

# N°222 / PC TOPIC(s) : (Chemo)enzymatic strategies / Enzyme discovery and engineering

# Degradation of PET microplastic to monomers in human serum by designer enzymes

# AUTHORS

Ximena LOPEZ LORENZO / KTH ROYAL INSTITUTE OF TECHNOLOGY, TOMTEBODAVAGEN 23, SOLNA David HUETING / KTH ROYAL INSTITUTE OF TECHNOLOGY, TOMTEBODAVAGEN 23, SOLNA Eliott BOSSHARD / KTH ROYAL INSTITUTE OF TECHNOLOGY, TOMTEBODAVAGEN 23, SOLNA Corresponding author : Per-Olof SYREN / per-olof.syren@biotech.kth.se

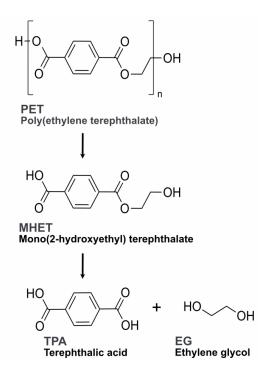
## PURPOSE OF THE ABSTRACT

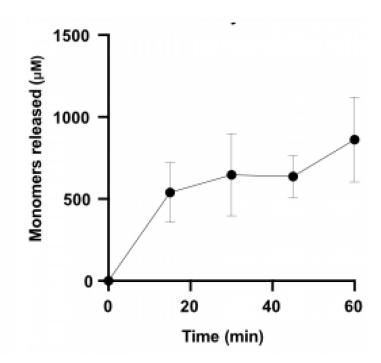
400 Mt of plastic years is generated per year [1,2] with a large amount of this plastic entering to bodies of water. Aa a consequence, around 3 million tons of plastic debris enters our oceans from rivers which humans are exposed daily to hundreds of microplastics (plastic particles with a size < 5 mm) upon inhalation and digestion. Due to its small size, this microplastics can translocate from the gut to body fluids and all the way to the organs [3,4]. Recently, studies have shown that plastic particles of  $\geq$ 700 nm in diameter are present in human whole blood [5]. Accordingly, the way synthetic polymers are discarded could pose a health risk to humans. Moreover, because of the prolonged exposure to these microplastic particles are found in human blood and other bodily fluids. As of now, there is a shortage of data regarding the hazards that could come due this exposure. Despite a lack of toxicity studies regarding microplastics, harmful effects for humans seem plausible and cannot be excluded.

Since the possible health risks associated with microplastics can't be determined due to lack of exposure data, there are no conclusions to which extent these microplastics represent a risk [4]. However, the European Environment Agency issued a report called Late Lessons from Early Warnings, highlighting how inaction regarding environmental issues due to a lack of risk data can pose a danger to society [6]. For this reason and based on the recent study by Leslie et. al. showing occurrence of microplastics in blood , in this study first steps towards a possible therapeutic measure to depolymerize microplastics in human serum was explored. In this study, we investigated the use of an enzyme-based treatment of serum that could constitute a promising avenue to clear synthetic polymers and their responding oligomers by degradation into monomers. Still, the activity of plastic degrading enzymes in serum remains unknown.

Herein, we report how engineered PETases can depolymerize microplastic-like particles of the commodity polymer polyethylene terephthalate (PET) into its non-toxic monomer terephthalic acid (TPA) in human serum at 37°C. As WT PETase is active at ambient temperature with a melting point close to 40°C [7,8], this enzyme was chosen as a model system to investigate enzymatic activity under blood-like conditions. Therefore, we sought to test the activity of PETases in human serum, still representing a relatively simple model of blood without complex clotting factors and blood cells. Our results demonstrate that PETases are highly active in serum, especially over the initial timepoints. Moreover, these initial results are exciting yet further research is needed regarding the interaction of this bacterial enzyme with the blood components to try and avoid the enzyme to be neutralised when introduced to the bloodstream. By developing an efficient method to depolymerize microplastics in vitro, our work takes a step closer to find a solution to the problem that microplastics in the bloodstream may pose in the future.

## **FIGURES**





# FIGURE 1

#### Degradation of PET

Depolymerization steps of PET into its monomers terephthalic acid (TPA) and ethylene glycol (EG) by PETase. For simplicity, only the intermediate MHET is shown.

## FIGURE 2

#### PETase activity in human serum

S238A activity in serum. For each reaction 1.5 ug mL-1 of enzyme was added to 1 mL of human serum with a substrate concentration of 2 mg mL-1. The samples were incubated at 37C. All reactions were stopped by heat inactivation (at 95C). Samples were take

# **KEYWORDS**

Enzyme engineering | Sustainability | Biocatalysis | Chemical biology

# BIBLIOGRAPHY

[1] Jonsson, C. et al. Biocatalysis in the Recycling Landscape for Synthetic Polymers and Plastics towards Circular Textiles. ChemSusChem 14, 4028-4040 (2021).

[2] Geyer, R., Jambeck, J. R. & Law, K. L. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782-e1700782 (2017).

[3] Yong, C. Q., Valiyaveettil, S. & Tang, B. L. Toxicity of Microplastics and Nanoplastics in Mammalian Systems. Int. J. Environ. Res. Public Health 17, 1509 (2020).

[4] Vethaak, A. D. & Legler, J. Microplastics and human health. Science 371, 672-674 (2021).

[5] Leslie, H. A. et al. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199 (2022).

[6] Leslie, H. A. & Depledge, M. H. Where is the evidence that human exposure to microplastics is safe? Environ. Int. 142, 105807 (2020).

[7] Guo, B. et al. Conformational Selection in Biocatalytic Plastic Degradation by PETase. ACS Catal. 12, 3397-3409 (2022).

[8] Guo et al. Fast depolymerization of PET bottle mediated by microwave pre-treatment and an engineered PETase. (2023). Under Revision