Reaction Networks Analysis and Kinetic Modeling of BHET Depolymerization as a (Sub-)Network of PET

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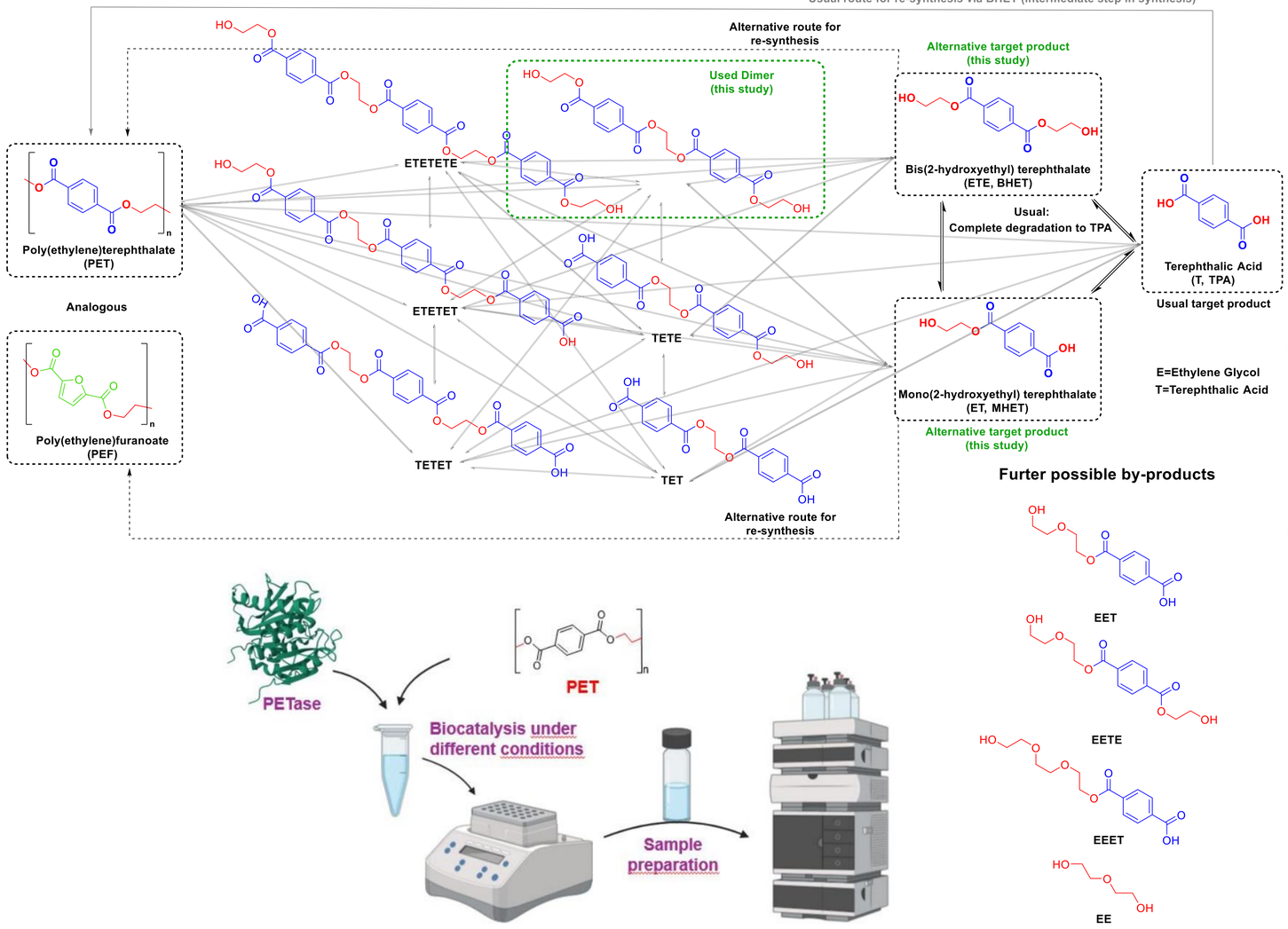
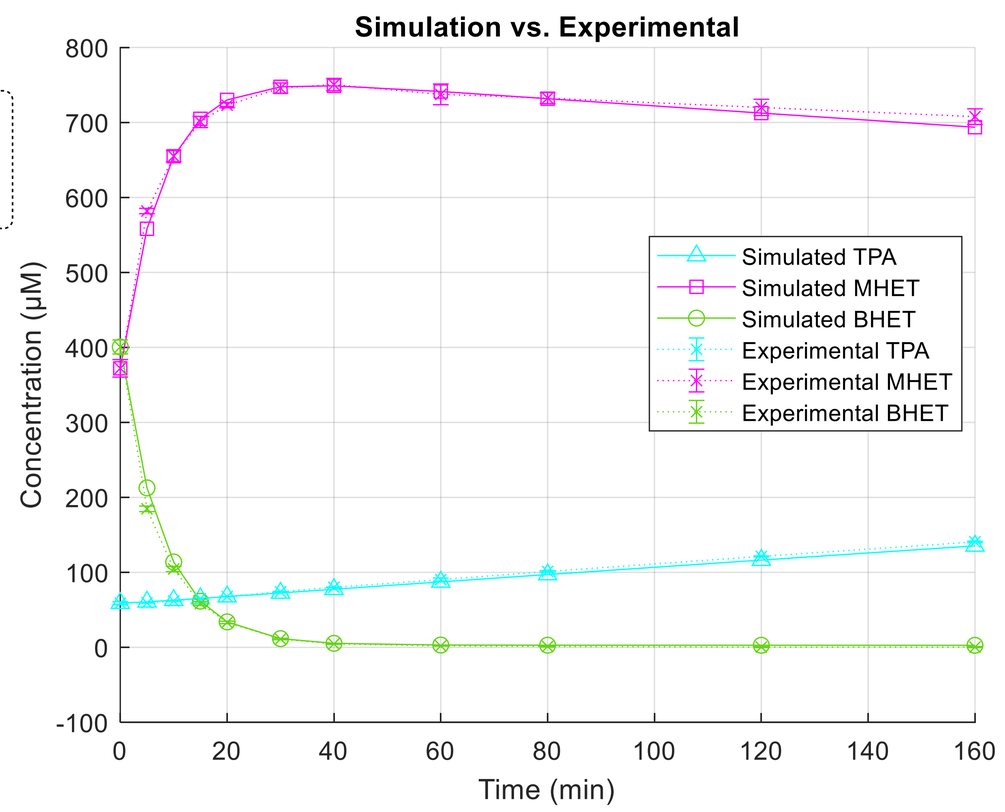
Introduction

