β-glucosidase immobilization on chemically modified chitosan support: Adsorption Kinetics studies

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ABSTRACT

In this study, β-glucosidase from Aspergillus niger was immobilized on a heterofunctional support prepared by activating chitosan (Chit) hydrogel with glutaraldehyde (GA), then functionalizing it with glycine (Chit–GA–Gly). Then adsorption kinetics studies were evaluated to elucidate the immobilization process. The pseudo-first-order model showed more adequate to explain the adsorption process (R2 = 0.9816), indicating a physisorption (reversible) processes due to ionic interactions. Thus, after 30 minutes almost 95% of enzyme activity were immobilized compared with a 15% of total protein, suggesting that occurred a partial purification, since was observed a preferential immobilization of β-glucosidase among all proteins contained in the raw extract.

Keywords: Ionic interactions 1. Adsorption studies 2. Fungal enzymes 3. immobilization 4.

1 INTRODUCTION

B-glucosidase (E.C.3.2.1.21) are hydrolases enzymes that cleaves β 1,4 glycosidic linkages [1] present in glycosidic substrates and play important role in plant defense, aroma releasing in beverages, detergent, pharmaceutical and biofuels industry [2, 3]. As these enzymes are present in several segments of industrial, they have an important space in the global market, being responsible for approximately 11% in 2021 [4;5]. Even these enzymes being important, their use in a soluble form in the industrial process are high cost and present poor stability in harsh conditions [6]. The immobilization strategies are an important tool to mitigate these problems [6;7;8;9]. Chitosan is derived from chitin a biopolymer abundant in shrimp shells, having application in medical, pharmaceutical, agricultural, immobilization support and waste treatment industry [10;14]. The modification in chitosan surface improve the bond stability, and the diversity of possibility of interactions due to groups addition [8].

2 MATERIAL & METHODS

Materials

Aspergillus niger LBA 02 was obtained from the Culture Collection of Food and Biochemistry Laboratory, University of Campinas, Campinas-SP, Brazil and wheat bran (Nattuday, Formiga, MG, Brazil) was purchased from a local store, bovine serum albumin (BSA), and *ρ*-nitrophenyl β-D-glucopyranoside (*ρ*-NPG) were all acquired from Sigma-Aldrich (St. Louis, MO, USA). Shrimp chitosan (powder form, with a 75–85% degree of deacetylation and a molecular mass distribution between 50 and 190 kDa) was also obtained from Sigma-Aldrich. Glutaraldehyde solution—GA (25% $v \cdot v^{-1}$); glycine (Gly); and sodium dodecyl sulfate (SDS) were purchased from Synth® (São Paulo, SP, Brazil). All other chemical reagents and organic solvents used in this study were of analytical grade and obtained from Synth®.

β-glucosidase production and enzyme extraction

The enzyme production and extraction were prepared according to Figueira et al. [7] with few modifications.

Preparing the Heterofunctional Supports

The supports were prepared according Miguez et al. [8]. The heterofunctional support (Chit–GA–Gly) was prepared by suspending 10 g of Chit–GA in 90 mL of a 0.5 $mol \cdot L^{-1}$ glycine solution at pH 8.0 at 25 °C maintained under orbital agitation (200 rpm) for 48 h. The resulting support was filtered in a Buchner funnel with Whatman filter paper N° 41 and thoroughly washed with distilled water. The support was stored in a 70% ethanol solution ($v \cdot v^{-1}$) at 4 °C until use.

Immobilization of β-glucosidase on Chit–GA–Gly Hydrogel

The β-glucosidase immobilization procedure on the supports was conduced according Miguez [8] with modificantions. The immobilization process involved preparing a suspension by combining the enzymatic solution, previously mixed with a 5 $mmol \cdot L^{-1}$ buffer solution pH 5.0 and the support in a 1:20 ($m \cdot v^{-1}$) proportion (support/ β-glucosidase solution). The immobilization suspension was then incubated at 25 °C on an orbital shaker (200 rpm) with 6 h of contact time. The heterogeneous biocatalysts were prepared by offering initial activity loading of 5 $U.g^{-1}$ of support. Once the enzyme was immobilized, the prepared biocatalysts

were vacuum filtered using a Buchner funnel with Whatman filter paper N° 41 and thoroughly washed with distilled water. The adsorption process was monitored by measuring the decrease in protein concentration and hydrolytic activity in the supernatant. Subsequently, the prepared biocatalysts were stored at 4 °C in the refrigerator for 24 h.

Determination of β-glucosidase activity

β-glucosidase activity was determined in accordance with Matsuura et al. [11], but with a few modifications [7]. The amount of *ρ*-nitrophenol release was determined based on the standard curve of *ρ*-nitrophenol ranging from 5 to 300 µmol. A unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of *ρ*-nitrophenol per minute of reaction in the experimental conditions described previously.

Determination of protein concentration.

Total protein concentration was determined through Bradford's protein assay [12] using BSA as standard.

