The 14th International Symposium of The Romanian Catalysis Society

July 9-11, 2025, Cluj-Napoca, Romania

Summer School
"Catalysis at the crossroads between biology and chemistry"
06-08 July 2025

https://www.chimie.unibuc.ro/romcat/

Improved Methodologies for Monitoring PETase Activity

Blanka-Eszter Nagy*, Krisztina Kelemen, Raluca Bianca Tomoiagă, Róbert Tőtős, Monica Ioana Toşa, Csaba Paizs, László Csaba Bencze*
Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania
**Corresponding authors: blanka.nagy@ubbcluj.ro, laszlo.bencze@ubbcluj.ro*

Introduction

Polyethylene terephthalate (PET) accumulation presents a serious environmental treat due to its durability and widespread use in consumer products [1]. Enzymatic hydrolysis of PET, via PET degrading enzymes -PETases- particularly by cutinases such as leaf-branch compost cutinase (LCC), offers a green and efficient alternative to conventional plastic recycling, converting PET into its monomers: bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), terephthalic acid (TPA), and ethylene glycol (EG) [2]. However, the development of high-performance PET-degrading enzymes depends not only on protein engineering and expression systems but also on robust and precise analytical tools to assess catalytic efficiency.

Experimental

A recombinant expression system was developed for efficient production of LCC using the pET-21a(+) vector in *E. coli* Rosetta pLysS. To evaluate enzymatic PET hydrolysis, two complementary analytical methods, HPLC and UV-Vis spectroscopy, were optimized. For the HPLC assay, caffeine was introduced as an internal standard, significantly improving quantification accuracy of TPA and related products. Calibration curves were established over a wide concentration range (µM to low mM), enabling sensitive and reproducible detection of PET hydrolysis products.

The UV-Vis assay was refined by adjusting detection wavelengths and recalculating the medium extinction coefficients of the aromatic degradation products. Cross-validation between the two methods ensured consistency and reliability of measurements [3].

Results and discussion

The enhanced HPLC method demonstrated improved sensitivity, precision, and reproducibility compared to traditional setups, with a limit of detection below reported PETase activity thresholds. Product concentration measurements showed low variability and minimal deviation from theoretical values across replicates. The optimized UV-Vis method provided a faster yet reliable alternative, validated against the HPLC results. Using these upgraded analytical tools, time-resolved monitoring of LCC-mediated PET hydrolysis revealed distinct degradation profiles for each monomer, Fig. 1., offering insights into reaction dynamics and enzyme performance under varying conditions.

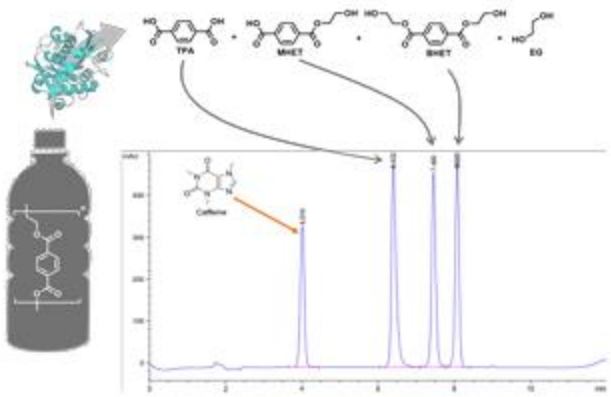

Fig. 1. HPLC analysis of the biodegradation products (TPA at 6.4 min, MHET at 7.8 min and BHET at 8 min) of PET using caffeine (at 4 min) as internal standard.

Conclusions

The refined analytical assays developed in this study enhance the sensitivity, accuracy, and consistency of PETase activity measurements. These improvements are critical for evaluating biocatalyst efficiency and support ongoing efforts to develop enzyme-based solutions for PET biodegradation and sustainable plastic recycling.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, UEFISCDI: Immobilized PET-degrading enzymes for microplastic control (detection and removal), project number PN-IV-P1-PCE-2023-2007.

References

[1] M. Oliveira, M. Almeida, Trends Anal Chem. 114 (2019) 196.
[2] V. Tournier, C.M. Topham, A. Gilles, B. David, C. Folgoas, E. Moya-Leclair, E. Kamionka, M.L. Desrousseaux, H. Texier, S. Gavalda, M. Cot, E. Guemard, M. Dalibey, J. Nomme, G. Cioci, S. Barbe, M. Chateau, I. Andre, S. Duquesne, A. Marty, Nature 580 (2020) 216.
[3] K. Boros, B.E. Nagy, R.B. Tomoiagă, R. Tötös, M.I. Toşa, C. Paizs, L.C. Bencze, Sci. Rep. 15 (2025) 1877.