,46] Our results suggested the potential of BIND-PETase for biocatalytic removal of PET microplastics in advanced wastewater treatment applications.

Enzymatic depolymerization is regarded as a promising green route for plastic waste recycling.[47] Herein, we evaluated the effectiveness of BIND-PETase in depolymerization of postconsumer PET materials into monomers for plastic recycling. To mimic the micronization pretreatment of plastic waste in industrial recycling practices,[24,48] PET microplastics generated from discarded drinking water bottles and disposable egg trays were used as feedstock. BIND-PETase could depolymerize the postconsumer PET into monomers TPA and MHET with the total degradation product concentration ranging from 5179.9 to 5216.2 $\mu$M after 7 days (Figure 3B and Figure S9). After reaction for 7 days, the degradation efficiency of commercial PET was 4.79%, while the degradation efficiency of the PET waste materials reached 7.43% for drinking water bottles and 7.48% for disposable egg trays. To explain such difference, we determined the crystallinity of the these PET materials (Section S9), a crucial property governing the enzymatic degradability of plastics.[47] The crystallinities were 36.6%, 27.7%, and 25.2% for the commercial PET, drinking water bottles, and disposable egg trays, respectively (Figure S10), which could explain the degradation variations of these PET samples given the negative relationship between plastic enzymatic degradability and crystallinity.[49] Addition of 0.02% STS could enhance the degradation efficiency by 22.1% for the plastic from drinking water bottles relative to the STS-free reaction (Figure 3C). Notably, a degradation efficiency reached 9.1% for highly crystalline PET (crystallinity > 20%) under the mild conditions at 30 °C used here, while a chemical method using more harsh conditions (100 vol % EtOH with 10 wt % NaOH at 50 °C) had PET degradation efficiency of 11.9%.[50] There is still room for improvement of BIND-PETase compared with another study on enzymatic PET depolymerization (which reported 8% depolymerization at 25 °C in 3 days).[34] Collectively, our results demonstrated the applicability of the BIND-PETase biocatalyst in depolymerizing highly crystalline PET waste, which is promising for plastic recycling applications.

