



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

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Background

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 (fig. 1).

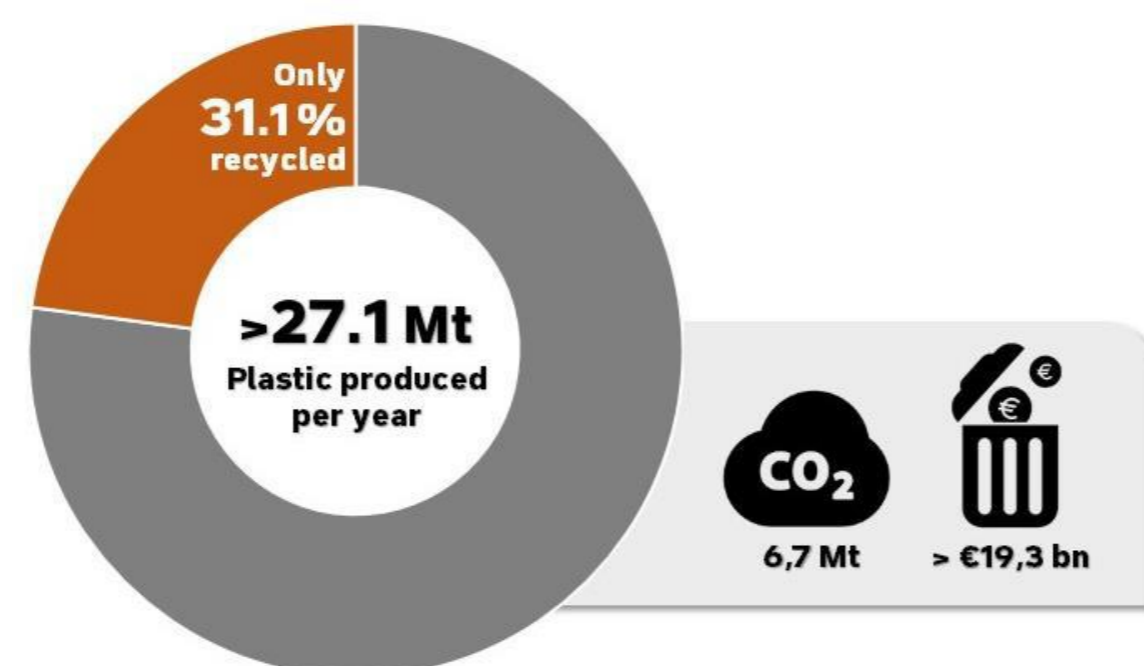


Figure 1. Production and fate of plastics in the EU28+NO/CH in 2017 (PlasticsEurope. Plastics – the Facts 2018).

Objective

One of the main types of plastic is **polyethylene terephthalate (PET)**. Mechanical recycling is widely used to reshape PET bottles into new PET products. However, this technology is not applicable to common PET multilayer packaging waste. Within the ENZYCLE project, we aim to develop an **enzymatic recycling** process targeting **PET-polyolefin multilayer waste** and demonstrate it at pilot scale.

Process overview

PETase: Leaf compost cutinase (LCC) produced in *Pichia pastoris*, leading to N-glycosylation and higher thermostability¹. (Crude broth)