- Polyethylene terephthalate (PET) is one of the most widely used synthetic polymers, but highly problematic as it does not decompose naturally and thus tends to accumulate as plastic waste in the environment
- PET-hydrolyzing enzymes provide a more environmentally friendly alternative to established chemical and physical/mechanical depolymerization techniques
- **PET is commonly decomposed via solvolysis (T↑, solvents) into terephthalic acid (TPA) and ethylene glycol (EG), which are then used to re-synthesize PET**
- **The intermediates MHET and BHET are of interest for efficient repolymerization**

Reaction-Network Analysis

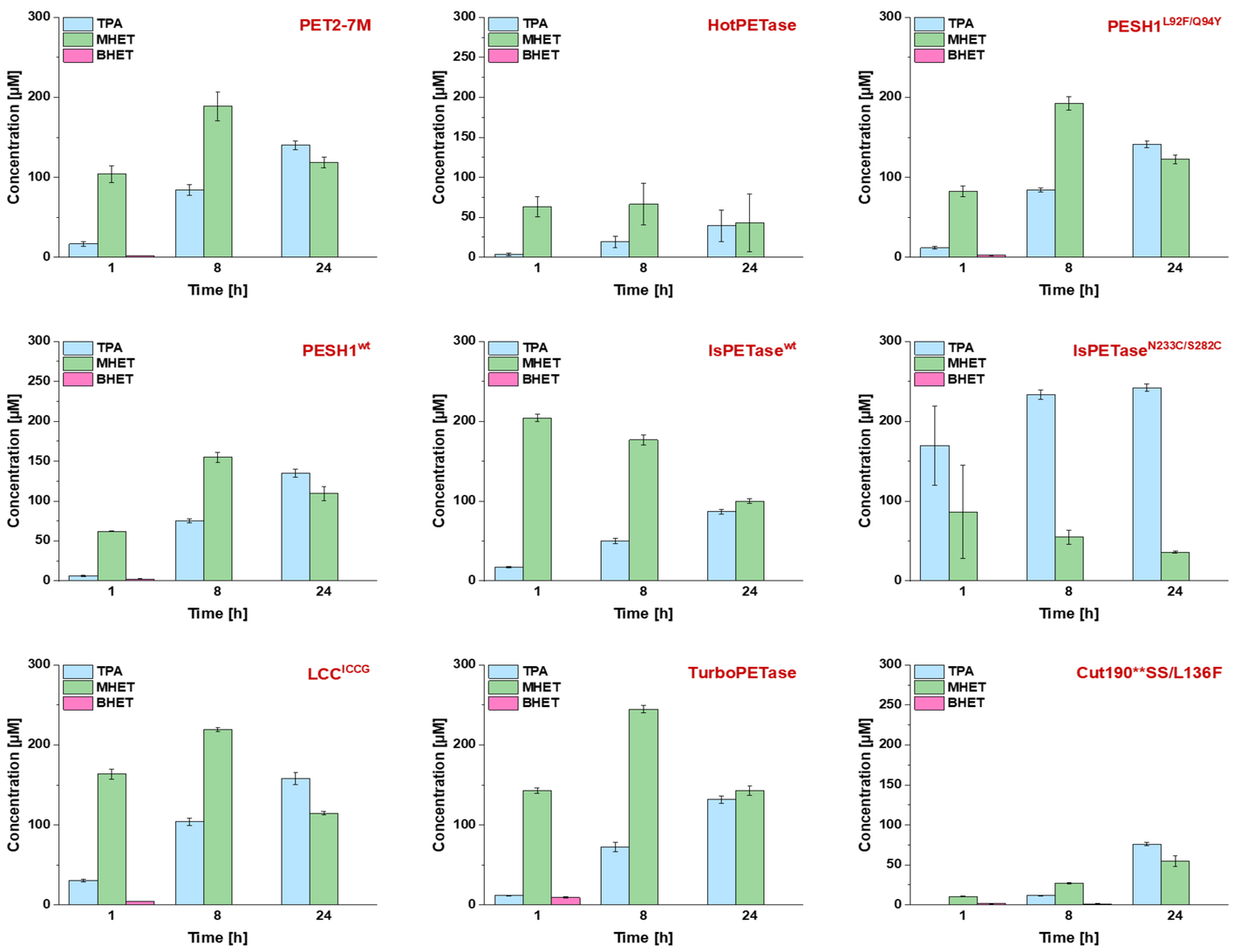


Kinetic Modelling of (Sub)-Network by Michaelis-Menten Kinetics



Standard: pH 7.5, 60 °C, 0.1 µM enzyme; 0.4 mM BHET; 0.4 mM MHET

Screening of 9 suitable PETase-hydrolyzing enzymes



Standard conditions for comparison: pH 7.5, 60°C, 24 hours; 0.1 µM enzyme; 0.161 mg/mL (350 µM) Nano-PET

- **TPA**: Increasing concentration over time, predominant product in the case of IsPETase$^{N233C/S282C}$
- **MHET**: Intermediate observed for LCC^{LCCG} and TurboPETase
- **BHET**: Detected in LCC^{LCCG} and TurboPETase
- ✓ **Seleted Enzym**: LCC^{LCCG} and ISPETase for kinetic modeling
- ✓ Proposed reaction network evaluated