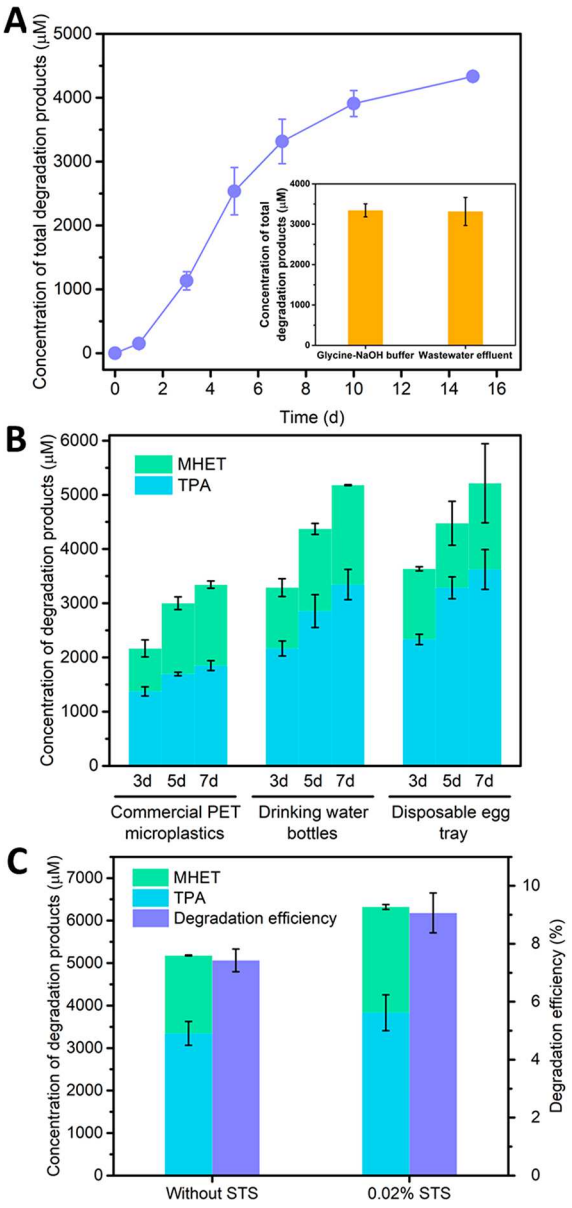


**Figure 3.** Degradation of PET microplastics in wastewater effluent and degradation of postconsumer PET waste by BIND-PETase. (A) PET degradation in secondary wastewater effluent sample (pH 9) with 1.2 U/mL of BIND-PETase and 20 mg of PET microplastics at 30 °C. Inset: Generation of total degradation products in glycine-NaOH buffer and wastewater effluent, respectively, with 1.2 U/mL of BIND-PETase and 20 mg of PET microplastics at 30 °C for 7 days. (B) Degradation of PET from commercial sources, discarded drinking water bottles, and disposable egg trays. The experiments were performed in 50 mM glycine-NaOH (pH 9) with 1.2 U/mL of BIND-PETase and 20 mg of PET microplastics at 30 °C. (C) Degradation of PET from discarded drinking water bottles in the presence of STS surfactant. The experiments were performed in 50 mM glycine-NaOH (pH 9) with 1.2 U/mL of BIND-PETase, 20 mg of PET, and 0.02% STS at 30 °C for 7 days. All experiments were conducted in triplicate, and values represent the mean ± standard error. Error bars are not visible when smaller than the symbol size.

This work leveraged synthetic biology to construct a new type of biocatalyst, BIND-PETase, by functionalizing the *E. coli* curli with PETase for PET degradation for the first time. BIND-PETase can be readily generated and renewed through simple cell cultivation. This biocatalyst can be reused and


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remain stable during storage. Importantly, the capability of BIND-PETase in PET microplastic degradation in a complex wastewater effluent matrix would contribute to developing a biocatalytic strategy for advanced wastewater treatment to mitigate microplastic pollution. Furthermore, the depolymerization of postconsumer PET waste by BIND-PETase provides an opportunity for recycling the plastic waste. For example, the degradation product TPA can be a feedstock for synthesizing new PET or for upcycling to value-added chemicals and materials.[24,51−53] It is noteworthy that the modular BIND platform can be used to create more efficient biocatalysts if engineered with more active PETase variants[54] or with both PETase and MHETase (a MHET-hydrolyzing enzyme) to harness their synergy to accelerate PET degradation.[55] This study opens a new horizon for developing efficient and sustainable biocatalytic approaches to address the critical challenge of plastic waste treatment and recycling.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00332.

Details about chemicals, reagents, and bacterial strains (Section S1). Cultivation and induction conditions for BIND-PETase production (Section S2). Conge red biding assay (Section S3). Western blot analysis (Section S4). Enzyme activity assay (Section S5). Analytical methods for PET degradation products (Section S6). Production of free PETase (Section S7). Degradation of PET by free PETase (Section S8). Measurement of PET plastic crystallinity (Section S9). Bacterial strains and plasmids used in this study (Table S1). Characteristics of the secondary wastewater effluent sample (Table S2). Maps of the constructed recombinant plasmids for the BIND system (Figure S1). Congo red binding assay for BIND-PETase (Figure S2). HPLC chromatogram for the degradation products of PET plastics (Figure S3). Degradation of PET plastics by BIND-PETase at different temperatures (Figure S4). Degradation of PET by free PETase (Figure S5). Degradation efficiency of various mass loads of PET by BIND-PETase (Figure S6). Degradation efficiency of PET by BIND-PETase in the presence of STS surfactant (Figure S7). Storage stability and reusability of BIND-PETase (Figure S8). Degradation efficiency of PET from postconsumer plastic product waste by BIND-PETase (Figure S9). Crystallinity for the PET samples (Figure S10) (PDF)

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### Author Contributions

#Q. Ye and Y. Seo are co-second author.s


### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Wierckx, N.; Narancic, T.; Eberlein, C.; Wei, R.; Drzyzga, O.; Magnin, A.; Ballerstedt, H.; Kenny, S. T.; Pollet, E.; Avérous, L.; O'Connor, K. E.; Zimmermann, W.; Heipieper, H. J.; Prieto, A.; Jiménez, J.; Blank, L. M. Plastic biodegradation: Challenges and opportunities. In *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation*, Steffan, R., Ed.; Springer International Publishing: Cham, 2018; pp 1−29.