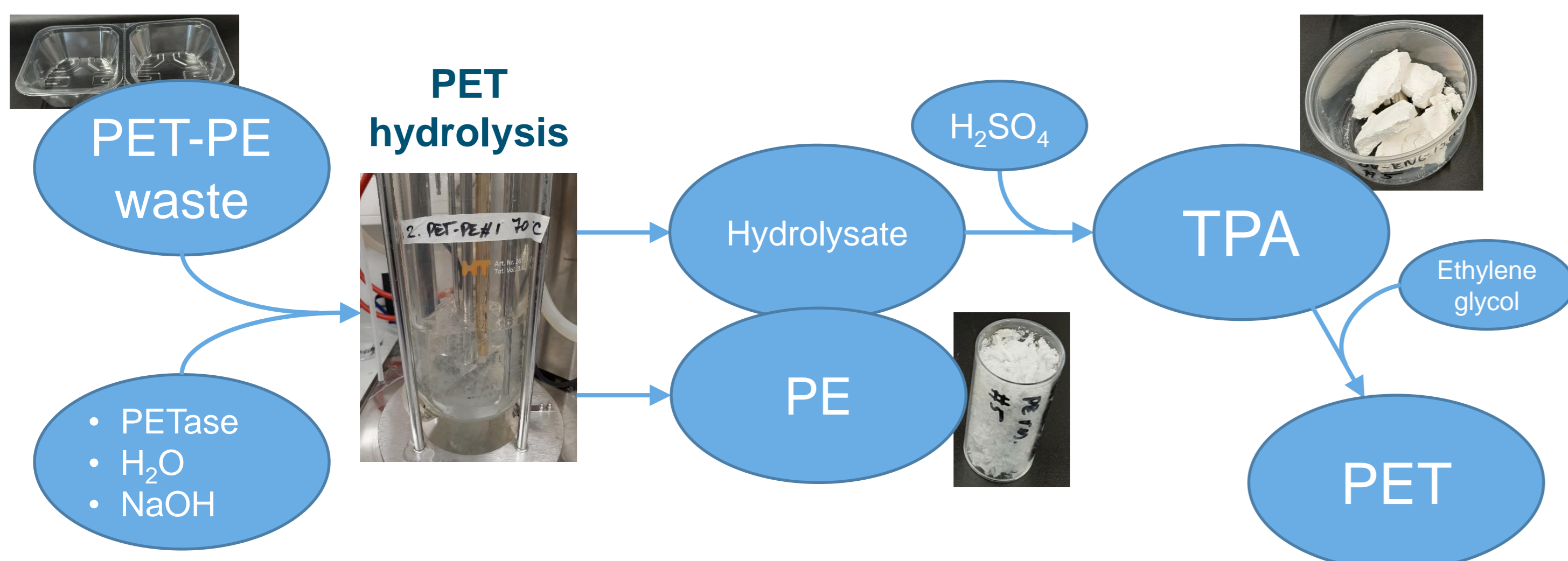


Figure 2. Process flowchart of enzymatic PET-PE recycling.

Clamshell trays

Clamshell trays and containers such as depicted above are a common type of PET-PE multilayer waste, which is currently not recycled.

Substrate: trays (Termoformas), 96-97% amorphous PET, 3-4% PE
Rate: ~0.8 μmol_{TPA}/h/U_{LCC}, half the rate of PET-only substrate (fig. 3).
Removal: 97% PET hydrolysis of 10 g_{PET-PE}/L at 65°C in 115 hours.
PET-PE loading: increased to 200 g/L with >97% hydrolysis (fig. 4).
TPA yield: 77-101%

