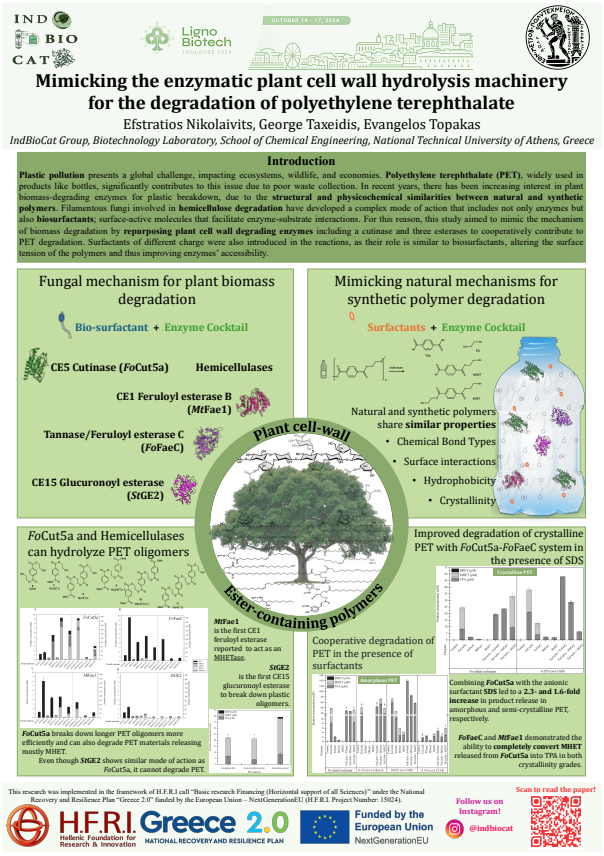
D3.4 Publication and/or presentation in International Conference on the selective degradation of PET & PLA polymers

The results from the investigation of the selective degradation of PET and PLA, which formed the core focus of the experimental activities within WP3, were disseminated at 4 international conferences through a total of 5 poster presentations, and one scientific publication at a peer-reviewed journal.

Poster presentations

- **LignoBiotech 2024,** Toulouse, France (14-17/10/2024), attended by over 250 participants.

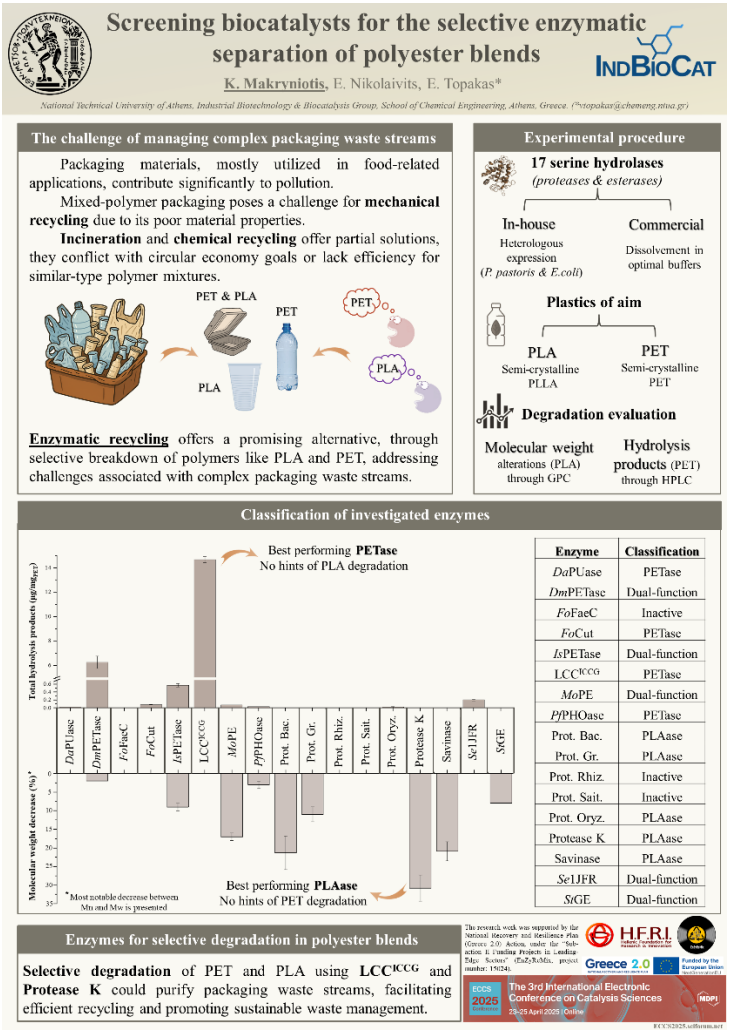
1) "Mimicking the enzymatic plant cell wall hydrolysis machinery for the degradation of polyethylene terephthalate" by Dr. Efstratios Nikolaivits.