(2) Zhang, J.; Wang, L.; Halden, R. U.; Kannan, K. Polyethylene terephthalate and polycarbonate microplastics in sewage sludge collected from the United States. *Environ. Sci. Technol. Lett.* **2019**, *6* (11), 650−655.

(3) Wang, L.; Zhang, J.; Hou, S.; Sun, H. A simple method for quantifying polycarbonate and polyethylene terephthalate microplastics in environmental samples by liquid chromatography-tandem mass spectrometry. *Environ. Sci. Technol. Lett.* **2017**, *4* (12), 530−534.

(4) Moller, J. N.; Loder, M. G. J.; Laforsch, C. Finding Microplastics in Soils: A Review of Analytical Methods. *Environ. Sci. Technol.* **2020**, *54* (4), 2078−2090.

(5) Long, Z.; Pan, Z.; Wang, W.; Ren, J.; Yu, X.; Lin, L.; Lin, H.; Chen, H.; Jin, X. Microplastic abundance, characteristics, and removal in wastewater treatment plants in a coastal city of China. *Water Res.* **2019**, *155*, 255−265.

(6) Liu, H.; Kwak, J. I.; Wang, D.; An, Y. J. Multigenerational effects of polyethylene terephthalate microfibers in *Caenorhabditis elegans*. *Environ. Res.* **2021**, *193*, 110569.

(7) Song, Y.; Cao, C.; Qiu, R.; Hu, J.; Liu, M.; Lu, S.; Shi, H.; Raley-Susman, K. M.; He, D. Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (*Achatina fulica*) after soil exposure. *Environ. Pollut.* **2019**, *250*, 447−455.

(8) Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J. H.; Abu-Omar, M.; Scott, S. L.; Suh, S. Degradation rates of plastics in the environment. *ACS Sustain Chem. Eng.* **2020**, *8* (9), 3494−3511.

(9) Semba, T.; Sakai, Y.; Sakanishi, T.; Inaba, A. Greenhouse gas emissions of 100% bio-derived polyethylene terephthalate on its life