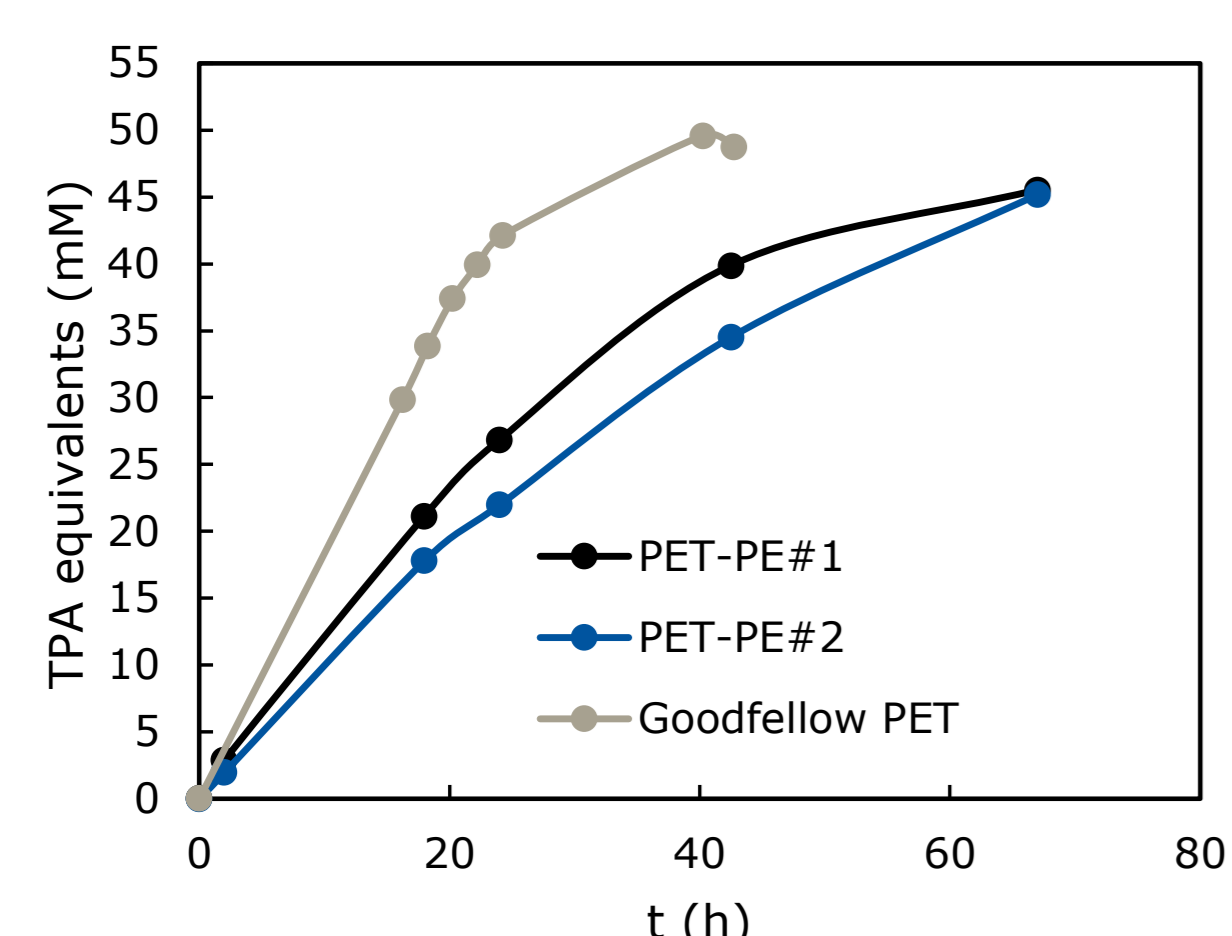


Figure 3. Enzymatic hydrolysis of amorphous Goodfellow PET sheet and two types of PET-PE clamshell trays (10 g_{PET-PE}/L, 75 U_{LCC}/g_{PET-PE}, 70°C).

