CONTINUOUS SYSTEM TO FRUCTOOLIGOSACCHARIDES PRODUCTION WITH LOOFA SPONGE AS AN IMMOBILIZATION MATRIX

Isabela A. A. Taramelli[1], Crislaine K. Amaral[1], Ana C. Vieira[1], Paula C. Leite[1], Maria L. A. N. Teixeira[1], Sara I. C. Silvério[2], Alfredo E. Maiorano[3], Sergio A. V. Morales[4], Michelle C. A. Xavier[4] & Rafael F. Perna[1]*

[1] *Graduate Program in Chemical Engineering, Institute of Science and Technology, Federal University of Alfenas, Poços de Caldas, Brazil.*
[2] *Centre of Biological Engineering, University of Minho, Campus de Gualtar,, Braga, Portugal.*
[3] *Bionanomanufacturing Center, Institute for Technological Research, São Paulo, Brazil.*
[4] *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) production is conducted in batch bioreactors, a slow and costly process. Therefore, it is necessary to implement continuous reaction systems in fixed bed reactors (PBR) that increase the volume of FOS production and decrease its operating costs. This work evaluates the transfructosylation activity of *Aspergillus oryzae* IPT-301 cells immobilized in loofa sponge as a biocatalytic bed of a PBR reactor and your potential in the continuous production of FOS. The immobilization of *A. oryzae* IPT-301 cells in loofa sponges occurred by submerged culture using synthetic culture medium, pH 5.5, 30 ºC and 200 rpm for 28h. The reactor was operated for 180min at 50 ºC, concentration of 400 g/L of substrate and pH 5.75, with 40 loofa sponge cubes containing 0.874 ± 0.035 g of immobilized microbial cells. The transfructosylation activity was stable during the time of reaction, reaching 156.09 ± 1.38 U g^{-1} at steady state. The experimental results indicated the potential of the biocatalytic bed for operation in a continuous FOS production system, as the enzymatic activity profile throughout the process remained stable.

Keywords: Loofa Sponge. Packed bed reactor. Fructosyltransferase. Biocatalytic cells. Immobilization.

1 INTRODUCTION

Prebiotics are food ingredients used selectively by host microorganisms conferring a health benefit [1]. Fructo-oligosaccharides (FOS) are prebiotic, low-calorie, non-cariogenic sugar that help reduce total serum cholesterol levels and treat diseases such as anemia, hypertension and renal failure [2]. To meet the growing global market demand for FOS, the industry needs to achieve its large-scale and low-cost production, however, current strategies to improve FOS production still depend on the development and improvement of the production process [3]. The main route of commercial production of FOS occurs through the transfructosylation reaction of sucrose molecules, catalyzed by enzymes of microbial origin such as fructosyltransferase (FTase) [4,5,6]. The FTases are synthesized, mostly, by fungi which can be secreted to the culture medium (extracellular FTase) and/ or adhered to the cellular walls of the microorganism (mycelial FTase). Specifically, *Aspergillus oryzae* IPT-301 has been reported as a potentially producing source of both FTases, with high transfructosylation activity, among several strains of filamentous fungi evaluated [7]. The innovative application of microbial cells in a continuous flow chemical reactor combines the advantages of an easy-to-produce biocatalyst with process intensification technology [8].

Experiments with batch reactor showed activity of fructosyltransferase from *Aspergillus oryzae* IPT-301. After 72h of culture, mycelial FTase presented 524.55 ± 177.10 U g^{-1}, within pH ranges (6.0 – 8.0) and temperatures ranges (30 – 40 °C) [9]. However, packed bed reactors (PBR) with cells of *A. oryzae* IPT- 301, under the same conditions of temperature and substrate concentration as the batch reactor, presented constant operational stability and expressive enzymatic activity (660 U g^{-1}), in 25 min of reaction. This demonstrates the potential implementation of PBR on industrial scale for the production of FOS at low costs [10]. Research on enzymatic immobilization on organic supports has revealed interesting results, pointing to greater advantages compared to free cells. Loofa (*Luffa cylindrica*) sponge is a material considered cheap, non- toxic, physiologically inert, widely available and highly accessible organic material. Studies have proven that relative activity was enhanced after immobilization of pectinase on loofa sponge crosslinked glutaraldehyde, compared to others organic materials [11]. *Agrobacterium sp.* cells adhered physically at fibers surface of loofa sponge, retained its metabolic activity over a period of 300 days of storage, it showed that the efficacy in the use of loofa sponge as an immobilization matrix [5]. Thus, this work evaluates the transfructosylation activity of immobilized cells from *A. oryzae* IPT-301 in loofa sponge as a biocatalytic bed of a PBR aiming at the continuous production of FOS.

