(Sub-)Network analysis of the enzymatic depolymerization of PET

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Highlights

- PET depolymerizing enzymes produce different amounts of MHET, BHET and TPA
- (Sub-)Networks, inhibitions and kinetics identified
- Product distribution during depolymerization can be adjusted by reaction engineering
- The hydrolysis intermediate MHET can be forced by adapting reaction conditions

1. Introduction

In contemporary society, plastics have become an indispensable part of our daily live, with Polyethylene Terephthalate (PET) being the most notable example due to its versatile applications in bottles, fibers, and films [1]. Despite its widespread use, PET poses a significant environmental threat as it does not naturally decompose, leading to the accumulation of plastic waste in the environment [2]. To date, chemical and mechanical depolymerization of PET is well-established and is generally conducted to obtain the end products terephthalic acid (TPA) and ethylene glycol (EG), which are subsequently isolated to re-synthesize PET into recycled PET [3]. Since the discovery of enzymes capable of depolymerizing PET 10 years ago, more environmentally friendly biocatalytic approaches are emerging to replace the conventional processes at lower temperature. Several enzymes have been identified and were partly further improved by enzyme engineering to obtain highly active, selective and stable enzymes, even at temperatures required for efficient PET depolymerization [4]. Furthermore, both approaches were applied to identify enzymes that lack inhibition of the reaction intermediate mono-(2-hydroxyethyl)-terephthalic acid (MHET), as it causes low reaction rates and product yields due to enzyme inhibition, which was very successful so far [5].

In contrast to the present practice of completely hydrolyzing PET bio catalytically to TPA and EG, this project aims to depolymerize PET only to the functional intermediate MHET, which should be separated during the process. This would simplify product extraction as only one product has to be isolated instead of two, which can also be used for PET re-synthesis, while MHET inhibition is also reduced due to a constant removal. Therefore, suitable enzymes and appropriate conditions have to be found by a reliable reaction kinetics to produce more MHET than TPA during the process. The experiments are accompanied by kinetic studies of the underlying reaction network in order to obtain a better understanding of the individual reaction rates and its influencing factors and thus to be able to influence them in a targeted manner to maximize the yield.

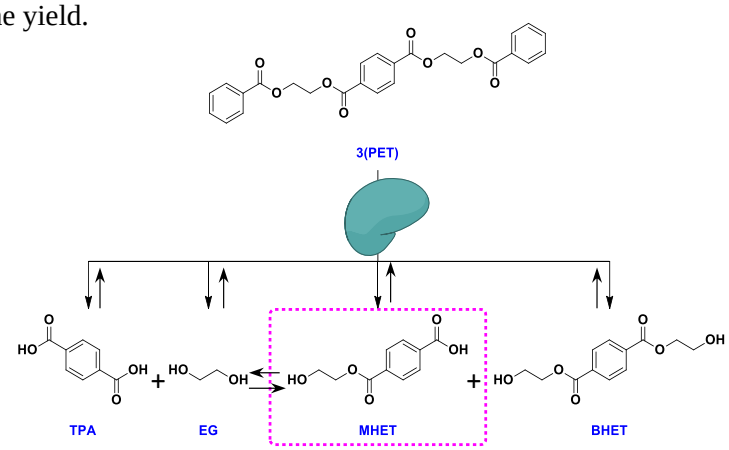

Figure 1. Simplified biocatalytic depolymerization of 3(PET). The model substrate 3(PET) is depolymerized by a hydrolase to the main products mono-(2-hydroxyethyl)-terephthalic acid (MHET), bis(hydroxyethyl)terephthalate (BHET), terephthalic acid (TPA) and ethylene glycol (EG). MHET is targeted as the main product, which should be extracted during bio catalysis.

2. Methods

All methods used in this study were adapted from the well-established literature in the field of biocatalytic PET depolymerization. In particular, all enzymes were expressed in *E. coli*, purified by His Tag purification and applied in biocatalytic reactions to follow the hydrolysis of the well-defined model substrate 3(PET) (PET-trimer). Samples of all species were analyzed at different reaction times via HPLC, which allowed the quantification of the hydrolysis products BHET, MHET, TPA and thus, the resulting main and sub-networks. The ratio of MHET to TPA was thereby a crucial factor to assess the efficiency of different enzymes and different conditions during bio catalysis. In addition, the model substrates will be extended to PET powder and nano PET as more realistic substrates to verify the model approach. MHET and 3(PET) were chemically synthesized according to established protocols in literature and analyzed via HPLC and NMR to obtain a non-commercially available HPLC standard and model substrate, respectively.

3. Results and discussion

A total of 15 different enzymes were applied in initial screenings to hydrolyze the model substrate 3(PET) in order to identify the most suitable enzymes that produces more desired MHET than TPA. In general, the production of MHET was highest at the beginning, which was then decomposed to TPA over time depending on the enzyme used, the corresponding intermediates and the individual reaction rates. Subsequently, 4 enzymes were selected producing significantly higher amounts of MHET than TPA. These enzymes were applied in detailed reaction kinetic studies with different conditions (initial, intermediate concentration, temperature, pH value) in order to derive and parametrize kinetic models, respectively. All investigated reaction conditions significantly affected the ratio of all hydrolysis products produced (i.e., BHET, MHET and TPA). Thus, depending on the conditions, the product spectra can be adjusted and MHET can be targeted as the desired product. Notably, low concentrations of unconventional media were often beneficial, whereas increasing concentrations resulted in lower productivities (i.e., lower product concentrations), most probably due to destabilized enzymes. In summary, conditions could be determined that force MHET as the main product of 3(PET) hydrolysis.

4. Conclusions

In this project, 15 enzymes were studied regarding their activity and selectivity to produce MHET over TPA. The performed (sub-)network and kinetic analysis of the time depended concentration courses and reaction rates revealed MHET as desired but intermediate product. The MHET/TPA ratio of the selected enzymes was very high compared to established ones. For model based optimizing the MHET production process a detailed description of the individual reaction rates including inhibition effects of substrate, intermediates and products of the identified network is required and presented. This behavior will soon be validated for more realistic PET-substrates (i.e., PET powder and nano PET), which have shown promising results in preliminary experiments.

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Keywords

PET-Depolymerization; Enzyme Screening; Selectivity; Reaction Network Analysis; Kinetics