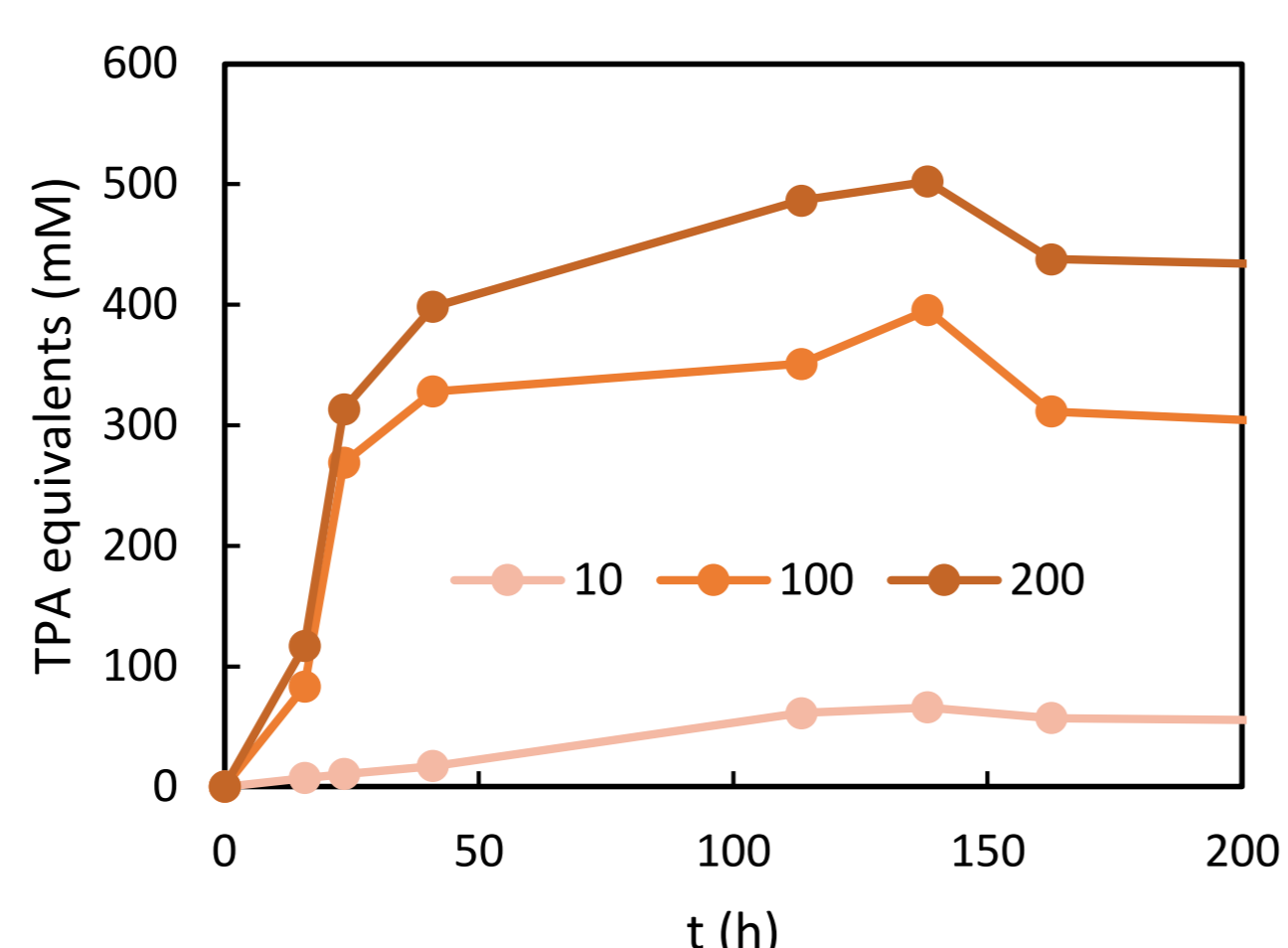


Figure 4. Enzymatic hydrolysis of different loadings of PET-PE clamshell material (10/100/200 g/L) with 55 U_{LCC}/g_{PET-PE} at 65°C.

PET-PE production waste

Substrate: PET-PE cuttings (Aliplast), 91% amorphous PET, 9% PE

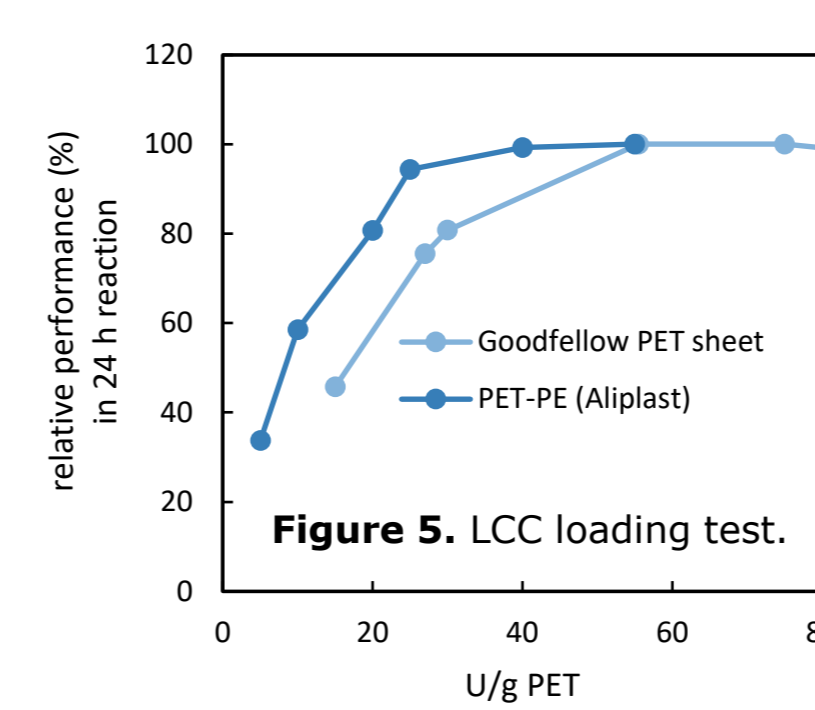


Figure 5. LCC loading test.

