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Short communication

A novel process for poly(ethylene terephthalate) depolymerization via enzyme-catalyzed glycolysis

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ABSTRACT

The discovery and improvement of processes for effective recycling of poly(ethylene terephthalate) (PET) packages meet high-priority and short-term demands for the implementation of a circular economy.

One of the most interesting approaches is enzyme-catalyzed depolymerization, which to date has been exclusively reported as hydrolysis reactions. In this paper, we describe a novel route for PET depolymerization using enzymes, via glycolysis reactions. *Humicola insolens* cutinase showed to act better at 60 °C, whereas *Candida antarctica* lipase B yielded more products at 37 °C. A comparison with hydrolysis reactions at the same conditions (temperature, enzyme loading) revealed that glycolysis favors the accumulation of more esterified end-products, whereas hydrolysis led to more end-products with free carboxyl groups. Molar fractions of the diesterified compound bis(2-hydroxyethyl terephthalate) of up to 0.88 was observed during glycolysis, revealing that it was the main depolymerization product. These findings add new relevant knowledge for the integration of depolymerization products in existing PET production industrial plants.

1. Introduction

Poly(ethylene terephtahalte) (PET) is one of the most important plastics for the global economy, being annually produced in amounts over 50 million tons [1]. The development of feasible processes to increase its yet low recycling rate (14%) is of paramount importance from environmental, social and economic aspects [2]. PET recycling via thermal/mechanical processes are more disseminated, but led to rapid changes in plastic characteristics (e.g. ductility, color) that restrict its adoption to very few cycles and to the production of lower value products [3] and are very dependent on efficient sorting to remove contaminants which impact the processing [4]. Chemical recycling includes a variety of routes (e.g. alcoholysis, glycolysis, hydrolysis, ammonolysis, aminolysis) and usually led to high yields, but low specificity to end products and severe reaction conditions are often reported, and the reuse or separation of catalysts is still challenging [4–6]. The biotechnological recycling, particularly using in vitro enzyme-catalyzed depolymerization reactions, is a promising alternative that has been increasingly regarded as efficient and selective for monomers [7,8].

To date, enzyme-catalyzed PET depolymerization has only been described via hydrolysis reactions (namely monomerization), yielding terephthalic acid (TPA) and often the enzyme-inhibitor mono(2-hydroxyethyl) terephthalate (MHET) as main products [8,9]. Once being considered in a new PET synthesis process, these products need to return to the first reaction vessel (esterification step) in order not to impact the process efficiency when co-processed with virgin raw materials [10]. One interesting alternative to save energy and costs is to obtain depolymerization products that can be fed into a posterior reactor, without impacting the final polymer quality, such as the diesterified compound bis(2-hydroxyethyl terephthalate) (BHET). BHET can be obtained via glycolysis reaction with monoethylene glycol (MEG), which currently has been described with the use of chemical catalysts, but under high temperature (170–220 °C) [11,12]. In this way, a BHET-rich mixture can enter in the PET synthesis process in the prepolymerization reactor (Fig. 1).

Therefore, the objective of this work is to report the proof of concept of an enzyme-catalyzed glycolysis reaction for PET depolymerization, aiming at to add knowledge and open up new opportunities for the development of a promising,

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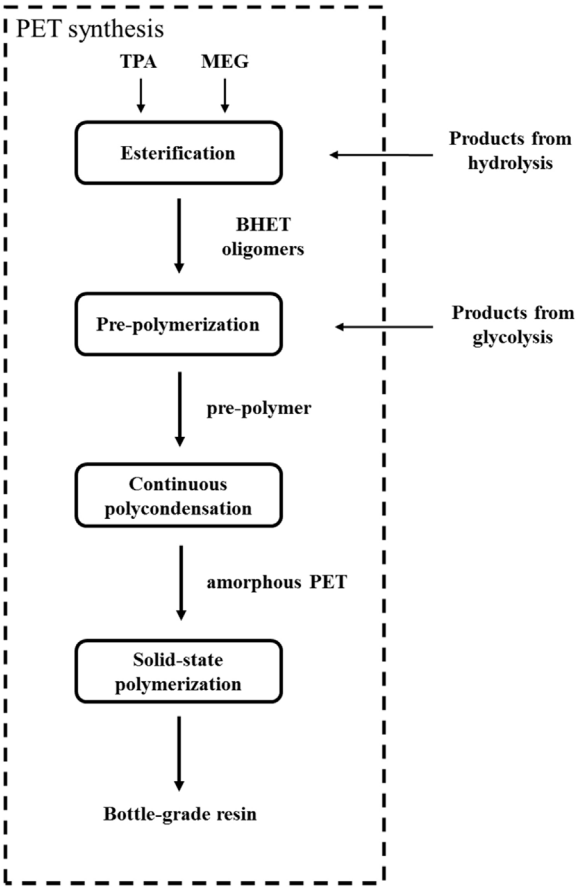

Fig. 1. Block diagram of a PET synthesis process and feeding points of products from hydrolysis or glycolysis reactions. Esterification step may comprise up to two vessels. PET diagram was elaborated based on [10].

environmentally-friendly and effective technology for PET recycling.

