IMMOBILIZATION OF MICROBIAL CELLS ON LOOFA SPONGE FOR ENZYMATIC SYNTHESIS OF FRUCTOOLIGOSACCHARIDES

Isabela A. A. Taramelli[1], Ana C. Vieira[1], Amanda P. Cavini[1], Érica D. Santos[1], Luís R. P. Filho[1], Kalebe F. Furtado[1], Elda S. Silva[3], Sergio A. V. Morales[1,2], Michelle C. A. Xavier[2] & Rafael F. Perna[1]*

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

ABSTRACT

Fructooligosaccharides (FOS) are classified as prebiotic sugar and industrially synthesized by transfructosylation reaction in sucrose molecules catalyzed by fructosyltransferase enzymes adhered to microbial cells. The immobilization of microbial cells in support materials make it possible to obtain robust heterogeneous biocatalysts resistant to adverse conditions of the reaction medium. Loofa (*Luffa* cylindrica) sponges are suitable for immobilization because they have size and distribution of pores that enable the diffusion of gas and substrate, essential for microbial growth. Therefore, this work aimed to evaluate the production of cells of *Aspergillus oryzae* IPT-301 by submerged cell culture and their concomitant immobilization in a loofa sponge. The production occurred in a synthetic culture medium, pH 5.5, 200 rpm at 30 ºC. The microbial growth curve showed that the best cell culture time occurred after 28 h of process, obtaining a transfructosylation activity of 559.96 ± 40.37 U g^{-1}. The results represent the beginning of a sequence of studies about immobilization cells in loofa sponge and its biotechnological application for the enzymatic synthesis of FOS.

Keywords: Fructooligosaccharides. Sponges. Submerged culture. Microbial cell immobilization. Prebiotics.

1 INTRODUCTION

Fructooligossaccarides (FOS) are fructose oligomers that are beneficial to human health and nutrition such as low-calorie, prebiotic and anticariogenic properties, help the control of cholesterol, and prevent anemia, obesity, osteoporosis, hypertension, diabetes, lactose intolerance, renal failure and colon cancer [1,2]. 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 [3,4,5]. Cells of *Aspergillus oryzae* IPT-301 have been reported as a potentially producing source of FTases, with high transfructosylation activity, among the seventeen strains of filamentous fungi currently evaluated [6]. The use of soluble enzymes requires the recovery and reuses of these to make the process economically viable [7]. In addition, soluble biocatalysts deactivate rapidly during immobilization, and the solid matrices can limit enzymatic activity by causing transport limitations to the molecules of substrate and products or by modifying the microenvironment of the enzyme [3,5]. Thus, microbial cell immobilization emerges as a strategy to overcome problems related to instability for large-scale application of enzymatic bioprocesses [8]. The operational advantage when compared to the use of soluble enzymes techniques can promote significant increases in activity and stability of the heterogeneous biocatalyst concerning to reuse and storage, which is generally higher than the soluble enzyme [9]. Therefore, the immobilization of microbial cells in support materials allows the acquisition of heterogeneous biocatalysts that are robust and resistant to the adverse conditions of the reaction medium [3]. Loofa (*Luffa cylindrica*) sponges are a typically tropical plant species that are abundantly found in African and Asian countries and almost all regions of Brazil. They are light, cylindrical in shape, and the makeup of an interconnecting void within an open network of matrix support materials [10]. As a result of their random lattice of small cross sections coupled with very high porosity, their potentiality as carriers for cell immobilization is very high, 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 [9]. Studies found in the literature reported great success in the use of loofa sponges as support materials [9,10,11]. Therefore, this work aimed to evaluate the production of cells of *A. oryzae* IPT- 301 by submerged cell culture and their concomitant immobilization in a loofa sponge. For this, the capacity of growth and adhesion of the microbial cells as a function of cultivation time, as well as their transfructosylation activity (A_T) were investigated to obtain robust and active biocatalyst for the synthesis of FOS.