2 MATERIAL & METHODS

The immobilization of *A. oryzae* IPT-301 cells in loofa sponges occurred by submerged culture using synthetic culture medium, pH 5.5, 30 ºC and 200 rpm for 28 h, according to adapted method [9]. The reactor was built in borosilicate glass with diameter and length of 1.2 and 20 cm, respectively, in which the immobilized microbial cells constituted its biocatalytic bed for enzyme activity assays. The thermal jacket present in the reactor maintained the test temperature at 50 ºC through the recirculation of water from a thermostatic water bath. The flow in 1 mL/min was given by the peristaltic pump which enabled the flow of the substrate solution (sucrose) and reactor feed at a concentration 400 g/L, pH 5.75 for 180 min. The biocatalytic bed consisted of 40 cubes of loofa sponge (1.0 x 1.0 x 1.0 cm) containing 0.874 ± 0.035 g of immobilized microbial cells, corresponding to a bed height of 12 cm. The reactor was operated for 180 min and samples were collected at predefined time intervals to obtain the transfructosylation enzymatic activity profile [9]. At the end of the enzymatic reaction assays, the wet immobilized cells (biocatalytic bed) were removed from the interior of the PBR reactor and washed abundantly with distilled water, filtered by vacuum, using the vacuum pump,

retained in filter paper (Whatman nº 1 with diameter of 90 mm) and kept in drying oven for 24 h at 60 ºC to obtain the dry mass of the cells used to determine the enzymatic activities of transfructosylation. The unit of activity of transfructosylation was defined as the amount of biocatalyst that transfers 1 µmol of fructose per minute in the chosen experimental conditions. The concentration of transferred fructose was determined by analytical methods [4,5].

3 RESULTS & DISCUSSION

The transfructosylation activities (A_T) of the cells from *A. oryzae* IPT-301 immobilized on loofa sponge showed a fast increase up to 30 min of reaction in the PBR (Figure 1). Then, it remained constant and reached maximum and statistically equal values from 30 to 180 min of reaction (140.81 ± 1.58 U g^{-1}). At the end of continuous process, the immobilized biocatalytic cells did not show indications of future activity loss, suggesting its applicability by longer time. Data reported the operational stability of glutaraldehyde-crosslinked *A. oryzae* IPT-301 cells used as a biocatalyst for the transfructosylation reaction in a PBR aiming at FOS production, which biocatalyst showed constant A_T of 75 U g^{-1} for 12 h of reaction in the PBR operating in continuous flow [4]. This stability was also observed in a study with *in nature* biocatalytic cells from the same microorganism presented A_T values around 253 U g^{-1} for the PBR operated at flow rate of 5 mL min^{-1} from 180 to 650 min of reaction [5].

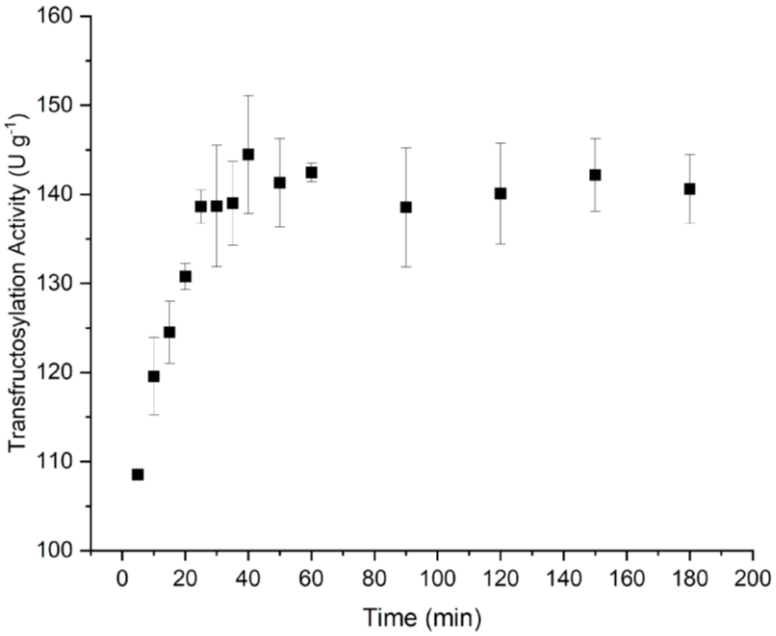

Figure 1 Transfructosylation activity profile for a continuous reaction system. Cells from *A. oryzae* IPT-301 immobilized on loofa sponge constituted the biocatalytic bed of the PBR. The reaction was carried out for 180 min, at 50 ºC, flow rate in 1 mL min^{-1}, 400 g L^{-1} of substrate solution and pH 5.75.