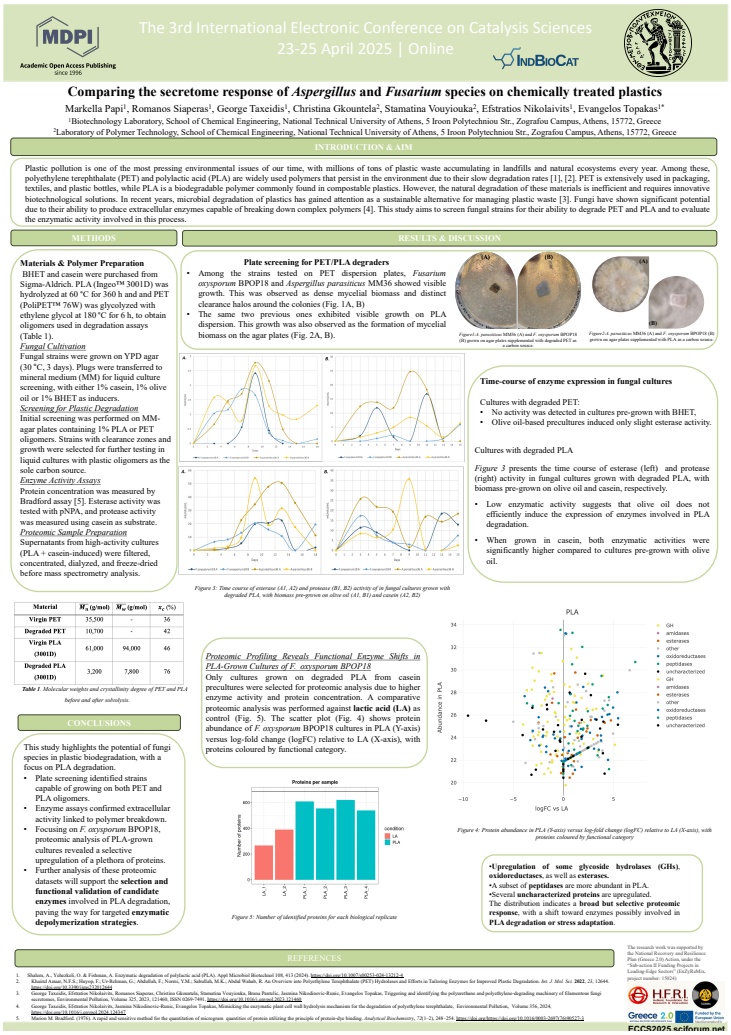
The poster described the use of fungal enzymes in a synergistic manner for the selective and complete enzymatic depolymerization of PET, as a part of WP3.

- **The 3rd International Electronic Conference on Catalysis Sciences,** online (23-25/04/2025), attended by over 500 participants.

2) "Screening biocatalysts for the selective enzymatic separation of polyester blends" by Mr. Konstantinos Makryniotis (PhD candidate).



