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STABILITY OF IMMOBILIZED AND CROSSLINKED MICROBIAL CELLS FOR THE PRODUCTION OF PREBIOTIC SUGARS

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ABSTRACT

Fructooligosaccharides (FOS) are prebiotic sugars beneficial to human health and can be produced by the transfructosylation of sucrose catalyzed by fructosyltransferase (FTase) enzyme. Many operational advantages can be obtained by using cells with transfructosylation activity (A_T) immobilized on porous supports such as the loofa sponge. Furthermore, greater operational stability can be achieved by cross-linking these cells. The objective of this work was to evaluate the effect of cross-linking on the operational stability of *Aspergillus oryzae* IPT-301 cells, immobilized on loofa sponge (*Luffa cylindrica*), during consecutive reaction cycles for the production of FOS. The A_T of biocatalysts was monitored for consecutive batch reaction cycles. It was observed that cell crosslinking promotes a significant increase in maximum A_T, since initial A_T equal to 707.13 ± 18.68 U g^{-1} e 947.97 ± 36.59 U g^{-1}, for immobilized and immobilized/crosslinked cells, respectively. Furthermore, for both biocatalysts evaluated, A_T remained statistically equal throughout the twelve cycles. This indication suggests that biocatalysts produced by the immobilization/cross-linking of microbial cells on loofa sponge, which showed greater operational stability compared to solely immobilized biocatalysts, can be considered promising for the production of FOS in operationally stable heterogeneous reaction systems with high A_T.

Keywords: Fructooligosaccharides. Fructosyltransferase. *Aspergillus*. Microbial cells. Immobilization.

1 INTRODUCTION

Fructooligosaccharides (FOS), composed of fructose oligomers, are beneficial prebiotic sugars for human an animal health. Their production is carried out by the sucrose transfructosylation reaction, catalyzed by the enzyme fructosyltransferase (FTase, E.C.2.4.1.9) [1]. Previous studies have highlighted the operational advantages offered by the use of whole cells in FOS synthesis [2]. The loofa *(Luffa cylindrica)* sponge, due to its renewability and biodegradability, has emerged as a promising substitute for conventional cells supports in immobilization processes [3]. Additionally, cells immobilized by cross-linking or encapsulation have shown remarkable improvements in operational parameters, such as the low decay rate of enzymatic activity [4]. Crosslinking between cells is defined as the formation of cell clusters that can occur naturally or artificially induced by chemical substances called crosslinking agents or bifunctional agents. This process involves the formation of covalent crosslinks between specific cellular ligand groups, promoted by the reactive ends of the cross-linking agent [4]. Among the commercially available cross-linking agents, glutaraldehyde is one of the most used, as they enable the production of biocatalysts by simple methods that have operational stability and high enzymatic activity [4]. In this context, this study aims to investigate the effect of crosslinking on the operational stability of *Aspergillus oryzae* IPT-301 cells immobilized in loofa sponge for FOS production.

2 MATERIAL & METHODS

Cells of *Aspergillus oryzae* IPT-301 with transfructosylation activity were produced by microbial growth in 50 mL of synthetic medium, pH 5.5, containing cubic supports of loofa sponge with edges of 1 cm, 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 28 h, inoculating 500 µL of a suspension at 1 x 10^7 spores mL^{-1} [1]. For crosslinking, the immobilized cells on loofa sponge were added to 200 mL of reaction medium containing 16.8 mL of 25 % (v v^{-1}) solution of glutaraldehyde and 183.2 mL of 0.2 mol L^{-1} tris-acetate buffer solution, pH 7.9. The crosslinking reaction was carried out at 25 ºC, 200 rpm for 45 min on a rotary shaker and stopped, for an additional 30 min, by adding 2 mL of 100 g L^{-1} $NaBH_4$ solution, previously dissolved in 1 x 10^{-3} mol L^{-1} NaOH solution. To determine the transfructosylation activity, 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 Dubnoff bath at 50 ºC, 190 rpm for 60 min. At the end of each batch cycle, corresponding to 60 min of reaction, the heterogeneous biocatalyst was removed from the reaction medium by vacuum filtration and then reintroduced into a new and similar reaction medium under the same experimental conditions for 12 cycles. 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 from the concentrations of reducing sugars and glucose obtained by the DNS (3,5-dinitrosalicylic acid) and GOD-PAP® methods, respectively. One unit (1U) of transfructosylation activity was defined as the amount of biocatalyst that transfers 1 µmol of fructose per minute under the established experimental conditions [4]. The data obtained for immobilized cells (IC) and immobilized/crosslinked cells (CC) were evaluated using the Tukey test, with a confidence level of 95 %.

