Influence of Surface Morphology on Enzymatic Plastic Degradation

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ABSTRACT

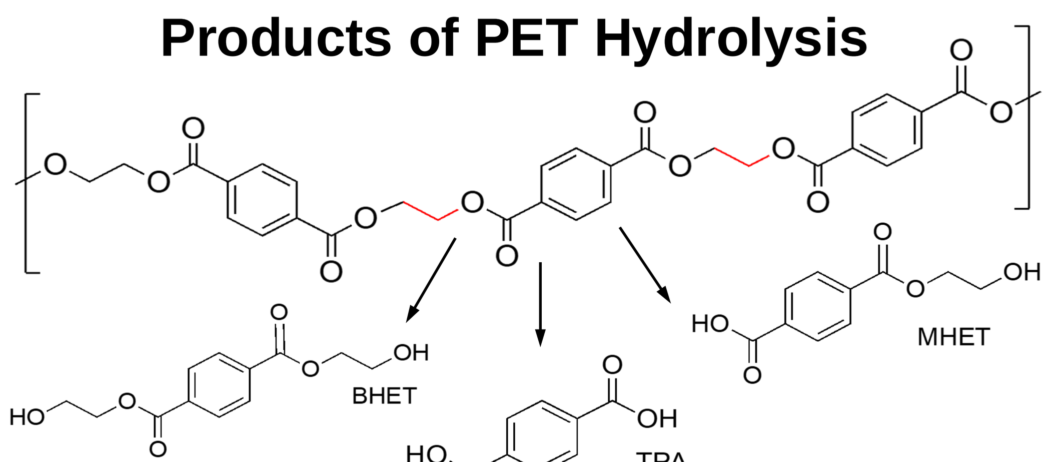
Polyethylene terephthalate (PET) is a widely used thermoplastic polymer, commonly found in single-use packaging due to its high chemical stability and resistance to degradation.[1] As a result, PET waste accumulates throughout the biosphere and significantly contributes to the global pollution crisis.[2] *Ideonella sakaiensis* is a bacterium capable of hydrolyzing PET into small molecules via the enzyme polyethylene terephthalate hydrolase (PETase).[3] Engineered variants, such as FAST-PETase, have been shown to degrade selected PET products, such as water bottles and food containers.[4] While factors such as crystallinity and surface morphology are known to influence the enzymatic depolymerization of plastics,[5] the specific impact of surface abrasion on FAST-PETase efficiency remains unclear. In this study, we produced high yields of recombinant FAST-PETase in *E. coli* and adapted a UV-vis spectroscopic assay to monitor product formation at 240 nm. We tested a range of PET substrates with varying surface morphologies. Our findings highlight the potential for further enhancing FAST-PETase activity to improve the degradation of diverse plastic products and address plastic waste accumulation.