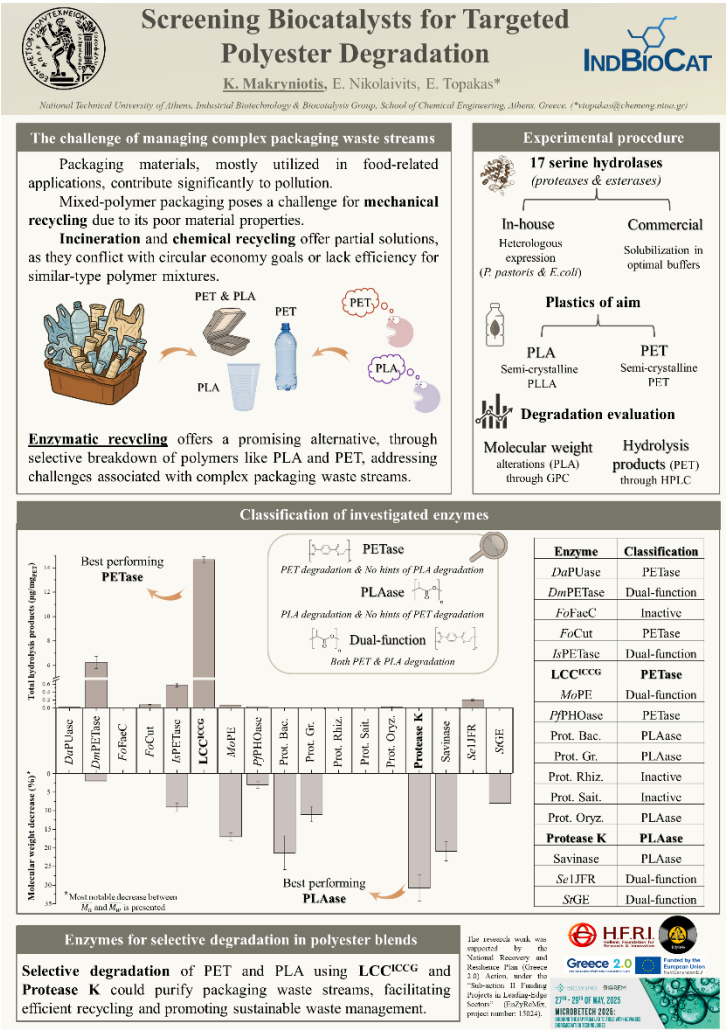
3) "Comparing the secretome response of *Aspergillus* and *Fusarium* species on chemically treated plastics" by Mrs. Markella Papi (PhD candidate).



This study was presented as a poster, showing the results of the comparison of the secretome responses of *Aspergillus* and *Fusarium* species when exposed to chemically treated plastics such as PET and PLA. Through plate screening and enzymatic assays, *F. oxysporum* BPOP18 and *A. parasiticus* MM36 demonstrated growth and polymer-degrading activity on both plastics. Proteomic analysis of *F. oxysporum* cultures grown on degraded PLA revealed upregulation of several enzymes, aiming to the discovery of novel PETases and PLAases for selective biodegradation.

- **MICROBETECH 2025,** online (27-29/05/2025), with an outreach of more than 100 participants.

