Short Communication

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Magnetic nanoparticles coated with polyaniline to β-galactosidase immobilization and lactulose production

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Abstract

Prebiotics are functional ingredients, undigested through the gastrointestinal tract, since they can withstand gastric pH mucosa and inhibit reproduction of pathogenic microorganisms. The lactulose assumes importance because it induces numerous beneficial to human healt. Lactulose synthesis using β -galactosidase immobilized on nanopaticulas magnetic coated with polyaniline (Mpani) was determined under optimal reaction conditionsc, such as pH and concentrations of lactose and fructose. The productivity of synthesis and the maximum lactulose produced and quantity was 2.1 g/L, using 200/L of fructose and lactose at pH 8.0 at 40 ° C. Thus, it can be concluded that a support is Mpani attractive and efficient for β-galactosidase immobilization and lactulose synthesis.

Introduction

Prebiotics are functional ingredients, whose functionality due not to digestion by the gastrointestinal tract, since they can resist to pH of the gastric mucosa, and selectively enhance the reproduction of bacteria of the microbiota, inhibiting the growth of pathogenic microorganisms (LONG et al., 2015).

The production of lactulose by pharmaceutical and food industry has increased due to its benefits for human health. This prebiotic is formed by the union of monosaccharides galactose and fructose in the glycoside portion of β-1,4 (NEVE et al., 2013). This disaccharide can be synthesized by chemical and enzymatic reactions, which are catalyzed by hydrolases glisosil (GH, EC 3.2.1.X), such as β-glicosidase and β-galactosidase (WANG et al., 2013).

The use of enzymes as biocatalyst is limited due to lack of stability and susceptibility to denaturation (HOMAEI et al., 2013). As a solution to this problem, enzymes have been immobilized in various typesof supports.

Iron nanoparticles possess stability, mechanical and chemical resistence, high surface area, high mobility and mass transfer (SOUZA; MOHALLEM; SOUSA, 2011; ROCHA, 2016). This material is produced from the co-precipitation of Fe2+ and Fe3+ and it has been coated with polymers, which allow the particles to disperse in homogeneous fluids increasing its stability and biocompatibility (ARAÚJO, 2011; MACIEL, 2012).

The present study aimed to lactulose synthesis by covalent β-galactosidase immobilized on coated magnetic nanoparticles with PANI.

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Material and Methods

Synthesis of iron oxide nanoparticles with PANI

Nanoparticles the iron oxide are synthesized according to the protocol described by Maciel et al., (2012).

Coating with PANI

Oxidative polymerization of aniline was carried out in the presence of magnetic nanoparticles by treated with KMnO4, according to NERI et al. (2009).

β-galactosidase immobilization

Nanoparticles coated with PANI (mPANI) were activated with glutaraldehyde in 2.5% (v/v). Then the particles were washed with distilled water and mM 50 potassium phosphate buffer (KPB) , pH 7.0. mPANI was incubated with β-galactosidade solution containing 500 µg/mL at pH 8,0 for 10 hours at 4 °C. To stimulate the amount of enzyme attached to the support and the retention of specific activity, protein concentration of the enzyme solution, the washed and supernatant was determined using the protocol described by Lowry et al. (1951).

Lactulose production

Lactulose production in presence of lactose and fructose by β-galactosidase was performed in 50 mM KPB , pH 8.0 and in 50 mM glycine-NaOH buffer , pH 9.5 a 40 °C for 5 hours. To investigate the influence of the amount sugar donor and recipient, early concentrations of lactose and fructose were tested of 100 and 200 g/L, varying the mixture ratio in the reaction medium. Thereafter, aliquots were withdrawn and heated at 100 ° C for 10 minutes and filtered. Lactose, galactose, glucose, fructose and lactulose concentration were determined by HPLC (Jasco AS-2057 Plus), using column Prevail Carbohydate ES (5 µm, 250 x 4.6 mm) (Alltech)

and evaporative light scattering detector Sedex 85 (Sedere). The mobile phase used was acetonitrile and water solution (70:30, v/v) at a flow rate of 0.9 mL.min-1 (Jasco PU-2080 Plus).

Results

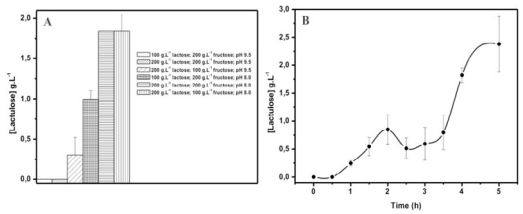

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Figure 1 – Analysis of donor and receptor aount and pH influence in enzymatic synthesis of lactulose (a). Time course of lactulose production from lactose and fructose. Reactions were conducted at 40ºC using lactose and frutose 200g.L -1 in 50mM potassium phosphate buffer pH 8.0 (b).

The increased production of prebiotic (1,84 g/L) occurred when IED (containing 3,5 U/mg specific activity), was incubated with equal amounts of lactose and fructose (200 g/L) at pH 8.0 at 40 °C (Figure A). Subsequently, the transgalactosylation reaction kinetics was evaluated. After 5h of reaction, the productivity of lactulose synthesis and the maximum amount produced was 0,5 g/L/h and 2,1 g/L, respectively, using 200 g/L of fructose and lactose at pH 8.0 at 40 °C (Figure B).

Discussion and Conclusion

This study aimed to evaluate the potential of lactulose synthesis using iron nanoparticles. The optimum pH and concentration of lactose and fructose synthesis lactulose were 8.0 and 200 g/L, respectively, and prebiotic amount produced was 2,1 g/L. The mPANI proved to be an attractive and efficient support for β-galactosidase immobilization and lactulose synthesis.

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