GALACTOOLIGOSACCHARIDES FROM PORUNGO CHEESE WHEY USING DIFFERENT BIOPROCESS STRATEGIES

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ABSTRACT

Different bioprocess engineering strategies can positively contribute to the production of biomolecules of interest, such as enzymes and prebiotics. This study aims to produce galactooligosaccharides (GOS) from porungo cheese whey using different bioprocess strategies. The β-galactosidase was produced by *Kluyveromyces marxianus* in the medium of porungo cheese whey (30 ºC, 200 rpm, pH 7.0), and the crude enzyme extract was used to immobilize the β-galactosidase on chitosan-genipin supports. Initially, GOS production was conducted in conical flasks from three different variables: enzyme concentration (50 U/mL - 150 U/mL), lactose concentration (200 g/L - 400 g/L), and temperature (37 – 43 ºC). The highest GOS yield (15.24%) occurred under intermediate process conditions (100 U/mL, 300 g/L, 40 ºC), reaching a GOS concentration of 27.04 g/L. Then, these better conditions were used to improve the bioprocess performance in packed-bed column bioreactor operated in batch mode, reaching highest yield (19.72%), lactose conversion (67.01%), and productivity (10.22 g/Lh).

Keywords: Galactooligosaccharides, β-galactosidase, cheese whey, immobilization.

1 INTRODUCTION

The need for a better life quality and individual well-being has motivated the food and pharmaceutical industries to develop technological strategies to obtain new biomolecules, such as galactooligosaccharides (GOS)[1,2]. GOS synthesis occurs by the transgalactosylation reaction catalyzed by β-galactosidases from different microorganisms and is influenced by variables such as substrate concentration, pH, and temperature[2]. Process improvements are continuously being investigated, and the technique of enzyme immobilization is a potentially promising strategy[3,4]. Additionally, sustainable GOS production has been reported from the use of waste, such as cheese whey[4]. Our research group is a pioneer in investigating the use of porungo cheese whey, which has been a potential raw material for the production of biomolecules of interest, of great commercial relevance and high economic value through of bioprocesses[4,5,6].

2 MATERIAL & METHODS

The production of GOS occurred from β-galactosidase produced by *K. marxianus* CCT 4086 under conditions previously optimized by our group[5]. The crude enzyme extract was immobilized on chitosan-genipin supports, and the GOS production was carried out in shaker, evaluating the variables temperature (37 ºC - 43 ºC), concentration of enzyme (50 U/mL – 150 U/mL) and of porungo cheese whey (200 g/L – 400 g/L). The tests were performed in 125 mL conical flasks, containing 30% of the reaction volume with the immobilized enzyme, under gentle agitation (50 rpm). Subsequently, GOS production in a packed-bed column bioreactor was conducted in a batch mode (40 ºC and 300 g/L of porungo cheese whey), where the bed was filled in 60% of its useful volume with the immobilized biocatalyst. All tests were performed in duplicate. The samples were collected, centrifuged (3000 × *g* for 15 min), and filtered in an acetate-cellulose membrane (0.22 µm) for further analysis of the monosaccharides, lactose, and galactooligosaccharides by HPLC.

3 RESULTS & DISCUSSION

The results demonstrated that the greatest production of GOS occurred at the temperature of 40 ºC, with a yield and lactose conversion of 9.21% and 55.58%, were obtained respectively, reaching GOS3 concentration of 23.91 g/L in 6h of reaction (Figure 1). At the temperatures of 37 ºC and 43 ºC, the GOS production were lower (19.97 g/L and 22.30 g/L), reaching yields of 7.96% and 8.63%, and lactose conversion of 40.47% and 53.21%, respectively. These results indicate that the ideal temperature for GOS production cannot be so high as to cause destabilization and reduction in the enzymatic activity, but it should also not be

so low as to make the transgalactosylation process ineffective. From these results, the temperature of 40 ºC was chosen for the subsequent tests of GOS production.

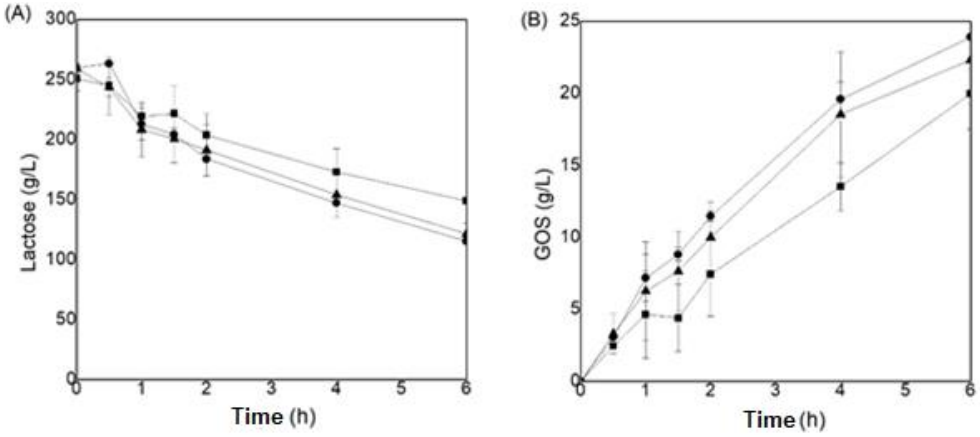

Figure 1. Kinetics of the consumption of lactose (A) and production of GOS (B) at different temperatures using the β-galactosidase immobilized on chitosan-genipin supports (50 U/mL), from porungo cheese whey (300 g/L), in a rotational incubator at 150 rpm for 6h of reaction. Temperature of 37 ºC (■), 40 ºC (●) and 43 ºC (▲).