INTRODUCTION

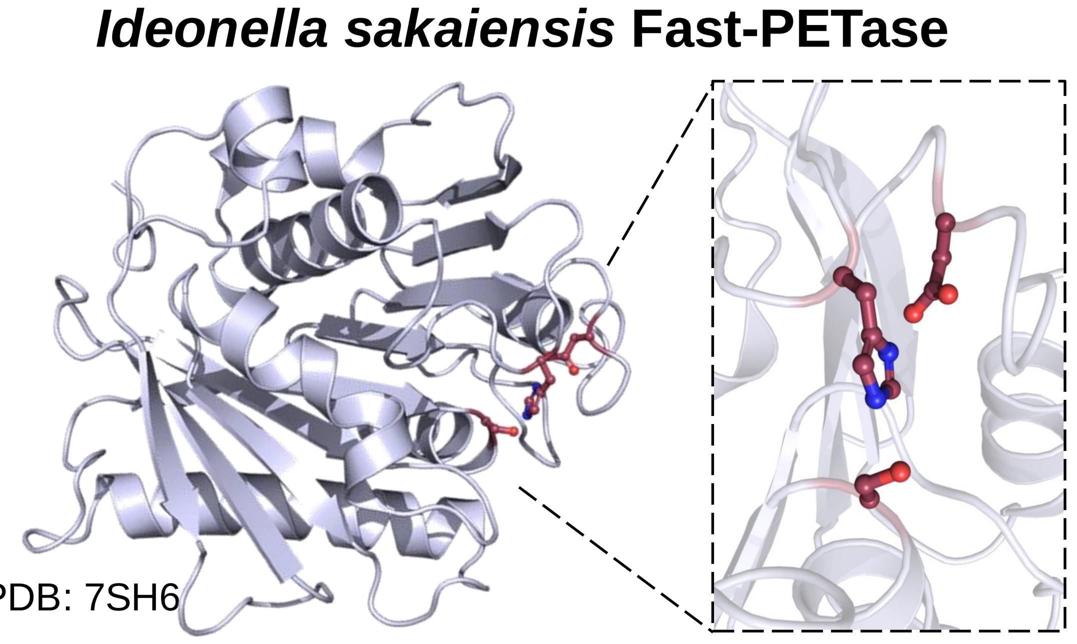
Various Plastic Materials

1 PETE – Polyethylene terephthalate (PET)
2 HDPE – High-Density Polyethylene (HDPE)
3 V – Polyvinyl chloride (V)
4 LDPE – Low-Density Polyethylene (LDPE)
5 PP – Polypropylene (PP)
6 PS – Polystyrene (PS)

