IMMOBILIZATION OF MICROBIAL CELLS ON TITANIUM OXIDE FOR FRUCTOOLIGOSACCHARIDE PRODUCTION

Érica D. Santos[1], Paula C. Leite[1], Ana C. Vieira[1], Bruna L. V. Quirino[1], Gustavo N. Teixeira[1], Suzana N. M. Alves[1], Sylma C. Maestrelli[1], Michelle C. A. Xavier[2], Rafael F. Perna[1]* & Sergio A. V. Morales[1,2]

[1]*Graduate Program in Chemical Engineering, Federal University of Alfenas, Poços de Caldas, Brazil.*
[2]*Graduate Program in Food Science and Technology, Federal University of Tocantins, Palmas, Brazil.*
**Corresponding author's email address: rafael.perna@unifal-mg.edu.br*

ABSTRACT

Fructooligosaccharides (FOS) are fructose oligomers which possess properties that improve the nutrition and health of humans and animals. FOS can result from the transfructosylation reaction of sucrose catalyzed by fructosyltransferase enzymes (FTase, E.C.2.4.1.9), which can be produced by microbial cells. Furthermore, highly active heterogeneous biocatalysts for FOS production can be produced by immobilizing these cells on porous materials. Thus, the aim of this work was to evaluate the immobilization of *Aspergillus oryzae* IPT-301 cells on a porous titanium oxide support, as well as its catalytic activity in the transfructosylation reaction of sucrose. The production and immobilization of the microbial cells on the oxide was performed in a synthetic culture medium, pH 5.5, at 200 rpm and 30 °C. The microbial growth curve and transfructosylation activity of the immobilized cells were evaluated as a function of cultivation time. The highest concentration of immobilized cells on titanium oxide was 0.94 ± 0.03 g_{cells} $g_{support}^{-1}$ after 40 hours of cultivation. The highest transfructosylation activity was shown by the cells after 32 hours of cultivation (174.39 ± 37.67 U g^{-1}). These results demonstrate the high potential of titanium oxide supports in cell immobilization for FOS production.

Keywords: *Aspergillus oryzae*. Fructosyltransferase. Titanium oxide. Immobilization.

1 INTRODUCTION

Prebiotic foods promote benefits to the host by stimulating the proliferation and activity of specific microorganisms in the intestine, such as bifidobacteria and lactobacilli [1]. Fructooligosaccharides (FOS) are important sweetener prebiotics, since they are low in calories, non-cariogenic, and can be safely consumed by diabetics [2]. FOS can be found naturally in the roots of certain plants, such as leeks, asparagus, chicory, wheat bran, and bananas, and they can also be produced enzymatically via the transfructosylation reaction of sucrose molecules using microbial-derived enzymes called fructosyltransferases (FTase; EC 2.4.1.9) [3], which can be synthesized by various fungal species. Especially, *Aspergillus oryzae* IPT-301 has been reported as a major producer of cells with high transfructosylation activity (A_T) [4]. Furthermore, several studies have demonstrated that the immobilization of *A. oryzae* IPT-301 is an important strategy for obtaining higher stability during FOS production [5]. Nonetheless, there is a lack of literature regarding cell immobilization on inorganic porous supports. In this sense, titanium oxide (TiO_2) is a promising support, commonly applied as a semiconductor material in solar cells, catalyst support and fuel cells. Therefore, this work aimed to immobilize *Aspergillus oryzae* IPT-301 cells on a titanium oxide support to obtain novel heterogeneous biocatalysts with high catalytic activity for the conversion of sucrose into FOS.

