

# Towards a Circular Textile Economy: Enzymatic Recycling of Textile Fibres

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## Flagship: Textiles

### Objective

Enzymatic back-to-monomer recycling of textile fibres offers a mild and sustainable strategy, but is challenging due to the crystallinity of the material. Here, we aim to investigate whether lytic polysaccharide monoxygenases (LPMOs) can aid in the depolymerisation of crystalline polyester fibres by hydrolase enzymes.

### Key Results

An increase in (amorphous) PET depolymerisation was observed when hydrolase was incubated with LPMO's (Table 1A). However, the LPMO is not catalytically active under the reaction conditions investigated. We hypothesized that this effect could be due to a surfactant effect the protein molecules exert, allowing the hydrolase to bind more effectively to the hydrophobic PET surface. This effect was also observed using an inactive control protein (Bovine Serum Albumin, BSA).

Treatment of crystalline PET fibres (obtained from a pillow) using the same enzymatic methods did not result in any significant depolymerization (Table 1C).

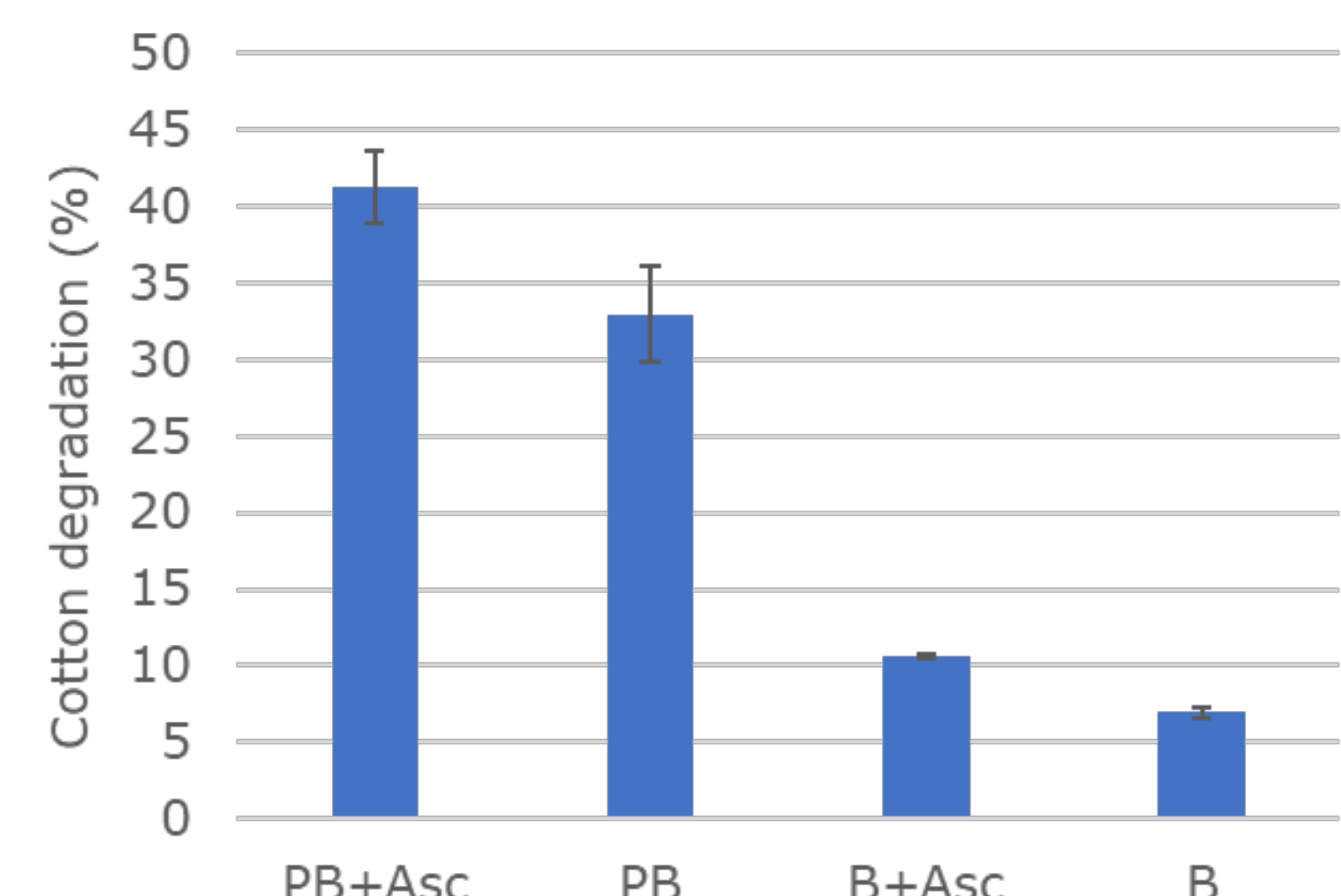
For subsequent experiments, we procured a textile material made out of 52:48 polyester:cotton with the aim to enzymatically degrade the cotton using a commercially available cocktail of polysaccharide degrading enzymes (CellicCtec3). The material was bead-milled to obtain small particles, which were then further refined into a fine powder using planetary ball milling. Gratifyingly, we observed the successful degradation of the cotton textile fabric (Figure 2). The utilization of planetary ball milling significantly enhanced the breakdown of cotton. Additional experiments are required to assess the selectivity of the reaction and to improve the efficiency of this process.