2 MATERIAL & METHODS

Cells of *A. oryzae* IPT-301 were produced and immobilized by submerged cell culture in 50 mL of synthetic and sterile culture medium, pH 5.5, containing cubic supports of loofa sponges with edges of 1.0 cm, according to adapted method[1 2]. The support materials used for the immobilization, were cut manually, submerged in distilled water, and heated in a water bath at 100 ºC for 30 min. The loofa sponges were previously washed with distilled water and, after 24 h, it was dried in a drying oven at 70 ºC for 6 h[13] (Figure 1A). The cultivation occurred in a rotary shaker at 30 ºC and 200 rpm for 50 h, inoculation 500 µL of a suspension at 1 x 10^7 spores mL^{-1} [4]. After cultivation, the culture broth was vacuum-filtered using a Whatman Nº 1 filter paper to retain the microbial cell immobilized (heterogeneous biocatalyst) with enzymatic activity (Figure 1B). Subsequently, immobilized cells were used for the construction of microbial growth curves. The amount of biocatalytic cells immobilized in the loofa sponge as a function of culture time was obtained by determining its dry mass, according to the adapted method[11]. For this, the mass of cells produced was calculated by the difference between the masses of dry cells and support contained in the culture medium ($g_{cells}\ g_{support}^{-1}$), respectively. The dry cell mass was obtained after the enzymatic activity assays in a drying oven at 40 ºC for 48 h. The mean ratio

of dry cell mass and support material values, with their respective standard deviations, were plotted as a function of the cultivation time for the construction of microbial growth curves. The transfructosylation activity was determined according to an adapted method [12]. For this, 3 cubic particles of heterogeneous biocatalyst were incubated in 3.7 mL of sucrose solution 47 % (m v^{-1}) and 1.2 mL of a tris-acetate buffer 0.2 mol L^{-1}, pH 5.5. The reaction was performed in a Dubnoff bath at 50 ºC, 190 rpm for 60 min and interrupted by the immersion of the reaction medium in boiling water for 10 min and an ice bath for 5 min for enzymatic inactivation. The reaction medium was vacuum-filtered and the concentration of reducing sugar and glucose were quantified by 3,5-dinitrosalicylic acid (DNS) and enzymatic Glucose kit (GOD-PAP®) methods, respectively [4]. The unit of transfructosylation activity was defined as the amount of enzyme that transfers one micromole of fructose per minute per gram of dry heterogeneous biocatalyst under the chosen experimental conditions [4,5,12]. All experiments were performed in triplicate. The analysis of the medium for the microbial growth curves and enzymatic activity assays was performed by Tukey's honest significance difference test, with a confidence interval of 95 %.

Figure 1 Loofa (*Luffa cylindrica*) sponges before cell immobilization (A). Cells of *A. oryzae* IPT-301 immobilized in loofa sponge under the conditions of cultivation in synthetic culture medium: pH 5.5, 30 ºC, 200 rpm, and 28 h of cultivation (B).

