INTEGRATION OF PROCESS STEPS FOR POLY(ETHYLENE TEREPHTHALATE) (PET) UPCYCLING TO PRODUCE HIGH-VALUE BIOPRODUCT

Nathália F. dos Santos[1*], Gabriella N. Ricarte[1], Aline M. de Castro[2], Bernardo D. Ribeiro[1] & Maria Alice Z. Coelho[1]

[1] *Department of Biochemical Engineering, School of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*
[2] *Petrobras Research, Development and Innovation Center (Cenpes), Rio de Janeiro, Brazil.*
** Corresponding author's email address: nath.ferreiras@eq.ufrj.br*

ABSTRACT

Poly(ethylene terephthalate) (PET) is the third most used polymer to produce plastic packaging, which is the main end segment for your use. Consequently, plastic recycling is the most sustainable approach to manage the huge quantities of post-consumer PET materials, recovering their monomeric units: terephthalic acid and ethylene glycol. However, current recycling methods are still expensive, leading to recovered PET monomers more expensive than virgin ones obtained from fossil sources. Recently, PET upcycling has emerged as a profitable strategy to value recovered monomers, converting them into molecules with greater added value, enhancing the viability of the recycling process. This study explored the integration of two powerful techniques: chemical depolymerization of PET combined with bio-upcycling of the PET intermediate. *Y. lipolytica* was able to grow in the presence of one of the PET intermediates obtained by chemical depolymerization, the bis(2-hydroxyethyl) terephthalate (BHET), and produce 22 mM glycolic acid.

Keywords: Upcycling 1. *Yarrowia lipolytica* 2. Glycolic acid 3. depolymerization 4. Poly(ethylene terephthalate) 5.

1 INTRODUCTION

Every day millions of tons of solid wastes and effluents are released in environment, causing great impact in our lives and in our planet. Many of these wastes are composed by polyesters, as poly(ethylene terephthalate) (PET), and are used in bottles, packaging and textile fibers.[1]

Mechanical, physical and chemical-based recycling technologies have been used to reprocess these materials. However, each one has advantages and disadvantages in its process.[2] Due to the high energy costs of PET depolymerization, the production of PET by chemical recycling has not shown economic merits. Therefore, it is necessary to improve the economics of PET recycling by converting the monomers obtained into products of higher value than PET.[3-6]

Glycolysis is a chemical method that occurs in the presence of a glycol degrading agent under high temperature, and since it is generally a slow process in the absence of catalysts, they are often applied. Generally, the degradation agent used is ethylene glycol (EG) which, at the end of the reaction, produces the monomer bis(2-hydroxyethyl) terephthalate (BHET) as the main product. The advantages of this route are simplicity, flexibility, low cost and high yield in BHET monomer. However, it does not provide significant amounts of the final PET monomers terephthalic acid (TPA) and EG.[3-4,7]

In addition to these, biological recycling has also been highlighted as a potential solution to the problem of plastics. Its main advantages are the use of milder reaction conditions and also the greater selectivity of biocatalysts.[8] *Yarrowia lipolytica* is capable of biodepolymerizing PET into its dimers and monomers comprising: BHET, mono(2-hydroxyethyl terephthalate) (MHET), TPA and EG and consumes them.[9]

Therefore, in this work, for the first time we experimentally validate the integration of two techniques and the biological valorization of one of the PET intermediates by *Y. lipolytica*. For this, chemical depolymerization was used to depolymerize the PET bottle into BHET in the presence of EG and a deep eutectic solvent (DES) (hydrogen bond donor (HBD)/metal salt) as catalyst, and the bio-upcycling of BHET into glycolic acid by *Y. lipolytica*.

2 MATERIAL & METHODS

Chemical glycolysis of PET was carried out according to Nunes et al. [10]. The PET was sieved to 0.5–1.18 mm size before addition to the glycolysis system. A 250 mL glass flask equipped with a reflux condenser and a heating jacket was used for the glycolysis reaction. PET and EG in a proportion of 1:4 (m/m; 5 g PET and 20 g EG) were added to the system, with 5% (m/m) of catalyst:PET. The experiments were carried out for 30 min under the reflux condition of EG (197.3 °C). After the reaction, the flask contents were passed through a 0.5 mm sieve to retain the unreacted PET granules, washed and dried until constant weight; finally, PET conversion was calculated as PET conversion (%) = ((Initial PET mass − Residual PET mass / Initial PET mass) x 100).

Then, the PET wash water and approximately 200 mL distilled water were added to the liquid phase, which was kept at 60–65 °C to avoid crystallization of the BHET and to ensure its solubility in the mixture. This solution was vigorously agitated to dissolve the BHET in water and was vacuum filtered in filter paper to remove oligomers, as they are insoluble in water at the cited temperature.

The filtered solution was refrigerated overnight (12 h) to crystallize BHET. The BHET formed was filtered again and dried in an oven at 60 °C and, after constant mass recording, it was weighed. The yield of BHET was calculated based on the recovered BHET (254.2 $g.mol^{-1}$) and the repeating units in the PET chain (192.2 $g.mol^{-1}$), as BHET yield (%) = ((Recovered BHET (mol) / PET repeating units (mol)) x 100).

