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Development of an Integrated Reaction Design for the Enzymatic Production of Lactulose

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Abstract : Galactooligosaccharides (GOS) are sugars with prebiotic function that can be synthesized chemically or enzymatically, and this last one can be promoted by the action of β-galactosidases. In addition to favoring the transgalactosylation reaction to form GOS, these enzymes can also catalyze the hydrolysis of lactose. A highly studied type of GOS is lactulose because it presents therapeutic properties and is a health promoter. Among the different raw materials that can be used to produce lactulose, whey stands out as the main by-product of cheese manufacturing, and its discarded is harmful to the environment due to the residual lactose present. Therefore, its use is a promising alternative to solve this environmental problem. Thus, lactose from whey is hydrolyzed into glucose and galactose by β-galactosidases. However, in order to favor the transgalactosylation reaction, the medium must contain fructose, due this sugar reacts with galactose to produce lactulose. Then, the glucose-isomerase enzyme can be used for this purpose, since it promotes the isomerization of glucose into fructose. In this scenario, the aim of the present work was first to develop β-galactosidase biocatalysts of Kluyveromyces lactis and to apply it in the integrated reactions of hydrolysis, isomerization (with the glucose-isomerase from Streptomyces murinus) and transgalactosylation reaction, using whey as a substrate. The immobilization of β-galactosidase in chitosan previously functionalized with 0.8% glutaraldehyde was evaluated using different enzymatic loads (2, 5, 7, 10, and 12 mg/g). Subsequently, the hydrolysis and transgalactosylation reactions were studied and conducted at 50°C, 120 RPM for 20 minutes. In parallel, the isomerization of glucose into fructose was evaluated under conditions of 70°C, 750 RPM for 90 min. After, the integration of the three processes for the production of lactulose was investigated. Among the evaluated loads, 7 mg/g was chosen because the best activity of the derivative (44.3 U/g) was obtained, being this parameter determinant for the reaction stages. The other parameters of immobilization yield (87.58%) and recovered activity (46.47%) were also satisfactory compared to the other conditions. Regarding the integrated process, 94.96% of lactose was converted, achieving 37.56 g/L and 37.97 g/L of glucose and galactose, respectively. In the isomerization step, conversion of 38.40% of glucose was observed, obtaining a concentration of 12.47 g/L fructose. In the transgalactosylation reaction was produced 13.15 g/L lactulose after 5 min. However, in the integrated process, there was no formation of lactulose, but it was produced other GOS at the same time. The high galactose concentration in the medium probably favored the reaction of synthesis of these other GOS. Therefore, the integrated process proved feasible for possible production of prebiotics. In addition, this process can be economically viable due to the use of an industrial residue as a substrate, but it is necessary a more detailed investigation of the transgalactosilation reaction.

Keywords : beta-galactosidase, glucose-isomerase, galactooligosaccharides, lactulose, whey
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