Medium engineering

	IsPETase^{wt}			LCC^{ICCG}		
	$\frac{MHET}{TPA}$	$\frac{BHET}{TPA}$	$\frac{TPA}{MHET}$	$\frac{MHET}{TPA}$	$\frac{BHET}{TPA}$	$\frac{TPA}{MHET}$
pH 7.5	**8.2**	**0.8**	**0.1**	**5.2**	**0.2**	**0.2**
10% DMSO	13.4	1.5	0.1	11.1	3.3	0.1
25% DMSO	22.1	4.4	0.1	26.8	10.2	0.05
10% EG	15.9	3.7	0.1	16.6	9.5	0.1
25% EG	37.0	25.3	0.1	86.0	123.4	0.01
pH 9.0	0.1	0.4	12.7	0.1	0.7	13.8

pH 7.5, 60 °C, 1 h (except pH 9.0: 24 h); 0.1 µM enzyme; 0.15 mg/mL Nano-PET

- **TPA**: Promoted by basic pH
- **MHET**: Promoted by increasing concentrations of DMSO or EG
- **BHET**: Promoted by high concentrations of DMSO or EG

Outlook: Mechanistic kinetic studies of enzymatic PET-depolymerization

- ❒ **Modeling of mechanistic kinetics & modelreduction**
- ❒ **Expansion of the reaction model by inhibition and autohydrolysis**
- ❒ **Benchmarking of reduced mechanistic kinetics models vs established kinetic approaches**
- ❒ **Extension of the kinetics to the trimer**

Associated references

Tobias Heinks, Katrin Hofmann , Igor Gamm, Simon Last, Luise Blach, Ren Wei, Uwe Bornscheuer, Christof Hamel, Jan von Langermann, Selective Modification of the Product Profile of Biocatalytically Hydrolyzed PET. *ChemSusChem* **2025**

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