Biochemical Engineering Journal 137 (2018) 239–246

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Enzyme-catalyzed simultaneous hydrolysis-glycolysis reactions reveals tunability on PET depolymerization products

Aline Machado de Castro[a,*], Adriano Carniel[b], Lys Sirelli[c], Marcos Lopes Dias[c], Sonia Maria Cabral de Menezes[d], Luiz Silvino Chinelatto Junior[d], Hercilio de Angeli Honorato[d]

[a] Biotechnology Division, Research and Development Center (Cenpes), PETROBRAS, Av. Horácio Macedo, 950, 21941-915, Rio de Janeiro, Brazil
[b] Falcão Bauer, R. Aquinos, 111, Água Branca, São Paulo, 05036-070, Brazil
[c] Institute of Macromolecules Professor Eloisa Mano (IMA), Federal University of Rio de Janeiro (UFRJ), Av. Horácio Macedo, 2030, 21941-598, Rio de Janeiro, Brazil
[d] Chemistry Division, Research and Development Center (Cenpes), PETROBRAS, Av. Horácio Macedo, 950, 21941-915, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 13 March 2018
Received in revised form 5 June 2018
Accepted 8 June 2018

Keywords:
Poly(ethylene terephthalate)
Glycolysis
Hydrolysis
PET recycling
Cutinase
Lipase

ABSTRACT

PET depolymerization via enzymatic catalysis is one of the most promising technologies in the context of plastics circular economy. So far, hydrolysis reactions accounted for almost the totality of studies, since the glycolysis route was only recently reported. In the present work, a comparative analysis of hydrolysis and glycolysis of PET oligomers catalyzed by *Humicola insolens* cutinase (HiC) and *Candida antarctica* lipase B (CALB) showed completely distinct profiles of the reaction products and rates. Then, simultaneous hydrolysis-glycolysis reactions with varied water and ethylene glycol concentrations revealed a gradual increase of the esterified products *mono*(2-hydroxyethyl) terephthalate (MHET) and *bis*(2-hydroxyethyl) terephthalate (BHET) as the reaction medium became more organic. Erosion patterns on the PET surface during hydrolysis and glycolysis reactions were also shown to be different. The results here reported open new opportunities for the use of task-specific solvents for biotechnological PET recycling, providing versatility for the depolymerization products in different reuse technologies.

1. Introduction

Environmental impacts associated to unappropriated plastics disposal are forecasted to grow at least 200% by 2050, unless effective actions are adopted [1]. As one of the major plastics produced worldwide (around 50 million tons annually) [2], post-consumer poly(ethylene terephthalate) (PET) packages have been investigated in a plethora of recycling routes, aiming to avoid or minimize their discharge in the ecosystems and improve the chain economics. The thermo-mechanical processes are the most mature, although more prone to downcycling of the processed material, as a consequence of changes in its thermal and visual properties [3]. The use of chemical catalysts (acids, alkalis, metal salts and oxides, ionic liquids) is reported for diverse depolymerization reactions (e.g. glycolysis, hydrolysis, methanolysis, ammonolysis, aminolysis) with usually short reaction times and medium-to-high yields, although performed under harsh conditions, commonly lacking near-quantitative selectivity and leaving catalysts in the products that can inactivate the polymerization catalyst [4]. Enzymatic depolymerization, on the opposite, releases slowly the monomers, but is greener (i.e. milder reaction conditions, biodegradable catalyst, no need of noble equipments, etc), usually high selective, and is more in line with the sustainable approach needed for this application [5,6].

When rethinking the future of plastics in the context of a circular economy, 50% of the priority actions for the global plastic packaging value chain are attributed to the improvement of process economics and products quality [7]. For depolymerization routes, this means to have a deep knowledge of the recycling mechanisms and to perform an extensive exploration of all potential technological alternatives for an efficient recycling, taking into consideration also the impact on the repolimerization process, in order to produce a polymer identical (or near identical) to its virgin peer (i.e., the depolymerization technology must deliver monomers for a 'drop in' application). The glycolysis route offers as the main advantage, the possibility to recycle the depolymerization products to the second or third stages of the polymerization process (depending on their purity), thus saving energy and increasing the plant throughput [8].

