**Development of biocatalysts for application in integrated process of lactose hydrolysis and glucose isomerization aiming the production of prebiotics**

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**Highlights**

* The coating of supports with polyethylenimine improves the immobilization parameters.
* The polyethylenimine biocatalysts were very effective in the hydrolysis of lactose.
* High conversion of glucose into fructose was obtained.

**1. Introduction**

β-galactosidase enzyme (E.C. 3.2.1.23) is widely used in the dairy industry for the hydrolysis of lactose, but over the years it has also increased its use as a catalyst for the synthesis of galactooligosaccharides (GOS) by favoring transgalactosylation reactions, having these GOS a recognized prebiotic function (Urrutia *et al.*, 2018). Besides that, studies have been carried out with the purpose of isomerizing the glucose formed in the hydrolysis reactions to fructose by glucose isomerase enzyme (GI) (E.C. 5.3.1.18) and the obtained fructose can be used as one of the substrates in the transgalactosylation reactions. These enzymes, when immobilized, can be much more effective because some enzymatic characteristics can be improved, such as the stability and activity, specificity or selectivity, and even its purity, besides the possibility of reusing the biocatalysts. The coating of supports has been proposed to extend its useful life, for example, the coating with a multifunctional polymer called polyethylenimine (PEI). This, in turn, involves the enzyme and suits its structure, giving greater stability, especially for multimeric enzymes like β-gal, and also protects from environmental variations, such as temperature, as well as to the aggressive effects of the activating agent (Bolivar *et al.*, 2009; Garcia-Galan, Barbosa and Fernandez-Lafuente, 2013). Therefore, the present work aims initially to evaluate the effects of coating the supports with PEI on the immobilization of β-gal from *Kluyveromyces lactis*, and then to determine the catalytic efficiency of these biocatalysts in the lactose hydrolysis. In parallel, it was evaluated the conversion of the formed glucose into fructose using GI from *Streptomyces murinus*, in order to obtain a product that can be used in the synthesis of prebiotics.

**2. Methods**

2.1. Immobilization of the β-galactosidase: β-gal (E.C. 3.2.1.23) from *Kluyveromyces lactis* was immobilized on chitosan gel (2 % w/v) activated with two different activation agents: glutaraldehyde (0.8 % v/v) according to Albuquerque *et al.* (2018) and glycidol adapted from Guisán (1988), and then the supports were coated with polyethylenimine - PEI (10 % w/v) adapted from Pessela *et al.* (2005). 10 mg of protein per gram of support was used in the immobilization and the process was performed at pH 7.0 and 25 °C in 100 mM potassium phosphate (KH2PO4) buffer, containing 0.1 mM MnCl2 and 0.2 mM MgCl2, under gentle agitation on a rotary shaker. The immobilization parameters were determined.

2.2. Catalytic Efficiency of Biocatalysts: The hydrolysis reactions were evaluated for 1.5 hours using a synthetic solution containing 66.7 g/L lactose, under stirring, pH 7.0 and 50 °C. After this process, the β-gal enzyme was removed and it was added 0.1 g of immobilized GI (E.C. 5.3.1.18) from *Streptomyces murinus*, 8 mL of KH2PO4 buffer, 2 mL of 0.5 mol/L MgSO4, 1 mL of 0.01 mol/L Co(NO3)2, and the isomerization reactions were evaluated for 4.5 hours under stirring, pH 7.5 and 70 °C. Samples were taken to determine the carbohydrate concentration (lactose, glucose, galactose and fructose) by High Performance Liquid Chromatography (Albuquerque *et al.*, 2018).

**3. Results and discussion**

The coating of supports with PEI considerably improved the immobilization parameters (Table 1), presenting higher results than those obtained by Albuquerque *et al.* (2018), which used the same immobilization conditions of the present study, but without coating the supports. It was expected since the PEI layer allows a very strong immobilization via multipoint adsorption, besides involving the enzyme and conforming to its structure, avoiding loss of activity due to conformational changes and thus conferring a greater stability to the biocatalyst (Pessela *et al.*, 2005).

Table 1. Immobilization parameters and efficiency of the integrated process of lactose hydrolysis and glucose isomerization by immobilized β-gal and immobilized GI, respectively.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Biocatalyst** | **Immobilization yield (%)** | **Activity offered (U/g)** | **Derivative activity (U/g)** | **Recovered activity (%)** | **Conversion of lactose (%)** | **Conversion of glucose (%)** |
| **A** | 97.45 | 210.28 | 95.26 | 45.30 | 63.96 | 36.02 |
| **B** | 92.74 | 175.36 | 142.96 | 81.52 | 71.72 | 21.18 |

β-gal immobilized on: (A) Chitosan activated with glutaraldehyde and coated with PEI; (B) Chitosan activated with glycidol and coated with PEI.

For the hydrolysis of lactose into glucose and galactose, the biocatalyst B showed the closest result at obtained by the soluble enzyme (88 %), after 2 hours of reaction and under the same conditions used (Albuquerque *et al.*, 2018). Biocatalyst B showed the highest conversion of lactose, which was expected since it had a superior enzymatic activity (142.96 U/g). At the end of this process, the reaction media containing the biocatalysts A and B showed concentrations of 44.9 g/L or 50.35 g/L of glucose/galactose and 24.04 g/L or 18.86 g/L of lactose, respectively. For the isomerization reactions, the highest conversion obtained was 36.02 %, an excellent result when compared to that obtained by Yu *et al.* (2011) (45 %), after 16 hours of reaction and using an enzyme dosage of 2 g. However, the reaction medium from biocatalyst B contained higher concentrations of glucose and galactose, and this may have affected the performance of the GI, leading to a lower conversion.

**4. Conclusion**

The coating of the supports with polyethylenimine improved the immobilization process of β-galactosidases. In addition, these biocatalysts were efficient for the hydrolysis of lactose, and it was possible to convert the obtained glucose into fructose through the isomerization reactions, thus proving that the use of this integrated process is possibly promising to produce prebiotics.

**References**

1. Bolivar, J. M. *et al.*, Biomacromolecules. (2009) 742–747.
2. Garcia-Galan, C., Barbosa, O., Fernandez-Lafuente, R., Enzyme and Microbial Technol. (2013) 211–217.
3. Guisán, J. M., Enzyme Microbial Technol. 10 (1988) 375–382.
4. Albuquerque, T.L *et al.,* Process Bioch. 73 (2018) 65–73.
5. Pessela, B. C. C. *et al.*, Enzyme and Microbial Technol. 37 (2005) 295–299.
6. Urrutia, P. *et al.*, Biological Macromol. (2018).
7. Yu, D. *et al.*, Process Bioch. (2011) 599-603.