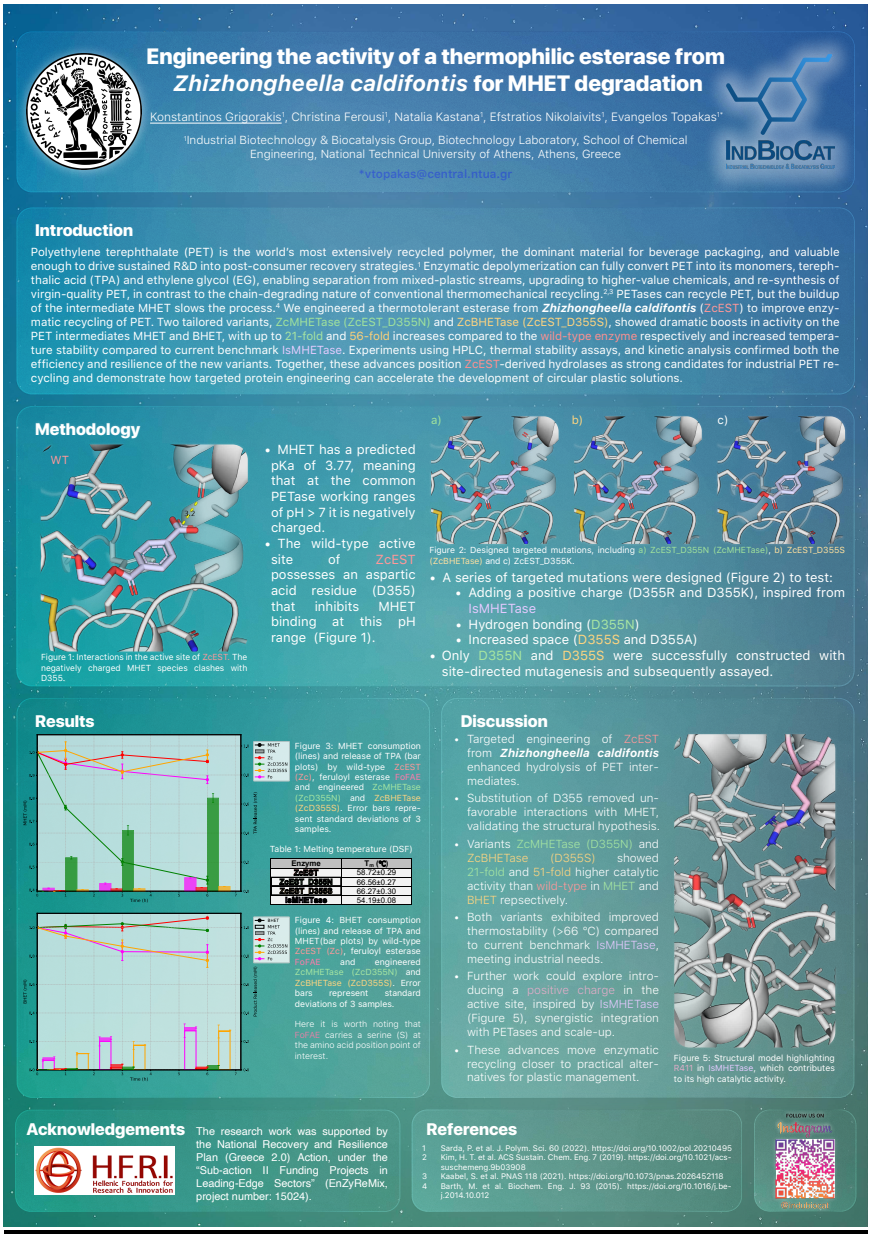
4) "Screening Biocatalysts for Targeted Polyester Degradation" by Mr. Konstantinos Makryniotis (PhD candidate).



Both posters presented results accomplished through WP3, focusing on the investigation of 17 serine hydrolases, including esterases and proteases, for their ability to selectively degrade project's key polyesters, PET and PLA. Through heterologous expression in *E. coli* and *P. pastoris*, followed by polymer degradation screening utilizing GPC and HPLC analyses, distinct substrate preferences were observed. Esterases exhibited limited polymer selectivity, while proteases favored PLA degradation. Notably, LCC^{ICCG} exhibited the highest PET hydrolysis activity (PETase), whereas Protease K and Savinase (PLAases) efficiently degraded amorphous and semi-crystalline PLA, respectively.

- **Joint International Conference of Mikrobiokosmos & CEESME,** Thessaloniki, Greece (22-24/09/2025), attended by over 200 participants.

5) "Engineering the activity of a thermophilic esterase from Zhizhongheella caldifontis for MHET degradation" by Mr. Konstantinos Grigorakis (PhD candidate).



Scientific publication

A review research paper was published on *Catalysts* journal in an open access format titled "Protein Engineering for Industrial Biocatalysis: Principles, Approaches, and Lessons from Engineered PETases" by K. Grigorakis, C. Ferousi and E. Topakas.

The paper explores the principles and methodologies of protein engineering, emphasizing rational design, directed evolution, semi-rational approaches, and the recent integration of machine learning. These strategies have significantly enhanced enzyme performance, even rendering engineered PETase industrially relevant. Insights from engineered PETases underscore the potential of protein engineering to tackle environmental challenges, such as advancing sustainable plastic recycling, paving the way for innovative solutions in industrial biocatalysis. More specifically, this work is correlated with making enzymes specific for specific polymers with high yields that make them industrially relevant.

Open Access | Feature Paper | Editor's Choice | Review

Protein Engineering for Industrial Biocatalysis: Principles, Approaches, and Lessons from Engineered PETases

by **Konstantinos Grigorakis**, **Christina Ferousi** and **Evangelos Topakas** *

IndBioCat Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Zografou Campus, 9 Iroon Polytech Str., 15772 Athens, Greece

* Author to whom correspondence should be addressed.

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