* Corresponding author.
E-mail address: alinebio@petrobras.com.br (A.M.d. Castro).

https://doi.org/10.1016/j.bej.2018.06.007
1369-703X/

The enzyme-catalyzed depolymerization is a very promising approach, but it has yet a wide range of aspects to be elucidated. Until recently, it had been only explored in hydrolysis reactions, in which the monomers terephthalic acid (TPA) and ethylene glycol (EG) are the main products, although some of the most efficient enzymes are inhibited by the key-intermediate *mono*(2-hydroxyethyl) terephthalate (MHET) [9,10]. As an alternative to the biocatalytic hydrolysis, we recently demonstrated for the first time the concept of enzyme-catalyzed glycolysis [11], thitherto seen only with chemical [12,13] and physical [14] methods. As a new route, there is much to understand about the action of enzymes during PET glycolysis, so that a robust decision can be taken for further developments.

Therefore, the present work aimed to deepen knowledge on PET depolymerization via glycolysis (EG as liquid phase and reagent) or hydrolysis (water as liquid phase and reagent) reactions by 1) investigating comparatively the time course of products formation using oligomers as substrates and 2) combining different water/EG proportions, to comprehend modulations when under simultaneous hydrolysis-glycolysis conditions.

2. Material and methods

2.1. Materials

Liquid preparations of *C. antarctica* lipase B (Lipozyme© CALB) (EC 3.1.1.3) and *Humicola insolens* cutinase (HiC, product Novozym© 51032) (EC 3.1.1.74) were kindly provided by Novozymes. These enzymes presented activities of 22.5 ± 0.5 U/mL and 367.6 ± 7.6 U/mL, respectively, on *p*-nitrophenyl butyrate, as previously reported [10]. Ethylene glycol (EG) was purchased from Isofar, whereas TPA and *bis*(2-hydroxyethyl) terephthalate (BHET) were purchased from Sigma-Aldrich, all of them of high purity. MHET (>99% purity) was obtained from near quantitative hydrolysis of BHET using HiC as biocatalyst, under conditions described by Carniel et al. [10]. Methanol (99.9% purity) was purchased from Tedia. MHET and BHET molecular formulas are $C_{10}H_{10}O_5$ and $C_{12}H_{14}O_6$, respectively.

PET oligomers 1 (O1), oligomers 2 (O2) and oligomers 3 (O3) samples were generously provided by the company PetroquímicaSuape (Suape, Brazil), and came from different stages of the polymerization process. Post-consumer PET (PC-PET) polymer (mixed packages without previous sorting, i.e. clear/colored, different thicknesses, etc.) was kindly supplied by a local industrial recycling plant (Rio de Janeiro, Brazil).

2.2. Hydrolysis and glycolysis reactions of PET oligomers

The performance of HiC and CALB was investigated in either hydrolysis or glycolysis reactions of different PET oligomers samples. Reactions were carried out in 20 mL of sodium phosphate buffer 200 mM pH 7.0 (for hydrolytic catalysis) or 20 mL of EG (for glycolytic catalysis), with 20 mg initial substrate and 0.2 $g_{protein}/g_{substrate}$ (determined according to [15]), in a shaking water bath (Innova 3100, New Brunswick Scientific) at 37 °C and 150 rpm. After withdrawal, samples were immediately diluted in methanol and filtered in 0.22 μm PVDF membranes.

