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Enzymatic recovery of terephthalic acid from PET-PE multilayer materials using crude glycosylated PETase

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PURPOSE OF THE ABSTRACT

Over the last 70 years, plastics have become a ubiquitous, indispensable part of society, but also a large waste stream. While about one-third of plastic waste is recycled, the remaining two-thirds is landfilled or incinerated, leading to unwanted accumulation in nature or CO2 emissions. One of the main types of plastic is polyethylene terephthalate (PET), which is a polyester consisting of ester-linked terephthalic acid and ethylene glycol. Although re-use or mechanical recycling is possible for products such as PET bottles, these solutions are not feasible for many other types of PET waste, such as common multilayer packaging materials. Recycling of PET within multilayer waste could be achieved by enzymatic hydrolysis and recovery of the monomers, making use of the intrinsic selectivity of enzymes. Within the EU-funded Horizon 2020 research project “ENZYCLE”, we aim to develop an enzymatic PET recycling process and to demonstrate the industrial feasibility at pilot scale. We selected two enzymes with high reported PETase activity: leaf-compost cutinase (LCC) and a recently described polyester hydrolase (PHL7). His6-tagged enzymes were heterologously expressed in the yeast Pichia pastoris, increasing their thermostability through glycosylation, and purified through affinity chromatography. At 70°C and pH 8 maintained by sodium phosphate buffer, the purified PETases efficiently hydrolysed amorphous PET film (40-50 mg) at a rate comparable to that of non-glycosylated LCC produced in Escherichia coli (1.2-1.4 mg PET µg-1 PETase d-1). We then explored the use of crude PETase in the form of P. pastoris culture filtrate for techno-economical purposes. The PETase activity of crude PHL7 was unaffected, whereas crude LCC showed partial PETase inhibition by an unknown component. Next, amorphous PET film hydrolysis was tested at 10 g scale (10 g/L) in pH-controlled bioreactors using crude PETase with and without sodium phosphate buffer, and monitored by HPLC analysis of monomer concentrations and by weight loss measurement of residual PET. Performance of crude LCC was unaffected by sodium phosphate concentration, whereas crude PHL7 required a high concentration (1 M) of sodium phosphate for thermostability at >60°C. Using crude LCC at 70°C, 98% of the PET was hydrolyzed in 43 hours at a constant rate (5.6 g L-1 d-1). We next tested two types of PET-polyethylene multilayer packaging materials (>96% w/w PET), which could both also be hydrolyzed almost completely (>96%) at 65°C in 92 hours, without any pretreatment required. Incubation at 70°C caused the PET in these materials to crystallize, lowering the final extent of hydrolysis. The substrate loading was raised to 200 g/L without loss of performance. Produced terephthalic acid was recovered by precipitation through addition of H2SO4 and filtration with yields of >77%, with the only contaminants being traces of mono(2-hydroxyethyl)terephthalic acid and 2 mol% isophthalic acid, which is commonly present in PET as co-monomer. Residual PET-polyethylene material still contained a significant fraction of PET (>35% w/w), which may be removable through hydrolysis with strong acid or base, enabling mechanical

recycling of the polyethylene fraction. In conclusion, we designed a promising enzymatic process for back-to-monomer recycling of PET from multilayer packaging material using crude LCC produced in P. pastoris as biocatalyst. Future efforts within the project will include further testing of the robustness, a techno-economic analysis, and upscaling to pilot scale.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

PETase | hydrolysis | recycling | plastics

BIBLIOGRAPHY