cycle compared with petroleum-derived polyethylene terephthalate. *J. Clean Prod* **2018**, *195*, 932−938.
(10) Rochman, C. M.; Browne, M. A.; Halpern, B. S.; Hentschel, B. T.; Hoh, E.; Karapanagioti, H. K.; Rios-Mendoza, L. M.; Takada, H.; Teh, S.; Thompson, R. C. Policy: Classify plastic waste as hazardous. *Nature* **2013**, *494* (7436), 169−171.
(11) Zhou, C.; Fang, W.; Xu, W.; Cao, A.; Wang, R. Characteristics and the recovery potential of plastic wastes obtained from landfill mining. *J. Clean Prod* **2014**, *80*, 80−86.
(12) Li, C. T.; Zhuang, H. K.; Hsieh, L. T.; Lee, W. J.; Tsao, M. C. PAH emission from the incineration of three plastic wastes. *Environ. Int.* **2001**, *27* (1), 61−67.
(13) Ragaert, K.; Delva, L.; Van Geem, K. Mechanical and chemical recycling of solid plastic waste. *Waste Manag* **2017**, *69*, 24−58.
(14) Rahimi, A.; García, J. M. Chemical recycling of waste plastics for new materials production. *Nat. Rev. Chem.* **2017**, *1* (6), 0046.
(15) Garcia, J. M.; Robertson, M. L. The future of plastics recycling. *Science* **2017**, *358* (6365), 870−872.
(16) Wei, R.; Zimmermann, W. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? *Microb Biotechnol* **2017**, *10* (6), 1308−1322.
(17) Ellis, L. D.; Rorrer, N. A.; Sullivan, K. P.; Otto, M.; McGeehan, J. E.; Román-Leshkov, Y.; Wierckx, N.; Beckham, G. T. Chemical and biological catalysis for plastics recycling and upcycling. *Nat. Catal* **2021**, *4* (7), 539−556.
(18) Lu, H.; Diaz, D. J.; Czarnecki, N. J.; Zhu, C.; Kim, W.; Shroff, R.; Acosta, D. J.; Alexander, B. R.; Cole, H. O.; Zhang, Y.; Lynd, N. A.; Ellington, A. D.; Alper, H. S. Machine learning-aided engineering of hydrolases for PET depolymerization. *Nature* **2022**, *604* (7907), 662−667.
(19) Singh, A.; Rorrer, N. A.; Nicholson, S. R.; Erickson, E.; DesVeaux, J. S.; Avelino, A. F. T.; Lamers, P.; Bhatt, A.; Zhang, Y.; Avery, G.; Tao, L.; Pickford, A. R.; Carpenter, A. C.; McGeehan, J. E.; Beckham, G. T. Techno-economic, life-cycle, and socioeconomic impact analysis of enzymatic recycling of poly(ethylene terephthalate). *Joule* **2021**, *5* (9), 2479−2503.
(20) Austin, H. P.; Allen, M. D.; Donohoe, B. S.; Rorrer, N. A.; Kearns, F. L.; Silveira, R. L.; Pollard, B. C.; Dominick, G.; Duman, R.; El Omari, K.; Mykhaylyk, V.; Wagner, A.; Michener, W. E.; Amore, A.; Skaf, M. S.; Crowley, M. F.; Thorne, A. W.; Johnson, C. W.; Woodcock, H. L.; McGeehan, J. E.; Beckham, G. T. Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115* (19), E4350−E4357.
(21) Yoshida, S.; Hiraga, K.; Takehana, T.; Taniguchi, I.; Yamaji, H.; Maeda, Y.; Toyohara, K.; Miyamoto, K.; Kimura, Y.; Oda, K. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* **2016**, *351* (6278), 1196−1199.
(22) Kawai, F.; Kawabata, T.; Oda, M. Current state and perspectives related to the polyethylene terephthalate hydrolases available for biorecycling. *ACS Sustain Chem. Eng.* **2020**, *8* (24), 8894−8908.
(23) Taniguchi, I.; Yoshida, S.; Hiraga, K.; Miyamoto, K.; Kimura, Y.; Oda, K. Biodegradation of PET: Current status and application aspects. *ACS Catal.* **2019**, *9* (5), 4089−4105.
(24) Tournier, V.; Topham, C. M.; Gilles, A.; David, B.; Folgoas, C.; Moya-Leclair, E.; Kamionka, E.; Desrousseaux, M. L.; Texier, H.; Gavalda, S.; Cot, M.; Guemard, E.; Dalibey, M.; Nomme, J.; Cioci, G.; Barbe, S.; Chateau, M.; Andre, I.; Duquesne, S.; Marty, A. An engineered PET depolymerase to break down and recycle plastic bottles. *Nature* **2020**, *580* (7802), 216−219.
(25) Cui, Y.; Chen, Y.; Liu, X.; Dong, S.; Tian, Y. e.; Qiao, Y.; Mitra, R.; Han, J.; Li, C.; Han, X.; Liu, W.; Chen, Q.; Wei, W.; Wang, X.; Du, W.; Tang, S.; Xiang, H.; Liu, H.; Liang, Y.; Houk, K. N.; Wu, B. Computational redesign of a PETase for plastic biodegradation under ambient condition by the GRAPE strategy. *ACS Catal.* **2021**, *11* (3), 1340−1350.