3 RESULTS & DISCUSSION

The cultivation and immobilization assays were performed to determine the best time of cultivation and immobilization concomitant in order to obtain a heterogeneous biocatalyst with a higher value of transfructosylation activity. Thus, it was possible to observe in Figure 2 the relationship between cultivation time, enzymatic activity, and biocatalytic cells produced and immobilized in loofa sponge. It was observed the exponential growth phase (or "log phase"), occurs immediately after the lag phase, at that time the cells adjust to their new environment and begin high cell proliferation, increasing the cell mass exponentially over time [15]. This phase extended over 50 h of culture, obtaining a peak concentration of immobilized cells equal to 2.08 ± 0.26 g_{cells} $g_{support}^{-1}$ (Figure 2). The latency phase (or "lag phase") that is characterized by the adaptation of cells to the new environment, being the period preceding the exponential growth of cells, was also not identified, since it possibly occurred before the first point collected, i.e., before 8 h of cultivation. Before 50 h of cultivation, the phases of stationery and cell death were not observed in this culture. The high concentration of immobilized cells derives mainly from the size and distribution of the pores of the support material used, which favors the diffusion of gases and substrate inside it [9]. It was verified that, for 28 h of cultivation, number of cells equal 0.64 ± 0.11 g_{cell} $g_{support}^{-1}$ were produced and immobilized with the highest transfructosylation activity values (559.96 ± 40.37 U g^{-1} defined as 100 % of the relative activity). The results showed that, although the cell mass is increasing over time after 32 h of culture it begins to reduce enzyme activity. It is important to emphasize that this cultivation time was the shortest cultivation time obtained in the literature involving biocatalysts for FOS synthesis and, therefore, this factor makes this work even more attractive for industry, which constantly seeks fast and efficient processes to optimize its productivity [4,5,12,14,16]. Other author [3,5] obtained the highest activities for 64 h of cultivation, under the same experimental conditions, but using a different immobilization technique involving only the microbial cells which underwent crosslinking with glutaraldehyde. Therefore, the results showed that the cells of *A. oryzae* IPT-301 with high A_T can be concomitantly produced and immobilized with a reduction of approximately 56 % in cultivation time and applied for the synthesis of FOS. Studies related production and immobilization of *Penicillium expansum* cells in loofa sponge matrices (3 x 3 mm) for the enzymatic synthesis of FOS [11]. The authors obtained the maximum concentration of immobilized cells of 0.28 g_{cells}. $g_{suporte}^{-1}$ after 48 h process and it defined as the best cultivation time, at 25 ºC and 160 rpm in synthetic culture medium. However, for the time 44 h of culture using the fungus *A. oryzae* IPT-301, this work obtained better results, in which immobilized microbial cell mass was 1.31 ± 0.05 g_{cells}. $g_{suporte}^{-1}$ [1].

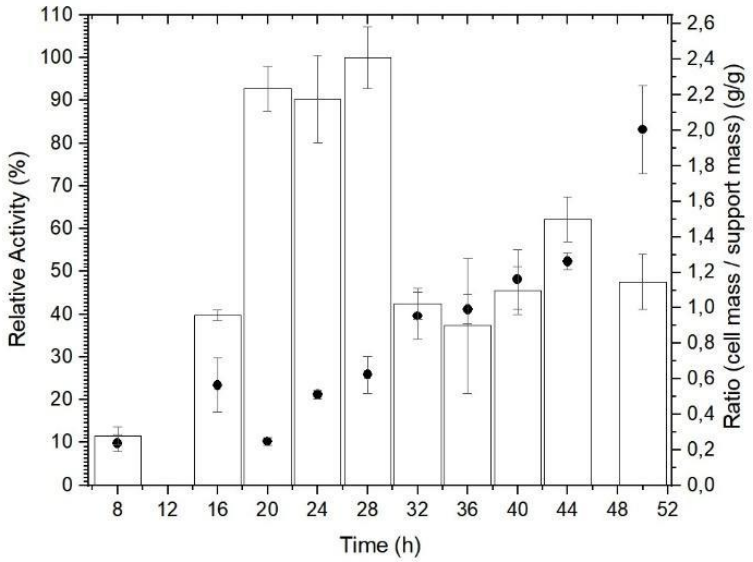

Figure 2 Influence of microbial cultivation time on the transfructosylation activity and concentration of cells of *A. oryzae* IPT-301 immobilized in loofa (*Luffa cylindrica*) sponge as a function of cell culture time. The maximum enzymatic activity (559.96 ± 40.37 U g^{-1}) was defined as 100% of the relative activity, which value is represented by bars. The ratio (cell mass/support mass) (g g^{-1}) is represented by points. Cultivation conditions in synthetic culture medium: pH 5.5, 30 ºC, 200 rpm, and 50 h of culture.

4 CONCLUSION

Through the microbial growth curve, it was observed that the immobilization in loofa sponge optimized the cultivation time resulting in the shortest time reported in the literature involving biocatalysts for FOS synthesis. The immobilization time of 28 h resulted in a catalytic activity of 559.96 ± 40.37 U g^{-1} and production of number of cells equal 0.64 ± 0.11 g_{cell} $g_{support}^{-1}$. Thus, the organic support proved to be a potential material for immobilization, since it promoted a link with biomass allowing it to remain active for application in transfructosylation reactions. This set of initial results shows the technological innovation present in this study both for the synthesis of FOS, as in the advancement of the bioproducts segment of industrial interest and the implementation of various bioprocesses.

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