2 MATERIAL & METHODS

Cubic particles of porous titanium oxide support with edge of 1.0 cm (Figure 1A) were produced from a slurry, using polyurethane particles with a porosity of 30 ppi (pores per inch) for its shaping. After obtaining the green piece, it was heated from 0 °C to 650 °C with a heating rate of 1 °C min^{-1} and 2 hours of plateau, followed by heating from 650 °C to 1500 °C with a heating rate of 0.5 °C min^{-1} and 2 hours of plateau. Then, the microbial cells were produced and immobilized on the titanium oxide support using a synthetic culture medium, pH 5.5, and distributed into Erlenmeyer flasks, each containing 50 mL of medium. The porous pieces were added to the flasks, and they were sterilized in an autoclave. After sterilization, 0.5 mL of a suspension of 10^7 spores mL^{-1} of the fungus *Aspergillus oryzae* IPT-301 was inoculated, followed by incubation in an orbital shaker for 48 hours at 30 °C and 200 rpm, collecting samples at predetermined times (Figure 1B). For the determination of the enzymatic activity, the biocatalyst was added to a reaction medium containing 3.7 mL of sucrose and 1.2 mL of TRIS-acetate buffer. The reaction was conducted in a stirred water bath at 50 °C and 190 rpm for 60 minutes. Subsequently, the biocatalysts were vacuum-filtered, and the reaction medium was subjected to colorimetric analyses for the quantification of the reducing sugars and glucose employing the methods DNS and GOD/PAP®, respectively. One unit (1U) of transfructosylation activity (A_T) was defined as the amount of biocatalyst that transfers 1 µmol of fructose (F_T) per minute [6]. All experiments were performed in triplicate. The analysis of the means for the microbial growth curves and enzymatic activity assays was performed by the Tukey's honest significance difference test, with a confidence interval of 95 %.

The calculation of the transfructosylation activity (A_T) was also performed using Equation 1:

$$A_T = \frac{F_T V_r}{t_r m_s} \quad (1)$$

Where: F_T is the amount of fructose transferred in µmol liter^{-1}, V_r is reaction volume in liters, t_r is reaction time in minutes, and m_s is the dry biomass mass produced in grams.

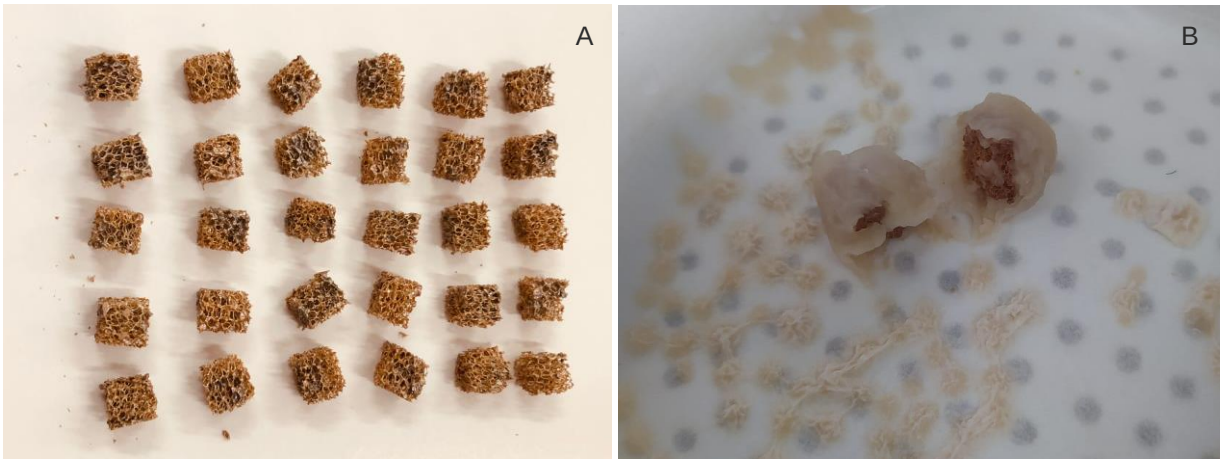

Figure 1 Porous titanium oxide supports (A). *A. oryzae* IPT-301 cells immobilized on titanium oxide (B).