(26) Bell, E.; Smithson, R.; Kilbride, S.; Foster, J.; Hardy, F.; Ramachandran, S.; Tedstone, A.; Haigh, S.; Garforth, A.; Day, P. Directed evolution of an efficient and thermostable PET depolymerase.. *ChemRxiv Preprint*, 2021.
(27) Zhu, B.; Chen, Y.; Wei, N. Engineering biocatalytic and biosorptive materials for environmental applications. *Trends Biotechnol* **2019**, *37* (6), 661−676.
(28) Nguyen, P. Q.; Botyanszki, Z.; Tay, P. K.; Joshi, N. S. Programmable biofilm-based materials from engineered curli nanofibres. *Nat. Commun.* **2014**, *5*, 4945.
(29) Chapman, M. R.; Robinson, L. S.; Pinkner, J. S.; Roth, R.; Heuser, J.; Hammar, M.; Normark, S.; Hultgren, S. J. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **2002**, *295* (5556), 851−855.
(30) Jiang, L.; Song, X.; Li, Y.; Xu, Q.; Pu, J.; Huang, H.; Zhong, C. Programming integrative extracellular and intracellular biocatalysis for rapid, robust, and recyclable synthesis of trehalose. *ACS Catal.* **2018**, *8* (3), 1837−1842.
(31) Tay, P. K. R.; Manjula-Basavanna, A.; Joshi, N. S. Repurposing bacterial extracellular matrix for selective and differential abstraction of rare earth elements. *Green Chem.* **2018**, *20* (15), 3512−3520.
(32) Vidal, O.; Longin, R.; Prigent-Combaret, C.; Dorel, C.; Hooreman, M.; Lejeune, P. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression. *J. Bacteriol.* **1998**, *180* (9), 2442−2449.
(33) Liu, C.; Shi, C.; Zhu, S.; Wei, R.; Yin, C. C. Structural and functional characterization of polyethylene terephthalate hydrolase from Ideonella sakaiensis. *Biochem. Biophys. Res. Commun.* **2019**, *508* (1), 289−294.
(34) Gercke, D.; Furtmann, C.; Tozakidis, I. E. P.; Jose, J. Highly crystalline post-consumer PET waste hydrolysis by surface displayed PETase using a bacterial whole-cell biocatalyst. *ChemCatChem.* **2021**, *13* (15), 3479−3489.
(35) Furukawa, M.; Kawakami, N.; Oda, K.; Miyamoto, K. Acceleration of enzymatic degradation of poly(ethylene terephthalate) by surface coating with anionic surfactants. *ChemSusChem* **2018**, *11* (23), 4018−4025.
(36) Schwaminger, S. P.; Fehn, S.; Steegmüller, T.; Rauwolf, S.; Löwe, H.; Pflüger-Grau, K.; Berensmeier, S. Immobilization of PETase enzymes on magnetic iron oxide nanoparticles for the decomposition of microplastic PET. *Nanoscale Adv.* **2021**, *3* (15), 4395−4399.
(37) Chen, Z.; Wang, Y.; Cheng, Y.; Wang, X.; Tong, S.; Yang, H.; Wang, Z. Efficient biodegradation of highly crystallized polyethylene terephthalate through cell surface display of bacterial PETase. *Sci. Total Environ.* **2020**, *709*, 136138.
(38) Sheldon, R. A.; Basso, A.; Brady, D. New frontiers in enzyme immobilisation: robust biocatalysts for a circular bio-based economy. *Chem. Soc. Rev.* **2021**, *50* (10), 5850−5862.
(39) Sun, J.; Dai, X.; Wang, Q.; van Loosdrecht, M. C. M.; Ni, B. J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21−37.
(40) Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* **2016**, *50* (11), 5800−5808.
(41) Liu, W.; Zhang, J.; Liu, H.; Guo, X.; Zhang, X.; Yao, X.; Cao, Z.; Zhang, T. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. *Environ. Int.* **2021**, *146*, 106277.
(42) Ziajahromi, S.; Neale, P. A.; Rintoul, L.; Leusch, F. D. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. *Water Res.* **2017**, *112*, 93−99.
(43) Piccardo, M.; Provenza, F.; Grazioli, E.; Cavallo, A.; Terlizzi, A.; Renzi, M. PET microplastics toxicity on marine key species is influenced by pH, particle size and food variations. *Sci. Total Environ.* **2020**, *715*, 136947.
(44) Heindler, F. M.; Alajmi, F.; Huerlimann, R.; Zeng, C.; Newman, S. J.; Vamvounis, G.; van Herwerden, L. Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)-