Saturation of PET surface by LCC

– PET sheet ~55 U/g_{PET}

– PET-PE ~25 U/g_{PET}

Indicates PET-PE has less PET surface area

- Low stirring (100 RPM) is sufficient
- Ultrafiltration of crude LCC results in higher rate: 2 μmol_{TPA}/h/U_{LCC}

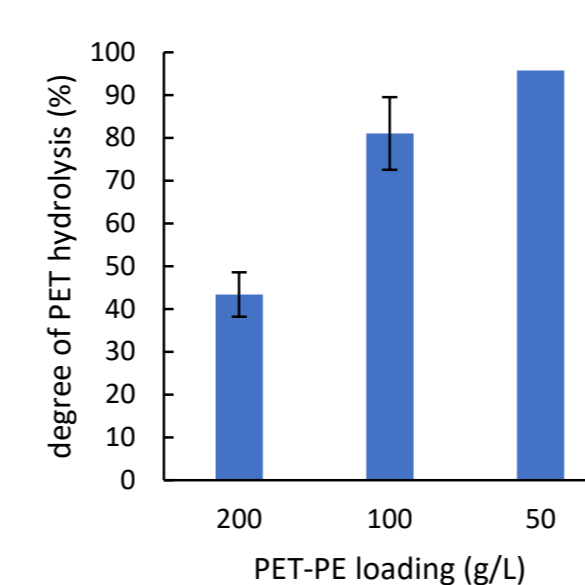


Figure 7. PET hydrolysis degrees at different loading of PET-PE cuttings.

200 g_{PET-PE}/L causes incomplete PET hydrolysis, probably due to release of inhibitors