The enzymatic activity profiles of cells immobilized in loofa sponge were obtained in a PBR operated at a low flow rate (Figure 1). High flow rates can provide shorter time for the contact between substrate and immobilized biocatalytic cells, limiting the efficiency of the biocatalyst [11]. Although low flow rates can contribute to the formation of a thick diffusional boundary layer around the biocatalyst particles, which limits the reaction by external mass transfer [4,5,12]. Low flow rates can reduce deformation and compaction of the biocatalytic bed, as loofa sponges have greater elasticity even though they are robust organic structures with good mechanical strength [13]. Therefore, the flow rate is an important parameter to obtain high A_T values in a reactor associated with high FOS productivity.

4 CONCLUSION

In this work, the microbial cells immobilized in loofa sponge showed a good stability when used as a biocatalytic bed in a PBR, which transfructosylation activity, at steady state, remained constant and reached its maximum values from 30 to 180 min of reaction. In addition, the results demonstrated that the implementation of a continuous reaction system, based on the application of biocatalytic cells immobilized in loofa sponge, can be promising for the continuous synthesis of FOS.

REFERENCES

[1] GIBSON, G. R., HUTKINS R., SANDERS, M. E., PRESCOTT, S. L., REIMER, R. A., SALMINEN, S. J., SCOTT, K., STANTON, C., SWANSON, K. S., CANI, P. D., VERBEKE, K., REID, G. 2017. Nat. Rev. Gastroenterol. Hepatol. 14 (8). 491-502.

[2] CORREA, A. C., LOPES, M. S., PERNA, R. F., SILVA, E. K. 2024. Carbohydr. Polym. 323.121396.

[3] MUTANDA, T., MOKOENA, M. P., OLANIRAN, A. O., WILHELMI, B. S., WHITELEY, C. G. 2014. J. Ind. Microbiol. Biotechnol. 41 (6). 893–906.

[4] RIBEIRO, B. M., JUNIOR, L. D. R. D. S., DIAS, G. D. S., XAVIER, M. D. C. A., ALMEIDA, A. F., SILVA, E. S., MAIORANO, A. E., PERNA, R. F., MORALES, S. A. V. 2024. Chem. Ind. & Chem. Eng. Q. 30 (2). 99-110.

[5] 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.

[6] PERNA, R. F., CUNHA, J. S., GONÇALVES, M. C. P., BASSO, R. C., SILVA, E. S., MAIORANO, A. E. 2018. Int. J. Eng. Res. 4 (3), 43-50.

[7] CUERVO-FERNÁNDEZ, R., OTTONI, C. A., SILVA, E. S., MATSUBARA, R. M., CARTER, J. M., MAGOSSI, L. R., WADA, M. A. A., RODRIGUES, M. F. A, MARESMA, B. G., MAIORANO, A. E. 2007. Appl. Microbiol. Biotechnol. 75. 87-93.
[8] KIRSCHNING, A., SOLODENKO, W., MENNECKE, K. 2006. Chem. Eur. J. 12. 5972-5990.
[9] CUNHA, J. S., OTTONI, C. A., MORALES, S. A. V., SILVA, E. S., MAIORANO, A. E. 2019. Braz. J. Chem. Eng. 36 (2). 657-668.
[10] MARTINEZ, C. O., RUIZ, S. P., FENELON, C., MORAIS, G. R., BAESSO, M. L., MATIOLI, G. 2016. J. Sci. Food Agric. 96 (7). 2410-2417.
[11] OLUWASEUN G. E., FREDRICK O. T., ANTHONY A. O. 2023. Appl. Food. Biotechnol. 10 (3). 177-190.
[12] LORENZONI, A. S. G., AYDOS, L. F., KLEIN, M. P., AYUB, M. A. Z., RODRIGUES, R. C., HERTZ, P. F. 2015. J. Mol. Catal. B Enzym. 111. 51-55.
[13] HA, T. T. V., VIET, N.M., THANH, P. T., QUAN, V. T. 2023. Environ. Technol. Innov. 32. 103265.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from CAPES, FAPEMIG (Proc. APQ-00085-21 and Proc. BPD-00030-22) and CNPq (Proc. 404912/2021-4 and Proc.421540/2018-4).