^{1}H-NMR and ^{13}C-NMR spectra were obtained from Bruker Avance III 500 MHz equipment. The solvent used to dissolve BHET was DMSO-d6, and 1,3,5-trioxane (99% purity, TCI America) was used as an internal quantitation standard.

Brazilian wild strain of *Y. lipolytica* IMUFRJ 50682, isolated from Guanabara Bay estuary in Rio de Janeiro[11] was used in all cultivation assays. Initially, *Y. lipolytica* was cultivated in YPD medium (1% yeast extract, 2% bacteriological peptone and 2% dextrose; all in w/v) at 28°C, 160 rpm, for 72 h. Then, 1 $g.L^{-1}$ of cells were inoculated in 200 mL YP medium supplemented with 50 mM of commercial BHET or BHET obtained from the chemical depolymerization of PET, and cultivated at 28°C, 250 rpm, for 96 h. Cultures were carried out in duplicate.

Quantification of cell concentration ($g.L^{-1}$) was done using a linear equation derived from the correlation between cell dry weight and optical density at 570 nm measured by an UV-1800 spectrophotometer (Molecular Devices, SpectraMax M2e). pH was determined in a digital pH meter (Tecnal), at room temperature (27 °C). For the quantification of TPA, MHET and BHET, high performance liquid chromatography (HPLC) (Shimadzu, Japan) was used. In these analyses the Eclipse Plus C18 column, 5 µm, 4,6×250 mm (Agilent, United States) was used. The injection volume of the sample was 10 µL, column temperature was maintained at 30 °C and the products were detected in a UV cell (254 nm). A gradient of acetonitrile and 0.05% (v/v) formic acid was used as mobile phase, at a flowrate of 0.5 $mL.min^{-1}$. The quantification of EG and glycolic acid were analyzed by a HPLC instrument (Shimadzu, Japan), equipped with Aminex® HPX-87H column (300 × 7.8 mm) and pre-column with cation-exchange resin (both from Bio-Rad Laboratories Ltd, United States). Detection was done in a refractive index cell at 40°C, column temperature was at 55°C, injection volume was 20 µL, and mobile phase was made of 5 mM H_2SO_4 (flow rate of 0.6 $mL.min^{-1}$).

3 RESULTS & DISCUSSION

First, PET needs to be depolymerized in its intermediate, BHET, before the PET can be valorized. In this work, the eutectic solvent, urea/$Zn(OAc)_2.2H_2O$, was use as catalysts in glycolysis reaction ([PET:EG] 1:4 (m/m); [Catalyst:PET] 4 (%m/m); 1 h) and could depolymerize PET, reaching 100% of conversion, although with a BHET yield of 54%. The BHET crystals recovered after 20 min were analyzed by ^{1}H-NMR, and ^{13}C-NMR. The purity achieved by ^{13}C-NMR was 98% (m/m) and by ^{1}H-NMR was 99.3% (m/m).

To experimentally validate the valorization of BHET from PET waste as raw material, the BHET sample obtained by chemical depolymerization of PET and commercial BHET was tested using *Y. lipolytica* IMFRJ 50682 to produce glycolic acid (Fig. 1 e 2). Glycolic acid (GA) is mainly used as an exfoliant in cosmetics, which corresponded to a 43.2% share of demand of GA in 2021.[12] The global GA market was valued at US$325 million in 2022 and is estimated to have a compound annual growth rate (CAGR) of 6.58% between 2023-2030.[13]

Y. lipolytica did not show inhibition of cell growth in the presence of BHET, both commercial and obtained from chemical depolymerization of PET, even showing a subtle increase in biomass compared to the control (without BHET) (Fig. 1).

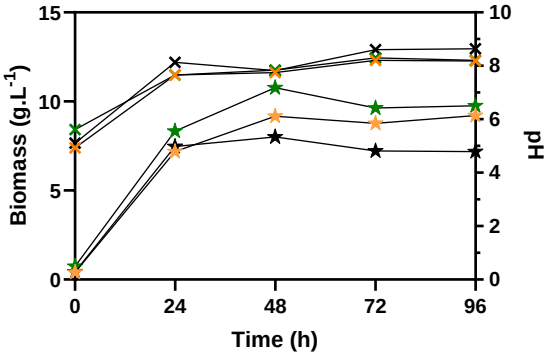

Figure 1 Cell growth kinetics of *Y. lipolytica* in YP medium supplemented with 50 mM of commercial BHET (orange), 50 mM of BHET obtained from chemical depolymerization of PET (green) and control (without BHET; black). (★): biomass; (×): pH.