3 RESULTS & DISCUSSION

Figure 1 shows the relative activity of IC and CC over the 12 cycles. In the first cycle, where activities were considered as 100 % for calculations of relative activities, transfructosylation activities equal to 707.13 ± 18.68 U g^{-1} e 947.97 ± 36.59 U g^{-1} were obtained for IC and CC, respectively. This indicates that CC promotes a significant increase in maximum A_T, compared to the biocatalyst produced only by immobilization.

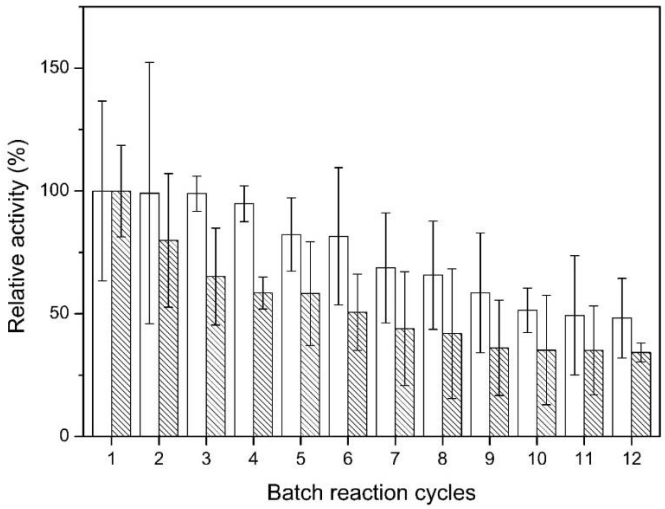

Figure 1 Operational stability of IC (hatched bar) and CC (unfilled bar) for 12 consecutive batch reaction cycles.

After 12 reaction cycles, the IC and CC retained relative transfructosylation activities equal to 34.16 ± 3.82 % and 48.22 ± 16.22 % of the initial enzymatic activity, respectively. For both types of experimental assays, there was a decrease in enzymatic activity as the number of cycles increased. However, this decline was less pronounced for the reticulated cells. After 12 cycles, the reticulated cells maintained 457.13 ± 153.85 U g^{-1} of activity, while the non-reticulated cells retained 241.56 ± 27.03 U g^{-1} of their activity. However, according to the Tukey test with a 95 % confidence level, the means of the relative A_T of the data referring to IC and CC did not show significant differences, which indicates that the activities evaluated are statistically equal. Thus, it can be considered that the evaluated biocatalysts presented satisfactory operational stability over 12 reaction cycles, since, statistically, A_T did not decrease. One study reported an increase in operational stability through crosslinking of *A. oryzae* IPT-301 cells with glutaraldehyde, where only a loss of 11.1 % of the initial activity was observed after 12 evaluated cycles [4]. In turn, other studies on operational stability showed that the transfructosylation activity of crosslinked *A. oryzae* IPT-301 cells encapsulated in calcium alginate remained up to 52 % of the initial activity, also over 12 evaluated reaction cycles [5]. These results are consistent with other research regarding the increase in cells operational stability due to the cross-linking of cells of *A. oryzae* IPT-301[4]. Therefore, crosslinking emerges as a promising alternative to enhance the reuse of heterogeneous biocatalysts based on immobilization of *A. oryzae* IPT-301 cells with high A_T for the enzymatic production process of FOS.

4 CONCLUSION

A crosslinking of immobilized *A. oryzae* IPT-301 cells on loofa sponge has proven to be a promising alternative for biocatalyst reuse in the FOS production process, as its operational stability and A_T was higher compared to cells solely immobilized on the same material support.

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