2. Material and methods

2.1. Materials

Liquid preparations of *Candida antarctica* lipase B (CALB, product Lipozyme© CALB L) and *Humicola insolens* cutinase (HiC, product Novozym© 51032) were generously provided by Novozymes. These enzymes were selected based on previous screening of biocatalysts for hydrolysis reactions [8].

MEG (99.8% purity) was purchased from Sigma-Aldrich. PET used was from non-carbonated mineral water bottles (brand Crystal©). It presented 0.1 mm thickness and was cut in squares of aprox. 0.5 cm.

2.2. Application of enzymes for PET glycolysis

The capability of both enzymes to catalyze glycolytic depolymeration of PET was studied, which included effect of enzyme loading, PET pretreatments and temperature. For enzyme loading assays, reactions were carried out in a shaking water bath (Innova 3100, New Brunswick Scientific) at 37 °C and 180 rpm in 5 mL MEG, 20 mg initial PET and enzyme loading of 0.01 and 0.05 $g_{protein}/g_{PET}$. Protein concentrations were determined according to Bradford method [13] for this test and all experiments of this work.

PET pretreatments (PT) were performed before enzyme addition to the reaction, and consisted of soaking 200 mg of PET particles in 5 mL MEG. PT-A was carried out for 22 h at 37 °C, based on the work by Brum et al. [14] while PT-B consisted of an incubation at 70 °C for same time as PT-A. After PET soaking, 0.01 $g_{protein}/g_{PET}$ was added and reactions were incubated in a shaking water bath at 37 °C and 180 rpm. These experiments were performed in duplicate.

Finally, the best pretreatment strategy was considered for reactions with 5 mL MEG and 200 mg initial PET, under higher temperature (60 °C) and an enzyme loading of 0.01 $g_{protein}/g_{PET}$. In order to allow a comparison between the nature of PET glycolysis and hydrolysis reactions, identical reactions as described above were performed, only substituting MEG for sodium phosphate buffer 200 mM pH 7.0 as reaction solvent. Control reactions were also performed, in which enzyme solution was not added. These experiments were performed in triplicate.

2.3. Analyses

TPA, MHET and BHET were measured by High Performance Liquid Chromatography (HPLC) provided of a C18 column an UV detector, under conditions described in details by Carniel et al. [8].

2.4. Calculations

Molar fraction of each reaction component (χ_i, where i = BHET, MHET or TPA) was expressed as a ratio of the molar concentration (C_i) of the component and the sum of molar concentrations of the three major depolymerization products, as shown in Eq. (1). Esterification degree (ED) was calculated as the ratio between (1) BHET and MHET Molar concentrations multiplied by the number of esterified carboxyl groups of each compound and (2) BHET, MHET and TPA molar concentrations multiplied by the number of possible ester bonds on carboxyl groups of these three molecules, as shown in Eq. (2).

$$\chi_i = \frac{C_i}{C_{BHET} + C_{MHET} + C_{TPA}} \quad (1)$$

$$ED = \frac{(1 \cdot C_{MHET} + 2 \cdot C_{BHET})}{2 \cdot C_{TPA} + 2 \cdot C_{MHET} + 2 \cdot C_{BHET}} \quad (2)$$

3. Results and discussion

In order to test the concept that PET glycolysis is possible via enzyme catalysis, a first set of experiments was carried out, considering two different enzyme loadings. Since the enzyme products used are presented as liquid preparations, water content in the reactions was consequently varied in these tests, so this investigation may be considered as a combined effect of both parameters. The results shown in Fig. 2 reveal that an increase in enzyme (and water) content reduces BHET content and increases TPA (in the case HiC was used) and MHET (when CALB was employed) contents. A water-free reaction was not intended, in order to allow the occurrence of the interfacial activation characteristic of lipases [15]. For reference, in these experiments, the water content varied in the range of 0.2–6.5% (p/p), depending on the condition used. The highest ED after 14d of reaction (61.4%) was observed when HiC was used at an enzyme loading of 0.01 g/g, which corresponded to the lowest water content investigated (0.2%). Interestingly, the highest χ_{MHET} was observed when CALB was used (up to 0.396), which is different of the behavior observed with this enzyme during BHET hydrolysis [8], suggesting a catalytic change modulated by the reaction medium.

As it has been described in chemically-catalyzed glycolysis of PET that a previous soaking in MEG can improve reaction efficiency (by the diffusion of the glycol between the PET chains, resulting in loosening effects) [14], two different soaking temperatures were investigated, being one at the same temperature of the glycolysis