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IMMOBILIZATION OF COMMERCIAL RESAMILASE ONTO SILLANIZED GLASS CAPILLARIES.

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1.INTRODUTION

Amylases are widely used in several industrial applications, such as animal feed, foods, textiles and paper pulp industry. The immobilization techniques offers a number of advantages over free enzyme, specially the possibility of their use repeatedly or in a continuous mode. This work describes the immobilization of a commercial *Bacillus subtillis* amylase (Resamilase - Novozyme) onto sillanized glass capillaries and some reaction parameters optimization. The amylase was covalently bound via glutaraldehyde.

2. MATERIALS AND METHODS

2.1.Enzyme Activity Determination

The amylase activity was measured as follows: to 100μL of a 0.5% (w/v) potato starch solution, were added 80μL of 0.1 mol L^{-1} acetate buffer pH 5.0 and 20μL of an enzyme extract diluted in a proportion of 1:1000 in the same buffer. The mixture was incubated at 40°C for 15 min and then, the reaction was interrupted by addition of 200μL of 0.1 mol L^{-1} acetic acid solution. 200μL of Iodine reagent was then added, the volume was completed to 10mL with distilled water and the amount of starch remaining was measured at 660 nm. The enzyme activity was expressed as percentage of starch hydrolysis, considering the initial concentration measured comparing the initial starch concentration (blank proof) with that remaining after enzyme hydrolysis. All tests were done in triplicate and results are presented as average plus standard deviation.

2.2.Optimum pH for free amylase activity

In the tests of optimum pH determination, the enzyme was diluted in different buffer solutions and activity determined as described above. The buffers used were 0.1 mol L^{-1} sodium acetate for pH values 4.0 and 5.5 and 0.1 mol L^{-1} phosphate buffer for pH 6.0-8.0.

2.3.Electrophoresis

The amylase extract was submitted to sodium dodecyl sulfate-polyacrylamide gel eletrophoresis (SDS-PAGE) under denaturing conditions using a 10% acrylamid gel, as described by Laemmli. The following molecular mass markers were used to determine the mass molecular of the enzyme

2.4. Immobilization of amylase

The amylase immobilization was carried out using three-step process of Weetall (1976) as shown. In the first step the glass capillaries were immersed in a 10% (v/v) 3-aminopropyl-triethosysilane (APTES) pH 4.0, at 75ºC for 2h. In the second step, 2.5% (v/v) glutaraldehyde solution in 0.1 mol L^{-1} phosphate buffer, pH 7.0, was coupled to amino group of alkylamine, at room temperature for 30 mim. After that, the

capillaries were washed with distilled water and 0.1 mol L^{-1} acetate buffer, pH 5.5. In the last step, the immobilization of α-amylase was done by filling the capillaries with 600 µL of varied dilutions of the amylase solution. The reaction was carried out at room temperature, by time intervals from 5 to 120 min. The pH of the enzyme solution was varied from 4.0 to 8.0 to optimize the immobilization parameters.
After immobilization procedure the capillaries were sequentially washed with 0.1 mol L^{-1} acetate buffer, pH 5.5, 0.1 mol L^{-1} glycine pH 5.5 and 1.0 mol L^{-1} NaCl solution to remove the unbounded enzymes.

2.5. Determination of immobilized enzyme activity

After enzyme immobilization, the activity was determined filling the capillaries with 600µL of 0.5% (w/v) potato starch solution, following by incubation at 40ºC in varied time intervals. An 200µL aliquot of the incubated starch solution was added to 200µL of 0.1 mol L^{-1} acetic acid and 200µL of Iodine reagent. The volume was completed to 10 mL with distilled water and the amount of starch remaining was measured at 660 nm. All tests were done in triplicate and results showed as average and standard deviation. Blanks were carried out using glass capillaries without enzyme.
The enzyme activity also was assayed by the determination of reducing sugar releasing using the 3.5-dinitrosalicylate method.

2.6.Optimum conditions for immobilized amylases activity

The pH profile for immobilized amylase was determined by varying the pH of the starch solution using the following buffers (0.1 mol L^{-1}): sodium acetate for pH values 4.0, 5.5 and phosphate buffer for pH 6.0-8.0).