BHET, both commercial and obtained from chemical depolymerization, were completely bioconverted into MHET and EG by *Y. lipolytica* after 48 h of cultivation (Fig. 2). These data corroborate the data obtained by da Costa et al. [9]. The released EG was simultaneously consumed by the yeast and bioconverted into GA, whereas within 48 h of cultivation the yeast had already consumed all the released EG and accumulated GA. On the other hand, the MHET formed was accumulated until the end of the cultivation, with no TPA formation (Fig. 2). Apparently, the yeast has enzymes that have a greater affinity for hydrolyzing BHET

than MHET. However, these enzymes produced by *Y. lipolytica* involved in this process have not yet been discovered. From 50 mM BHET, 22 mM GA was obtained by *Y. lipolytica*, and there was no significant difference between the consumption kinetics of commercial BHET and BHET obtained from chemical depolymerization of PET (Fig. 2).

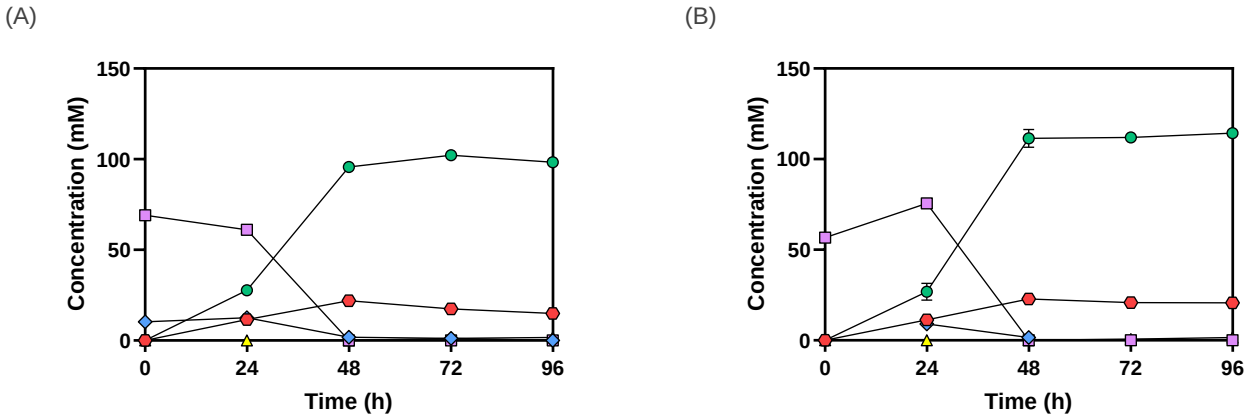

Figure 2 Consumption kinetics of commercial BHET (A) and BHET obtained from chemical depolymerization of PET (B). BHET (purple); MHET (green); EG (blue); TPA (yellow); GA (red).

4 CONCLUSION

This study explored the integration of two powerful techniques: chemical degradation of PET and bio-upclycling of the PET intermediate. BHET obtained by chemical depolymerization of PET was bioconverted by yeast *Y. lipolytica* into glycolic acid, which is a higher value product compared to the value of PET with relatively high molar conversion yields of 44%, as part of the EG was not released being trapped in the MHET molecule.

REFERENCES

1 Nisticò, R. 2020. Polymer Testing. 90. 106707.
2 Carniel, A., Valoni, É., Nicomedes, J., Gomes, A. C., de Castro, A. M. 2017. Process Biochem. 59. 84–90.
3 Tournier, V., Topham, C. M., Gilles, A., David, B., Folgoas, C., Moya-Leclair, E. et al. 2020. Nature. 580. 216–219.
4 Al-Sabagh, A. M., Yehia, F. Z., Eshaq, G., Rabie, A. M., ElMetwally, A. E. 2016. Egypt J Pet. 25. 53–64.
5 Hu, Y., Wang, Y., Zhang, X., Qian, J., Xing, X., Wang, X. 2020. J Macromol Sci. 57. 430–438.
6 Carniel, A., dos Santos, N. F., Buarque, F. S., Resende, J. V. M., Ribeiro, B. D., Marrucho, I. M., Coelho, M. A. Z., Castro, A. M. 2024. Green Chemistry.
7 Geyer, B., Lorenz, G., Kandelbauer, A. 2016. Express Polym Lett. 10. 559–586.
8 Wei, R., Zimmermann, W. 2017. Microbial Biotechnology. 10(6). 1308–1322.
9 da Costa, A. M., de Oliveira Lopes, V. R., Vidal, L., Nicaud, J. M., de Castro, A. M., Coelho, M. A. Z. 2020. Process Biochemistry. 95. 81-90.
10 Neves, G. R., Dias, M. L., Sirelli, L., Langone, M. A. P., de Castro, A. M., Coelho, M. A. Z., Ribeiro, B. D. 2021. Journal of Chemical Technology & Biotechnology. 96(11). 3237-3244.
11 Hagler, A. N., Mendonça-Hagler, L. C. 1981. Applied and Environmental Microbiology. 41(1). 173-178.
12 Transparency Market Research. Glycolic acid market. Available in: <https://www.transparencymarketresearch.com/glycolic-acid-market.html>.
13 RM, Research and Markets. Global Glycolic Acid Market Size, Tends, By Grade, By Application, and By Region: Global Opportunity Analysis and Industry Forecast, 2023-2030. Available in: <https://www.researchandmarkets.com/reports/5792453/global-glycolic-acid-market-size-trends-grade#tag-pos-4>.

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