Products of PET Hydrolysis



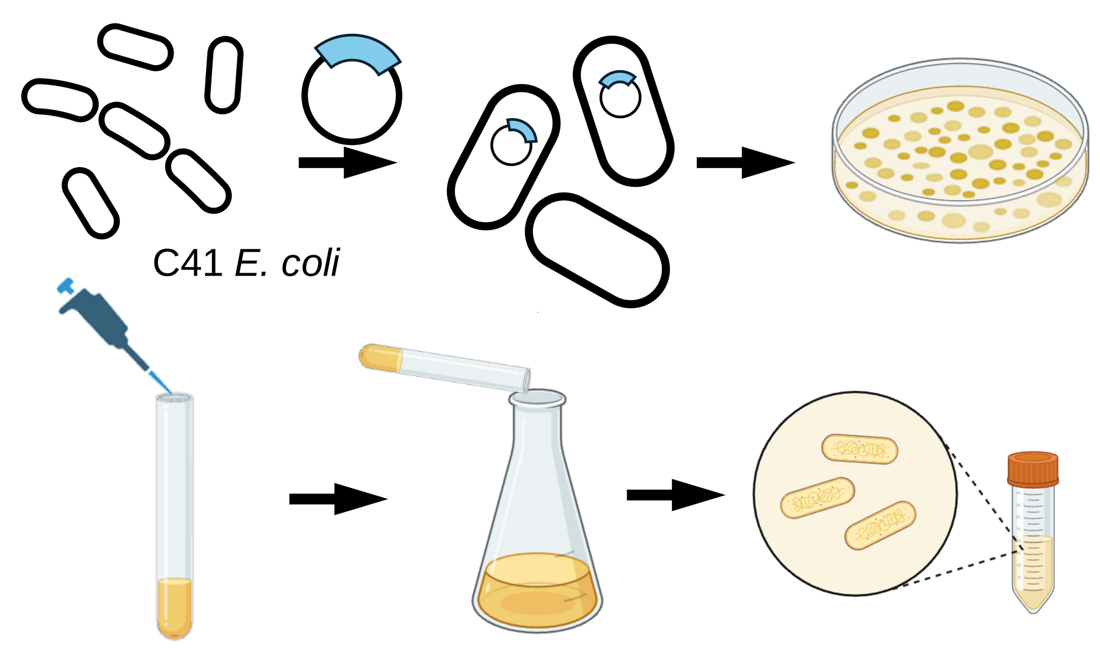
Ideonella sakaiensis Fast-PETase



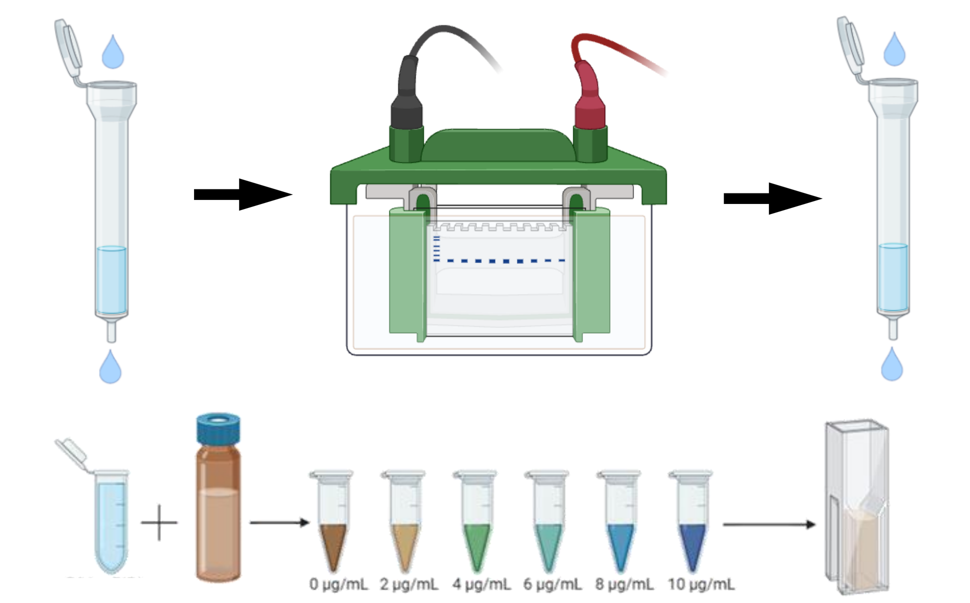
PDB: 7SH6

METHODS

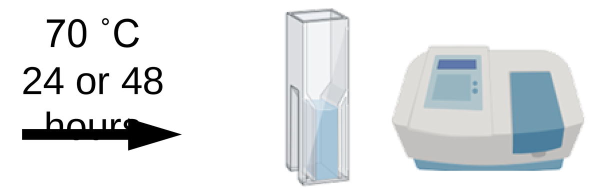
Enzyme Expression

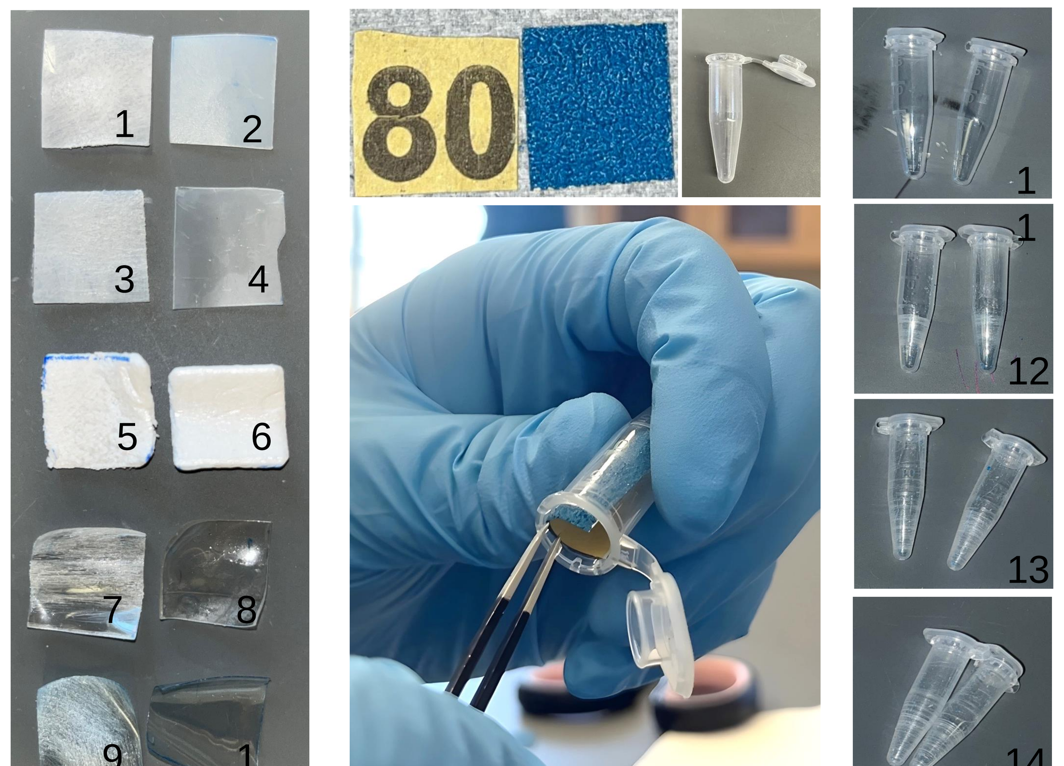


Enzyme Purification & Quality Control



Enzyme Activity Assay

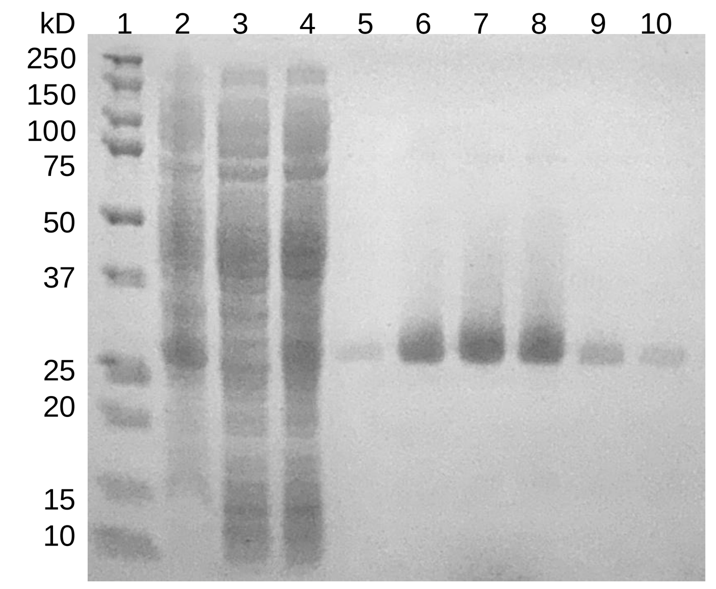




