Enzyme Selection, Optimization, and Production toward Biodegradation of Waste Poly(ethylene terephthalate) at Scale

DONGMING XIE[1], Ya-Hue Soong[1], Umer Abid[1], Allen C. Chang[1], Christian Ayafor[1], Akanksha Patel[1], Jin Xu[1], Carl W. Lawton[1], Hsi-Wu Wong[1], and Margaret Sobkowicz J[1]

[1]University of Massachusetts Lowell

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Abstract

Poly(ethylene terephthalate) (PET) is one of the world's most widely used polyester plastics. Due to its chemical stability, PET is extremely difficult to hydrolyze in a natural environment. Recent discoveries in new polyester hydrolases and breakthroughs in enzyme engineering strategies have inspired enormous research on biorecycling of PET. This study summarizes our research efforts toward large-scale, efficient, and economical biodegradation of waste PET, including PET hydrolase selection and optimization, high-yield enzyme production, and high-capacity enzymatic degradation of waste PET. First, genes encoding PETase and MHETase from *Ideonella sakaiensis* and the ICCG variant of leaf-branch compost cutinase (LCC) were codon-optimized and expressed in *Escherichia coli* BL21(DE3) for high-yield production. To further lower the enzyme production cost, a *pelB* leader sequence was fused to *LCC* so that the enzyme can be secreted into the medium to facilitate recovery. To help bind the enzyme on the hydrophobic surface of PET, a substrate-binding module in a polyhydroxyalkanoate depolymerase from *Alcaligenes faecalis* (PBM) was fused to the C-terminus of *LCC*. The resulting four different LCC variants (LCC, PelB-LCC, LCC-PBM, and PelB-LCC-PBM), together with PETase and MHETase, were compared for PET degradation efficiency. A fed-batch fermentation process was developed to produce the target enzymes up to 1.2 g/L. Finally, the best enzyme, PelB-LCC, was selected and used for the efficient degradation of 200 g/L recycled PET in a well-controlled, stirred-tank reactor. The results will help develop an economical and scalable biorecycling process toward a circular PET economy.

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