3 RESULTS & DISCUSSION

The concentration of *Aspergillus oryzae* IPT-301 cells immobilized on titanium oxide, as well as the transfructosylation activity over time, are shown in Figure 2. A rapid cell growth is observed up to 40 hours of cultivation, which probably corresponds to the exponential growth phase (or lag phase) in which the fungal cells have already adapted to the cultivation medium and multiply rapidly. After 40 hours of cultivation, the cell concentration does not show a significant difference, indicating that cell growth reached the stationary phase, in which the cell growth rate equals the cell death rate. The cultivation of the same fungus for 76 hours, but in its free form, presented the maximum biomass concentration (9.35 ± 1.26 g L^{-1}) at 48 hours of processing [2]. For the immobilization of *Aspergillus japonicus* on synthetic materials, the concentration of the immobilized cells was 1.13, 0.48, and 1.25 g_{cell} $g_{support}^{-1}$ in stainless steel sponge, polyurethane foam, and vegetable fiber, respectively, also for the time of 48 hours [7].

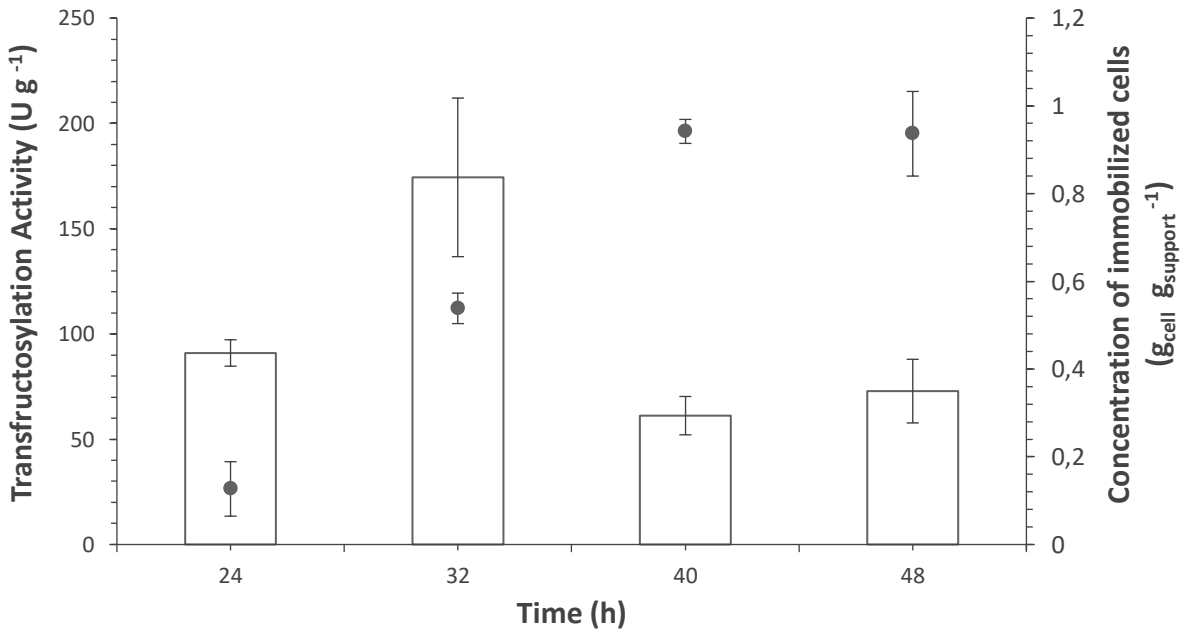

Figure 2 Concentration and transfructosylation activity of the cells of the fungus *A. oryzae* IPT-301 immobilized on porous titanium oxide particles (1.0 x 1.0 x 1.0 cm), as a function of cultivation time. The values of transfructosylation activity are represented by the bars, and the concentration of immobilized cells, by points.