1. HDPE 100#; 2. HDPE 0#; 3. LDPE 100#; 4. LDPE 0#; 5. PS 100#; 6. PS 0#; 7. PET-A 100#; 8. PET-A 0#; 9. PET-B 100#; 10. PET-B 0#; 11. Tube 0#; 12. Tube 25#; 13. Tube 50#; 14. Tube 100#.

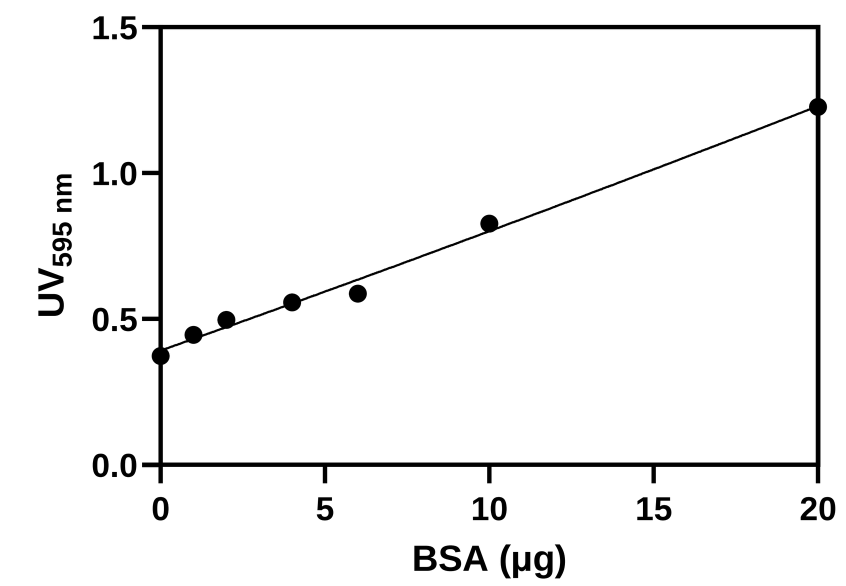
RESULTS

Enzyme Purification



1 – Protein marker
2 – Cell pellet
3 – Ni-NTA flowthrough
4 – Column wash 1
5 – Column wash 2
6 – Ni-NTA fraction 1
7 – Ni-NTA fraction 2
8 – Ni-NTA fraction 3
9 – Ni-NTA fraction 6
10 – Ni-NTA fraction 5