Enzyme	Ascorbic	Temp	pH	PET (mg)	Enzyme	Weight Loss (m
MtLPMO9B + MtAA16A	Yes	30	5	50	2 uM	0,3
MtLPMO9B + MtAA16A	No	30	5	50	2 uM	0,7
NcLPMO9C + NcLPMO9M	Yes	30	5	50	2 uM	0,6
NcLPMO9C + NcLPMO9M	No	30	5	50	2 uM	-0,9
LCC	No	70	8	50	25 ug	-22,3
LCC	Yes	30	5	50	25 ug	0,6
LCC	No	30	5	50	25 ug	0,7
LCC + All 4 LPMO's	Yes	30	5	50	25 ug + 2 uM	-0,3
LCC + All 4 LPMO's	No	30	5	50	25 ug + 2 uM	0
LCC + All 4 LPMO's	Yes	70	8	50	25 ug + 2 uM	-24,5
LCC + All 4 LPMO's	No	70	8	50	25 ug + 2 uM	-32,3
No enzyme	Yes	30	5	50		0
No enzyme	No	30	5	50		0,1
No enzyme	Yes	70	8	50		0,1
No enzyme	No	70	8	50		0,2
BSA	No	70	8	50	100 ug	-0,1
BSA + LCC	No	70	8	50	125 ug	-31,7

Enzyme	Ascorbic	Temp	pH	PET (mg)	Enzyme	Weight Lo
LCC	No	70	8	50	25 ug	-0,3
LCC + All 4 LPMO's	Yes	70	8	50	25 ug + 2 uM	-0,1
LCC + All 4 LPMO's	No	70	8	50	25 ug + 2 uM	0,5
BSA + LCC	No	70	8	50	125 ug	0,2
All 4 LPMO's then LCC	Yes	30	5	50	25 ug + 2 uM	-0,3
All 4 LPMO's then LCC	Yes	30->70	5	50	25 ug + 2 uM	0,6
All 4 LPMO's then LCC	Yes	30->70	5->8	50	25 ug + 2 uM	0,1
BSA then LCC	Yes	30->70	5->8	50	25 ug + 2 uM	0,9

**Figure 1: A)** Weight loss of amorphous PET film (Goodfellow) under several reaction conditions (pH, temperature and presence of ascorbic acid). **B)** Reaction scheme of enzymatic PET hydrolysis into terephthalic acid and ethylene glycol monomers. **C)** Weight loss of crystalline PET fibers under a variety of reaction conditions.



**Figure 2:** Degree of cotton degradation in a polyester/cotton textile material using CellicCtec3. Reaction conditions: 24 mg enzyme/g substrate, 100 mM NaAc buffer, pH 5.0, 50 °C, for 72h adding 1 mM ascorbic acid (Asc) every 24h. Degradation percentage based on glucose release. B = Bead milled, PB = Bead milled + Planetary ball milled.

### Lessons learned and readiness

Throughout the course of this project, our team acquired a better understanding of the complex nature of textile fibers, thereby becoming aware of the challenges associated with their recycling. This heightened awareness underscored the need for innovative solutions in the realm of textile waste management.

Moreover, the collaboration between our research groups proved very educational. We learned a lot about novel enzyme classes from our expert colleagues. Therefore, we hope this project was able to form the foundations of future collaborative research between our groups.

In terms of Technical Readiness Level, the project has made first steps towards a proof-of-concept. We were able to degrade a commercially available textile enzymatically to some extent (TRL3-4). Further experiments are required to elevate the TRL of this technology.

### Next steps

Additional planned experiments involve the determination of the crystallinity of the polyester textile material after planetary ball milling and incubation with CellicCtec3. This treatment might allow improved enzymatic ester hydrolysis, which will also be tested.

Based on our results, we recognize that further investigation of textile pretreatments to reduce crystallinity and enhance availability could prove beneficial to the success of enzymatic textile recycling.

In their natural function, LPMOs are highly evolved to act upon polysaccharide substrates. To allow these enzymes to target polyester materials might require rigorous optimization and enzyme engineering.

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