https://doi.org/10.1021/acs.estlett.2c00332
*Environ. Sci. Technol. Lett.* 2022, 9, 650−657

phthalate on the calanoid copepod, *Parvocalanus crassirostris*. *Ecotoxicol Environ. Saf* **2017**, *141*, 298−305.
(45) Djapovic, M.; Milivojevic, D.; Ilic-Tomic, T.; Ljesevic, M.; Nikolaivits, E.; Topakas, E.; Maslak, V.; Nikodinovic-Runic, J. Synthesis and characterization of polyethylene terephthalate (PET) precursors and potential degradation products: Toxicity study and application in discovery of novel PETases. *Chemosphere* **2021**, *275*, 130005.
(46) Staples, C. A.; Williams, J. B.; Craig, G. R.; Roberts, K. M. Fate, effects and potential environmental risks of ethylene glycol: a review. *Chemosphere* **2001**, *43* (3), 377−383.
(47) Wei, R.; Zimmermann, W. Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. *Microb Biotechnol* **2017**, *10* (6), 1302−1307.
(48) Awaja, F.; Pavel, D. Recycling of PET. *Eur. Polym. J.* **2005**, *41* (7), 1453−1477.
(49) Wei, R.; Breite, D.; Song, C.; Grasing, D.; Ploss, T.; Hille, P.; Schwerdtfeger, R.; Matysik, J.; Schulze, A.; Zimmermann, W. Biocatalytic degradation efficiency of postconsumer polyethylene terephthalate packaging determined by their polymer microstructures. *Adv. Sci. (Weinh)* **2019**, *6* (14), 1900491.
(50) Ügdüler, S.; Van Geem, K. M.; Denolf, R.; Roosen, M.; Mys, N.; Ragaert, K.; De Meester, S. Towards closed-loop recycling of multilayer and coloured PET plastic waste by alkaline hydrolysis. *Green Chem.* **2020**, *22* (16), 5376−5394.
(51) Sadler, J. C.; Wallace, S. Microbial synthesis of vanillin from waste poly(ethylene terephthalate). *Green Chem.* **2021**, *23* (13), 4665−4672.
(52) Tiso, T.; Narancic, T.; Wei, R.; Pollet, E.; Beagan, N.; Schroder, K.; Honak, A.; Jiang, M.; Kenny, S. T.; Wierckx, N.; Perrin, R.; Averous, L.; Zimmermann, W.; O'Connor, K.; Blank, L. M. Towards bio-upcycling of polyethylene terephthalate. *Metab Eng.* **2021**, *66*, 167−178.
(53) El-Sayed, E.-S. M.; Yuan, D. Waste to MOFs: sustainable linker, metal, and solvent sources for value-added MOF synthesis and applications. *Green Chem.* **2020**, *22* (13), 4082−4104.
(54) Zhu, B.; Wang, D.; Wei, N. Enzyme discovery and engineering for sustainable plastic recycling. *Trends Biotechnol* **2022**, *40* (1), 22−37.
(55) Knott, B. C.; Erickson, E.; Allen, M. D.; Gado, J. E.; Graham, R.; Kearns, F. L.; Pardo, I.; Topuzlu, E.; Anderson, J. J.; Austin, H. P.; Dominick, G.; Johnson, C. W.; Rorrer, N. A.; Szostkiewicz, C. J.; Copie, V.; Payne, C. M.; Woodcock, H. L.; Donohoe, B. S.; Beckham, G. T.; McGeehan, J. E. Characterization and engineering of a two-enzyme system for plastics depolymerization. *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117* (41), 25476−25485.





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