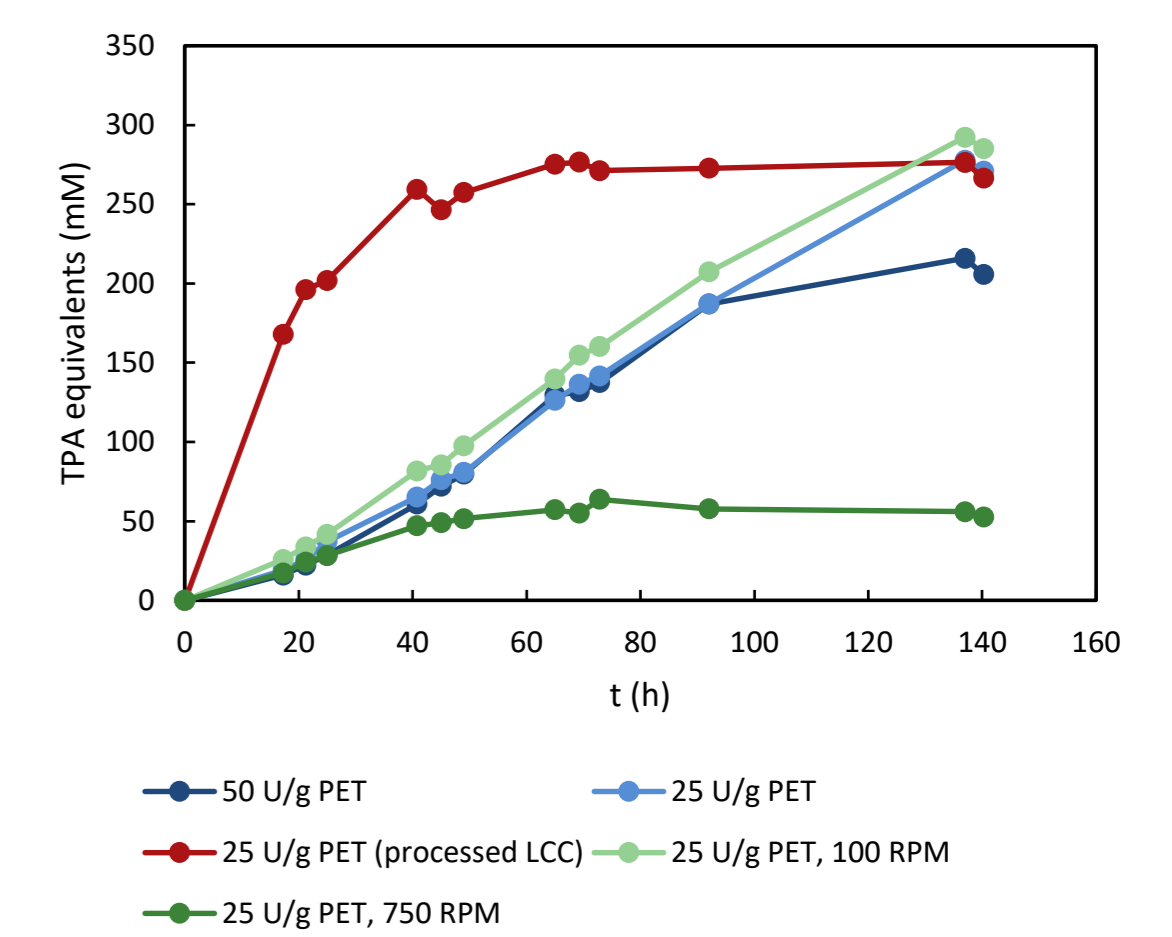


Figure 6. Enzymatic hydrolysis of PET-PE cuttings (200 g_{PET-PE}/L, 68°C) with 25 or 50 U_{LCC}/g_{PET-PE} and 100, 500 or 750 RPM stirring.

Terephthalic acid precipitation and purity analysis

After depolymerization of PET and filtering out residual plastic, TPA was recovered from the hydrolysate. The hydrolysate was clarified by centrifugation, and TPA was precipitated out of solution by addition of sulfuric acid (H₂SO₄), collected by filtration, washed, and dried.

The yield was 76-101%, with the cause for variation unclear.