It can also be observed that the highest A_T (174.39 ± 37.67 U g^{-1}) was shown by the cells cultivated for 32 hours. The cells immobilized after this time presented lower A_T despite the higher cell production. Some works have presented a maximum activity of 524.55 ± 177.10 U g^{-1} for 72 hours of processing [2], and obtained higher activities for *A. oryzae* IPT-301 cells cross-linked with glutaraldehyde after 64 hours of cultivation[8]. These results suggest that a highly active biocatalyst for FOS production can be obtained by the cultivation of *A. oryzae* IPT-301 on porous TiO_2 supports for 32 hours.

4 CONCLUSION

It is concluded that the production and immobilization of the cells of the fungus *Aspergillus oryzae* IPT-301 on porous titanium oxide support is feasible and allow the synthesis of a heterogeneous biocatalyst with high transfructosylation activity. The microbial growth curve indicated that the highest concentration of *Aspergillus oryzae* IPT-301 cells immobilized on titanium oxide was 0.94 ± 0.03 g_{cells} $g_{support}^{-1}$ after 40 hours of cultivation, and the highest transfructosylation activity was presented by the cells produced after 32 hours of cultivation, reaching 174.39 ± 37.67 U g^{-1}. These results corroborate the use of titanium oxide as a support for cell immobilization aiming at fructooligosaccharide production. These data correspond to the first application of porous TiO_2 particles in the immobilization of microbial cells for food production.

REFERENCES

[1] SAAD, S. M. I. 2006. Rev Ciênc Farm Básica Apl. 42 (1). 1-16.

[2] CUNHA, J. S., OTTONI, C. A., MORALES, S. A. V., SILVA, E. S., MAIORANO, A. E., PERNA, R. F. 2019. Braz J. Chem. Eng. 36 (2). 657-668.

[3] DIAS, G. S.; SANTOS, E. D.; XAVIER, M. C. A.; ALMEIDA, A. F.; SILVA, E. S.; MAIORANO, A. E.; PERNA, R. F.; MORALES, S. A. V. 2022. J. Chem. Technol. Biotechnol. 97 (10). 2904–2911.

[4] FERNANDEZ, R. C.; OTTONI, C. A.; DA SILVA, E. S.; MATSUBARA, R. M. S.; CARTER, J. M.; MAGOSSI, L. R.; WADA, M. A. A.; DE ANDRADE RODRIGUES, M. F.; MARESMA, B. G.; MAIORANO, A. E. 2007. Appl. Microbiol. 75 (1). 87–93.

[5] SOUZA, L. T. de A.; VERÍSSIMO, L. A. A.; JOÃO, B. C. P.; SANTORO, M. M.; RESENDE, R. R.; MENDES, A. A. 2017. Biotecnologia Aplicada à Agro & Indústria. (ed). 1nd ed. Blucher, São Paulo. 529–568.

[6] GONÇALVES, M. C. P., MORALES, S. A. V., SILVA, E. S., MAIORANO, A. E., PERNA, R. F. KIECKBUSCH, T. G. 2020. J. Chem. Technol. Biotechnol. 95 (9). 2473- 2482.

[7] GARCIA, R. L.; DIAS, G. S.; MORALES, S. A. V.; XAVIER, M. C. A.; SILVA, E. S.; MAIORANO, A. E.; TARDIOLI, P. W.; PERNA, R. F. 2021. Braz. J. Chem. Eng. 38 (2). 273–285

[8] MUSSATO, S. I.; AGUILAR, C. N.; RODRIGUES, L. R.; TEIXEIRA, J. A. 2009. Carb. Res. 344 (6). 795-800.

ACKNOWLEDGEMENTS

The research described in this work was supported by the Minas Gerais Research Foundation (FAPEMIG; Proc. APQ-00085-21 and Proc. BPD-00030-22) and National Council for Scientific and Technological Development (CNPq, Proc. 404912/2021-4). Érica Daniely Santos acknowledges scholarships financial support from and Coordination for the Improvement of Higher Education Personnel (CAPES) under finance code (88887.704175/2022-00).