Influence of Immobilization Time: Kinetic Adsorption Studies

The kinetics of β-glucosidase from *A. niger* adsorption on Chit–GA–Gly were studied under at initial activity loading of 5 $U.g^{-1}$ protein. The immobilization process was monitored by measuring the residual protein concentration in the supernatant of the immobilization suspension using the Bradford method [69]. Three conventional non-linear kinetic models, namely the pseudo-first-order (Equation (1)), pseudo-second-order (Equation (2)), and the Elovich models (Equation (3)) [13], were fitted to the experimentally obtained data:

$$q_t = q_e \times \left(1 - e^{-k_1 \times t}\right) \quad (4)$$

$$q_t = \frac{k_2 \times q_e^2 \times t}{1 \times k_2 \times q_e \times t} \quad (5)$$

$$q_t = \frac{\ln(\alpha + \beta)}{\beta} + \frac{\ln(t)}{\beta} \quad (6)$$

where q_t and q_e are the concentration of adsorbed β-glucosidase at a given time *t* and at equilibrium ($U \cdot g^{-1}$), respectively; k_1 (min^{-1}) and k_2 ($mg \cdot g^{-1} \cdot min^{-1}$) are the first-order and second-order adsorption rate constants, respectively; *α* is the initial adsorption rate ($mg \cdot g^{-1} \cdot min^{-1}$); *β* is the desorption constant ($g \cdot g^{-1} \cdot mg^{-1}$); and *t* is the contact time.

3 RESULTS & DISCUSSION

In this study, the influence of immobilization time on the enzyme immobilization process on Chit–GA–Gly was evaluated at initial activity loading of 5 $U.g^{-1}$. These tests were conducted in 5 $mmol \cdot L^{-1}$ sodium acetate at pH 5.0 and 25 °C, with mechanical stirring at 200 rpm. In previous work the β-glucosidase enzyme presented high stability in pH 5.0 after 3 hours remaining with 90 % of residual activity (Cunha, 2022).

As shown in Figure1 A–C, increasing the contact time increases the amount of enzyme adsorbed in the support.

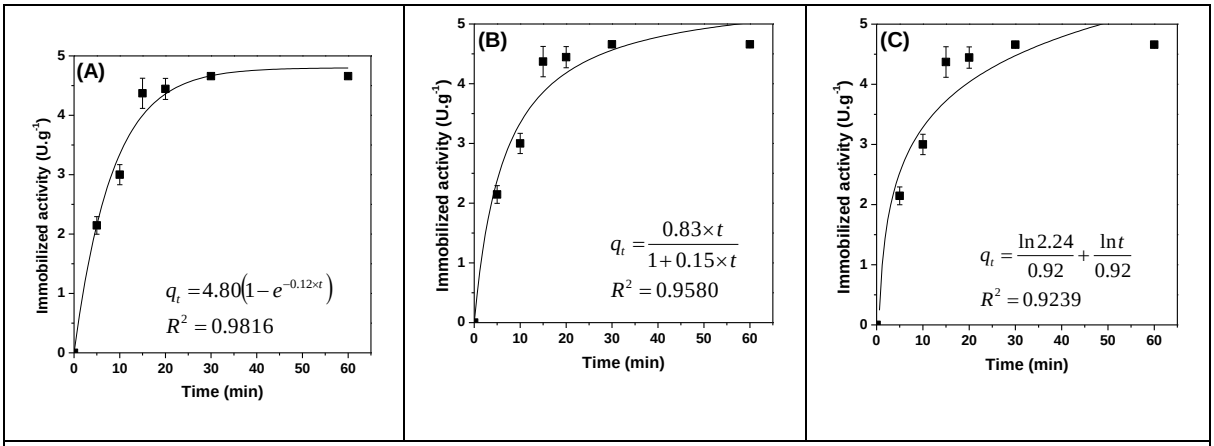

Figure 1. Non-linear adsorption kinetic models of pseudo-first-order (A); pseudo-second-order (B); and Elovich (C) plots for the adsorption of β-glucosidase from *Aspergillus niger* on Chit–Glu–Gly support at 25 °C, 200 rpm, and pH 5.0 (buffer sodium acetate at low ionic strength – 5 $mmol \cdot L^{-1}$), using an initial activity loading of 5 $U.g^{-1}$ of support.

Equilibrium in the adsorption process conducted at initial activity loading of 5 U.g−1 was reached after 30 min of contact. To elucidate the process of the enzyme adsorption on the prepared heterofunctional support, the non-linear kinetic pseudo-first-order, pseudo-second-order, and Elovich models were fitted to the resulting experimental data. The kinetic model that presented the higher correlation coefficient (R2) was selected as the most adequate to explain the adsorption process. As shown, the pseudo-second-order (Figure 1B) and Elovich kinetic model (Figure 1C) models produced the lowest R2 values for the protein loading evaluated (0.9580 ≥ R2 ≥ 0.9239). However, kinetic pseudo-first-order (Figure1A) the produced the highest correlation coefficient values (R2 = 0.9816) for at initial activity loading of 5 U.g−, and was thus selected to describe the kinetics of β-glucosidase immobilization on the selected support. The pseudo-first-order adsorption kinetic model has been consistently employee to describing physisorption (reversible) processes. In this study, the reversible adsorption of the β-glucosidase occurs via preferential Ionic interactions of the enzyme of the amino protonate groups (amino terminal of the enzyme and the lysine and arginine residues) and carboxylate (carboxy-terminal and those of glutamate and aspartate residues) with the ionic groups introduced on the surface of the support as carboxylate and amino protonate groups (reversible immobilization). Besides, in the conditions assayed almost 95% of enzyme activity was immobilized in the support while approximately 16 % of total protein, suggesting that the immobilization method promoted a partial purification contributing for a preferential immobilization of β-glucosidase in the support.

4 CONCLUSION

Kinetic studies demonstrated β-glucosidase from *A. niger*, was preferentially adsorbed via reversible interactions. A maximum immobilized activity amount of ·4.5 U.g−1 was obtained after 30 min of contact time in a batch mode under mild experimental conditions −25 °C, 200 rpm at low ionic strength (5 mmol·L−1) pH 5.0. The immobilization process promoted a partial purification mechanism, presenting a 95 % of enzyme activity immobilization and a 16% of total protein offered.

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