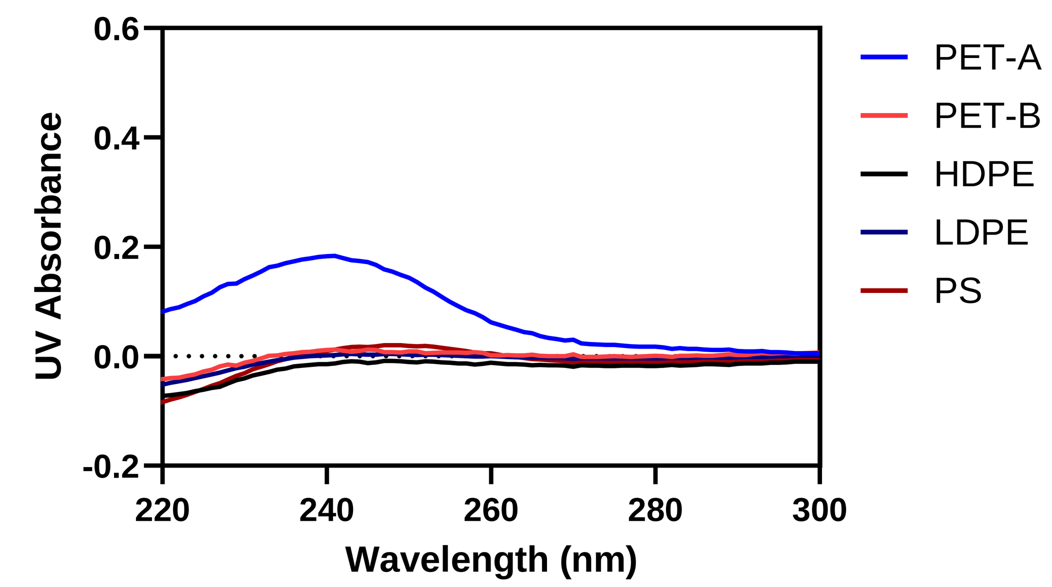
Bradford Assay Standard Curve



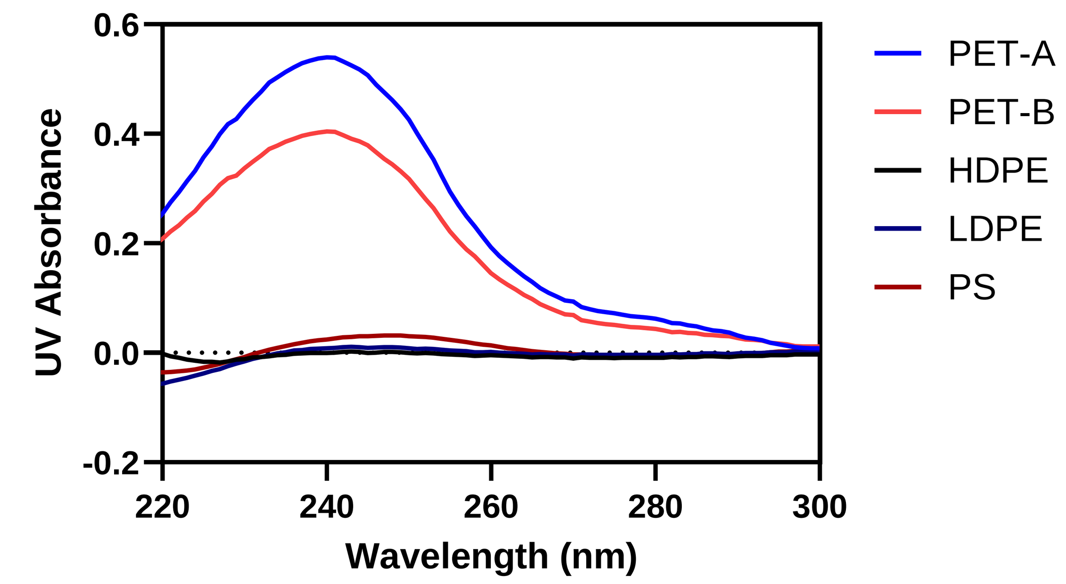
2 L of *E. coli* culture produces 1.25 ml 24mg/ml (800 μM) purified enzyme