The influence of the enzyme concentration on GOS production can be observed in Figure 2A. The highest GOS yields (15.24%) and lactose conversion (50.34%) were achieved at the concentration of 100 U/mL, and the lower values of these parameters were obtained for the concentration of 50 U/mL (10.22% and 21.41%), respectively. Moreover, for the greatest enzyme concentration (150 U/mL), intermediate values were obtained (12.57% and 32.66 %), indicating a possible limitation on the mass transfer on the surface of the matrix, probably due to the high enzyme load, which can hinder access to the substrate. Thus, the concentration of 100 U/mL was the one established for use in the subsequent experiments.

Regarding the influence of substrate concentration on GOS production (Figure 2B), the maximum GOS production (32.79 g/L) was obtained from a lactose concentration of 400 g/L, in 6 hours of reaction. Nevertheless, considering GOS yield and lactose conversion, the most effective strategy was for intermediate substrate concentration (300 g/L), in which GOS production of 27.04 g/L was obtained at 4 h of reaction, reaching the maximum GOS yield (15.24%) and lactose conversion (50,34%). In this research, it was observed that low substrate concentrations (200 g/L) led to the lowest yields (8.35%), which was 45.21% inferior than obtained at the lactose concentration of 300 g/L, and 35.72% lower for the concentration of 400 g/L (12.99%). Regarding lactose conversion, it was the highest (64.32%) for the lowest substrate concentration tested, possibly because of the conversion of not only GOS by transgalactosylation, but also undesirable products, such as glucose and galactose, possibly due to the simultaneous hydrolysis reaction. In this context, these results (40 ºC, 300 g/L of lactose and enzyme concentration of 100U/mL) were used for the subsequent experiments in the bioreactor.

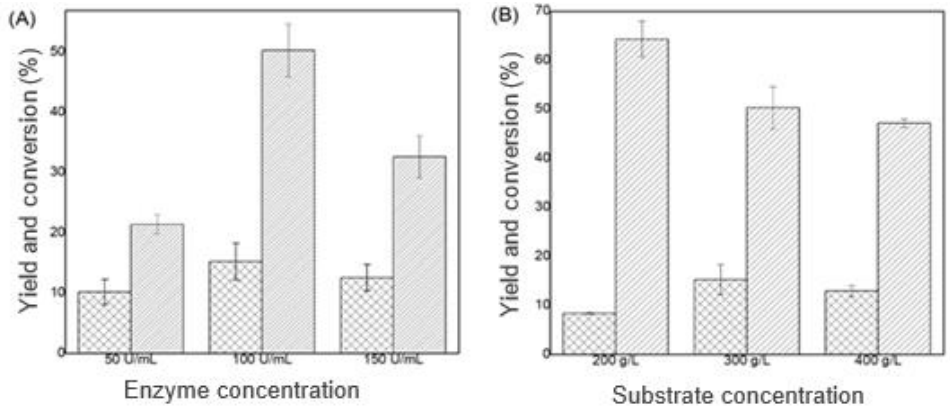

Figure 2. Influence of the immobilized enzyme concentration (A) and lactose concentration in porungo cheese whey (B) on GOS yield and lactose conversion, in a rotational incubator at 150 rpm for 4h of reaction. Yield (▨) and Conversion (▨).

Figure 3 presents the kinetic profile of lactose consumption for GOS production in a fixed-bed column bioreactor operated in batch. The GOS3 concentration was its maximum (20.45 g/L) in only 2h of reaction, with a consumption of 68.35% of the initial substrate. Furthermore, a maximum GOS3 yield of 19.72%, maximum lactose conversion of 67.01%, and productivity of 10.22 g/L.h was observed, being 62.49% superior to what was obtained in the shaker (6.29 g/L.h), possibly because of the more robust geometry and also due to the improve in the ratio of biocatalyst per volume in a bioreactor compared to a simpler geometry.

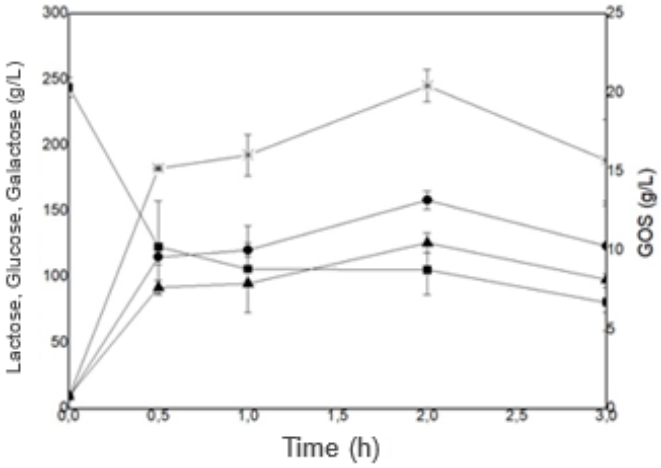

Figure 3. Kinetics of the conversion of porungo cheese whey (300 g/L), at 40 °C, from β-galactosidase immobilized in chitosan-genipin (100 U/mL) in a fixed-bed column bioreactor. Concentration of Lactose (■), glucose (●), galactose (▲) and GOS (*).

4 CONCLUSION

Porungo cheese whey demonstrates have a biotechnological potential for the production of enzymes and galactooligosaccharides, which contributes to a more sustainable production of biomolecules with a great commercial and world importance, and a high biotechnological interest in the food, pharmaceutical and agricultural industries.

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