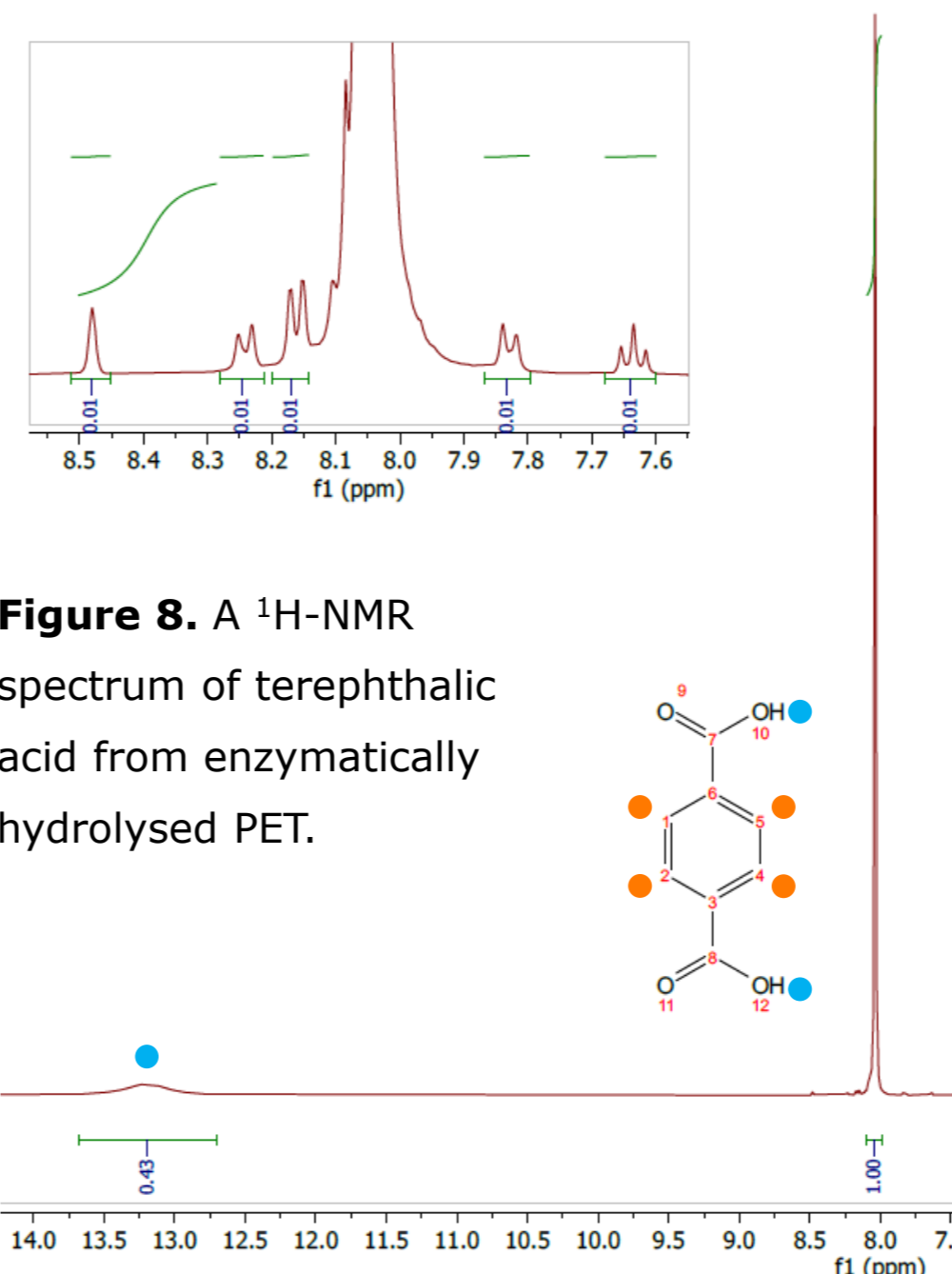


Figure 8. A ¹H-NMR spectrum of terephthalic acid from enzymatically hydrolysed PET.

TPA purity

- Main contaminants (1-3%) were isophthalic acid and mono-1-hydroxyethylterephthalate (fig. 8), both compatible with repolymerization to PET
- Trace impurities (<0.13%) of benzoic, para-toluic and trimellitic acid, dichlorobenzene and trichlorobenzene. PETase inhibitors?
- Presence of elements S, Na and P (0.18-0.57%) indicates salts and/or protein, which are not compatible with repolymerization and need to be removed

Residual (PET-)PE

Within the project, it is envisioned to recycle the PE layer of processed PET-PE waste. The residual plastic of the hydrolysed clamshell PET-PE still contained 35-58% w/w PET (fig. 9). Further processing is thus necessary to obtain pure residual PE suitable for mechanical recycling.

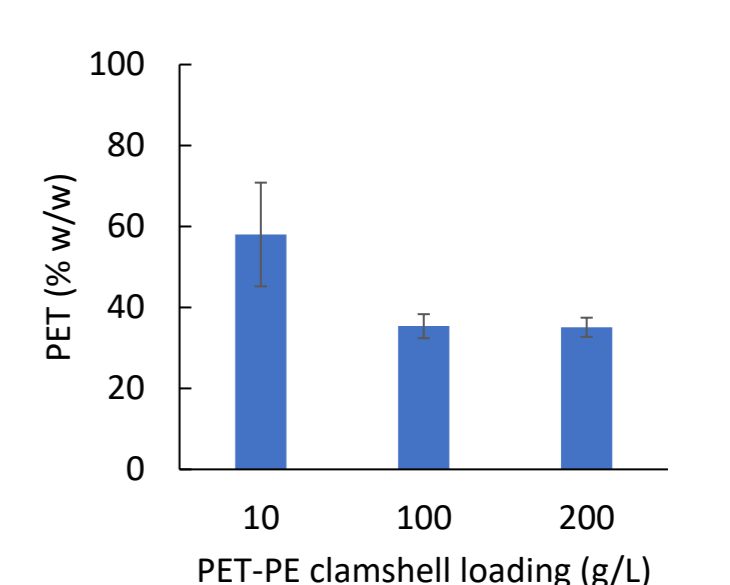


Figure 9. PET in residual PET-PE

Conclusions

Efficient recycling of PET-PE waste is possible via PET hydrolysis using crude *Pichia*-produced LCC. Future efforts will focus on upscaling, TPA purification and repolymerization into recycled PET.



References

1. Shirke, A. N., C. White, J. A. Englaender, A. Zwarycz, G. L. Butterfoss, R. J. Linhardt and R. A. Gross (2018). Stabilizing Leaf and Branch Compost Cutinase (LCC) with Glycosylation: Mechanism and Effect on PET Hydrolysis. *Biochemistry* 57(7): 1190-1200.