Substrate Preferences

48-hours Low Abrasion



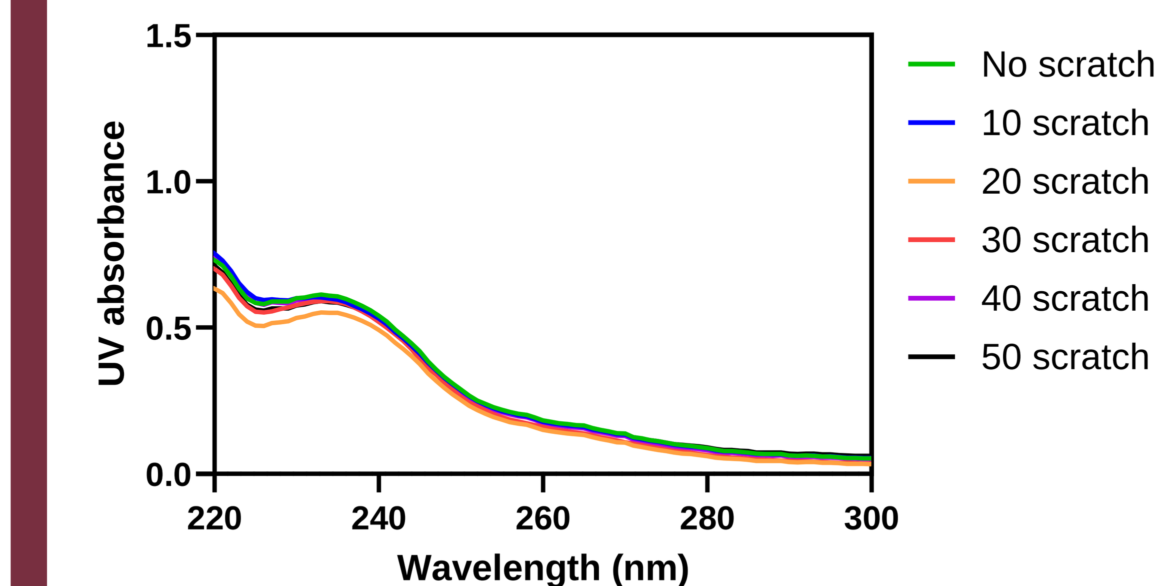
48 hours High Abrasion



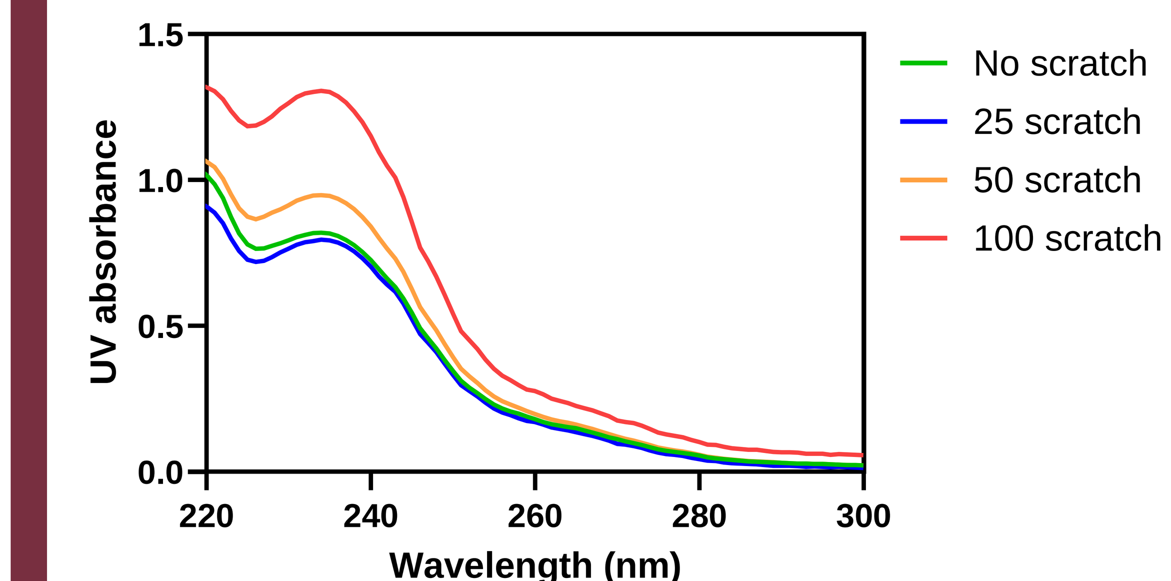
RESULTS

Time-dependence of PP degradation

24-hour PP Degradation



48-hour PP Degradation



CONCLUSIONS

- 30 mg of FAST-PETase was successfully produced from 2 Liters of *E. coli* culture.
- FAST-PETase selectively targets PET, showing minimal activity toward HDPE, LDPE, and PS.
- Increased surface abrasion enhances the efficiency of PET degradation.
- FAST-PETase also demonstrates activity against polypropylene (PP), a material commonly used in microcentrifuge tubes in the lab.
- The PP degradation product is distinct from known PET degradation products, such as BHET, TPA, or MHET.
- Extended incubation times correlate with higher levels of PP degradation.

REFERENCES

1. Ma J., *et al. Macromolecules.* **2019**; 52: 565-574.
2. Dhaka V., *et al. Environ Chem Lett.* **2022**; 20: 1777-1800.
3. Yoshida S., *et al. Science*. **2016**; 351: 1196-1199.
4. Lu H., *et al. Nature*. **2022**; 604: 662-667.
5. Thomsen T., *et al. N. Biotechnology.* **2023**; 78: 162-172.