3. RESULTS AND DISCUSSION

The commercial enzyme was characterized by electrophoresis (SDS PAGE), showing at least six proteins bands. (Figure 1)
Activity was measured by formation of reducing sugar using ADNS and starch disappearing by FUWA method. It was possible to obtain 98 % of starch hydrolysis when the immobilization conditions were 20 min at room temperature, pH 5.5 and 0.3 mg mL^{-1} (Figure2). The free enzyme showed an optimum reaction pH from 5.0 to 8.0. The immobilized counterpart showed a narrowed pH profile, with optimum reaction at pH 5.0, with 61% of activity maintenance until pH 7.0. (Figure 3).

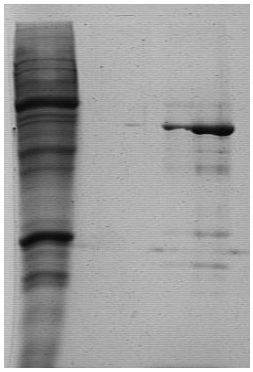

Figure 1. Gel eletrophoresis (SDS-PAGE) under denaturing conditions

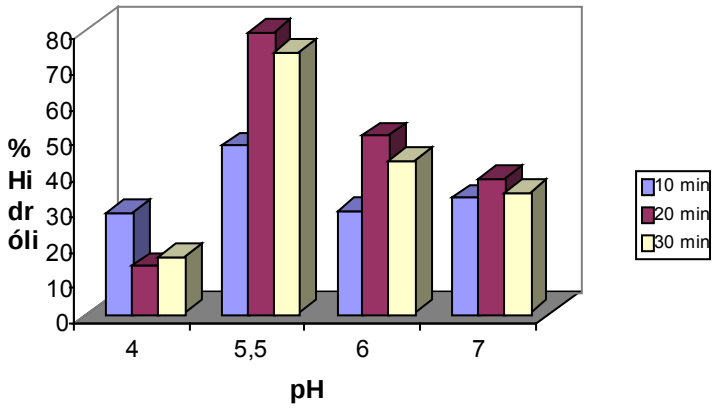

Figure 2 . Immobilization parameters of amylase onto sillanized glass capillaries.

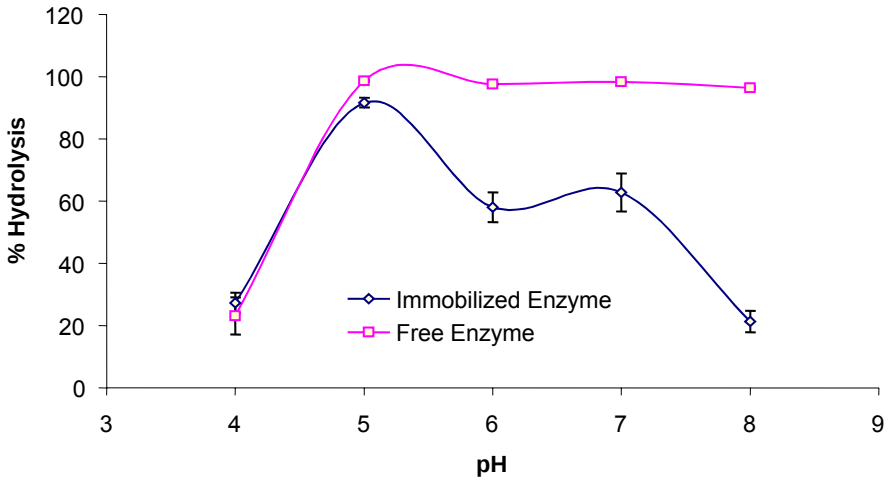

Figure 3. Effect of pH on activity of free and immobilized amylase. Buffer solutions:0.1mol L^{-1} sodium acetate (pH 4.0 and 5.5) and 0.1 mol L^{-1} phosphate buffer (pH 6.0-8.0).

4.CONCLUSIONS

This finding indicates the immobilization prevalence of one of the amylases on the commercial pool, probably due the presence of a superficial reactive group on this enzyme. The starch hydrolysis kinetic showed that 30 min was sufficient to obtain complete disappearing of starch and the same was observed for reducing sugar appearance. This immobilization method apparently had as result the preferential immobilization of one of the several amylases in the pool with high enzymatic activity compared to the complete pool of amylases.

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