2.3. Simultaneous hydrolysis-glycolysis reactions of PC-PET

As the most promising biocatalyst, HiC was employed in the investigation of simultaneous hydrolysis-glycolysis reactions of post-consumer PET. Based on previous studies in our labs (results under publication), these reactions were carried out in different proportions of Tris-HCl buffer (397 mM, pH 8.95) and EG (from 0 to 100% EG), with 404 mg initial substrate and 0.02 $g_{protein}/g_{substrate}$, in a hybridization incubator (Combi-D24, FINEPCR) at 62.6 °C and 25 rpm. Samples were treated after withdrawal as described in Section 2.2.

2.4. Analyses

Thermal properties were determined by Differential Scanning Calorimetry (DSC) using a TA Q1000 calorimeter with a heating and cooling rate of 10 °C/min from 30 to 300 °C under nitrogen flow. The sample was first heated at 10 °C/min, followed by a fast cooling run (quenching) at 70 °C/min to erase the polymer thermal history and obtain a predominantly amorphous material. A second heating run at 10 °C/min was performed, followed by a second cooling run at 10 °C/min and a final heating run at 10 °C/min. From this sequence of thermal treatment, the melt transition temperature (T_m) and degree of crystallinity (X_c) were taken from the first heating run to evaluate the influence of previous material thermal history, the glass transition temperature (T_g) and cold crystallization (T_{cc}) when present was taken from the second heating run after the fast cooling, and the crystallization temperature (T_c) was taken from the second cooling run at 10 °C/min. The temperature where 5% mass loss occurs ($T_{on\,set}$) and the temperature where the maximum weight loss takes plase (T_{max}) were obtained from the second cooling run. The influence of possible structural changes (mainly average molar mass) caused by the biodepolymerization process was evaluated from the third heating run (T_m and X_c). X_c was determined by the ratio between the melt enthalpies of the samples and 100% crystalline standard (140 J/g), computing the crystallization enthalpy in the case of cold crystallization.

Thermogravimetric analyses (TGA) were performed using a TA TGA Q500 Thermoanalyser. Measurements were carried out under nitrogen flow at a heating rate of 25 °C/min from 30 to 700 °C.

Gel permeation chromatography (GPC) was employed for the determination of the molecular weight and calculation of the degree of polymerization (DP), considering the molecular weight of the repeting unit as 192.2 g/mol. The analyses were performed in a Shimadzu LC 20 equipped with a differential refraction index detector RID-20 A and a linear Shim-Pack GPC-803 column. Analyses were carried out using 1,1,1,3,3,3-hexafluoro-isopropanol (HFIP) as eluent, flow rate of 0.5 mL/min, polymer concentration of 0.2 wt/v% and 2.0 μl of injection volume, at 30 °C. Monodisperse poly(methyl metacrylate) standards were used for the molar mass evaluation.

The analyses by Time domain NMR (TD-NMR) were carried out in a Bruker Minispec MQ20 spectrometer (0.47 T of magnetic field) at 20 MHz of hydrogen frequency. Transversal relaxation time (T_2) of oligomers and PET polymer were measured by CPMG (Carr-Purcell-Meiboom-Gill) sequence using 10 mm od tubes at 150 °C. 1024 echos spaced by 50 μs were used and 32 scans were accumulated. Non-linear regression by least squares was used to adjust the decay curves considering a bi-exponential function.

TPA, MHET and BHET concentrations in the samples taken from the reactions were measured in a Thermo Scientific Dionex UltiMate 3000 HPLC equipped with an Eclipse Plus C18 column, an Agilent Zorbax SB-C18 pre-column and an UV detector, under conditions previously described [16].

Water contents in each condition of combined hydrolysis-glycolysis reactions were determined according to ASTM E-203/01 method, using a Methron 870 Karl Fischer Titration Plus equipment.

At the end of reactions, PET samples were observed by Scanning Electron Microscopy (SEM), after metallization with Au/Pd in a Quorum Q150TES coater. Sample images were then captured in a Zeiss EVO-40 microscope (Oxford Instruments), under a voltage of 15 kV, using the software SmartSEM.