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PRODUCTION AND IMMOBILIZATION OF WHOLE CELLS OF *Aspergillus oryzae* IPT-301 IN POLYURETHANE FOR THE ENZYMATIC SYNTHESIS OF FRUCTOOLIGOSACCHARIDES

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1. INTRODUCTION

Fructooligosaccharides (FOS) are fructose oligomers that are beneficial to human health and nutrition for being prebiotic sugars. Their production occurs by a transfructosylation reaction in sucrose molecules catalyzed by fructosyltransferase enzymes (FTase, E.C.2.4.1.9) adhered to microbial cells (GARCIA et al. 2021). The immobilization of whole cells in support materials allows the acquisition of heterogeneous biocatalysts that are robust and resistant to the adverse conditions of the reaction medium (SOUZA et al. 2017). Polyurethane (PU) foams are supports indicated for immobilization, since they present thermal and mechanical stabilities, besides size and distribution of pores which enable the diffusion of gases and substrate, essential for microbial growth (MARTINEZ et al. 2015). Therefore, the aim of this work was to evaluate the production of whole cells of *Aspergillus oryzae* IPT-301 by submerged cell culture and their concomitant immobilization in PU sponges. For this, the capacity of growth and absorption of the cells as a function of cultivation time, as well as their transfructosylation activity (A_T), were investigated to obtain robust and active biocatalysts for the synthesis of FOS.

2. MATERIAL AND METHODS

Whole cells of *A. oryzae* IPT-301 were produced by submerged cell culture in 50 mL of synthetic and sterile culture medium, pH 5.5, containing cubic supports of PU with edges of 0.8 cm, and composed of (in g L^{-1}): sucrose 150, yeast extract 5.0, $NaNO_3$ 5.0, KH_2PO_4 2.0, $Mg_2SO_4.7H_2O$ 0.5, $MnCl_2.4H_2O$ 0.3 and $FeSO_4.7H_2O$ 0.01. The cultivation occurred at 30 ºC and 200 rpm for 64 h, inoculating 500 µL of a suspension at 1 x 10^7 spores mL^{-1}. To determine the A_T activity, 0.68 g of the cells immobilized in 3.7 mL of a sucrose solution at 47 % (m v^{-1}) and 1.2 mL of a tris-acetate buffer solution at 0.2 mol L^{-1}, pH 5.5, were incubated. The reaction was performed in a thermostated bath at 50 ºC, 190 rpm for 60 min, and interrupted by the immersion of the reaction medium in boiling water for 10 min. The reaction medium was vacuum-filtered, and the cake was stored in an oven at 60 ºC for 24 h to obtain the dry mass. The permeate was used to determine the concentration of transfructosylated fructose (F_T) from the concentrations of reducing sugars and glucose obtained by the methods DNS (3,5-dinitrosalicylic acid) and GOD-PAP®, respectively. One unit (1U) of A_T was defined as the amount of FTase that transfers 1 µmol of F_T per minute under the established experimental conditions (GARCIA et al. 2021).

3. RESULTS AND DISCUSSION

Figure 1 presents results of A_T and the concentration of whole cells of *A. oryzae* IPT-301 immobilized by absorption in a PU support, as a function of cultivation time. It was observed that, after 24 h of process, there was an expressive increase in the growth and absorption of the microbial cells with enzymatic activity in the immobilization supports. The high concentration of absorbed cells derives mainly from the size and distribution of the pores of the support used, which favor the diffusion of gases and substrate inside it (MARTINEZ et al. 2015). It was verified that, for 32 h of cultivation, 7.1 ± 2.4 g of cells/g of PU were produced and immobilized with the highest A_T values (177.6 ± 41.4 U/g). GARCIA et al. (2021) obtained the highest activities for 64 h of cultivation, under the same experimental conditions, but using a different immobilization method involving only the microbial cells which underwent crosslinking with glutaraldehyde. The results showed that the whole cells of *A. oryzae* IPT-301, with high A_T, can be concomitantly produced and immobilized, with a reduction of 50 % in cultivation time, and applied for the synthesis of FOS.

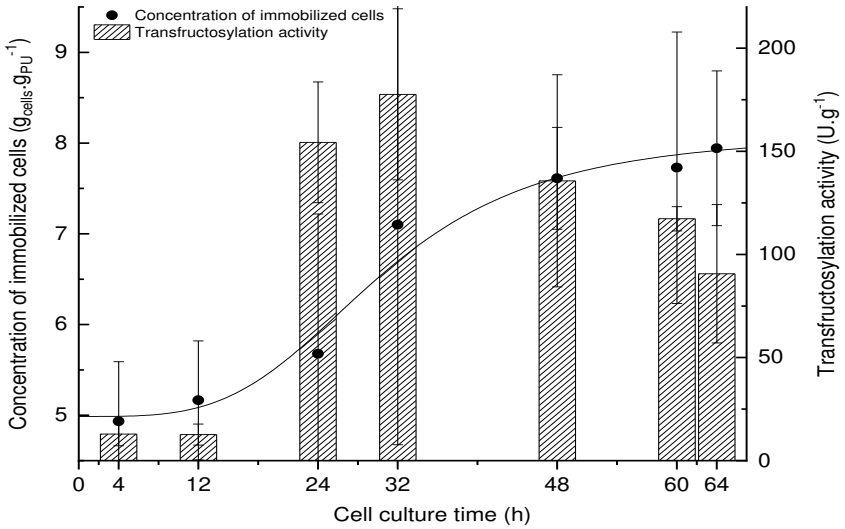

Figure 1. *Transfructosylation activity and concentration of whole cells of A. oryzae IPT-301, immobilized in PU support, as a function of cell culture time.*

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