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RESEARCH ARTICLE

IMMOBILIZATION Of *Phaseolus vulgaris* AMYLASE ONTO CHEMICALLY CHARGED WOVEN *Bombyx mori* SILK FABRIC

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ABSTRACT

Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal. They degrade starch into free glucose and limit dextrins. Hence, amylases have couple of applications in food & pharmaceutical industries too. Amylase extracted form *Phaseolus vulgaris* was immobilized on a *Bombyx mori* silk fabric activated by chlorination and diazotization. The 82 % of immobilization was done onto chemically charged B*ombyx mori* silk fabric. The optimum temperature, pH, time of incubation, substrate concentration and effect of calcium chloride concentration were studied. Thermal stability of enzyme was improved after immobilization up to 60°C which was 50°C for free enzyme. As well as immobilized enzyme was stable at least for 4 -5 months when stored at 1 M KCL solution at 4° C.

INTRODUCTION

Amylases are derived from animal, fungal and plant sources. Silks are fibrous proteins with remarkable mechanical properties form by silkworms. Recently regenerated silk solutions have been used to form a variety of biomaterials, such as gels, sponges and films, for medical applications. Silks can be chemically charged through amino acid side chains to alter surface properties or to immobilize enzymes and it is resistant to microbial attack too (Rani, 2012). Initially the term amylase was used originally to designate enzymes capable of hydrolyzing α-1,4- glucosidic bonds of amylose, amylopectin, glycogen andtheir degradation products (Bernfeld, 1955; Fisher and Stein, 1960; Myrback and Neumuller, 1950). They act by hydrolyzing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved. In recent years a number of new enzymes associated with degradation of starch and related polysaccharides structures have been detected and studied (Buonocore *et al*., 1976; Griffin and Fogarty, 1973). Physical entrapment of alpha-amylase in calcium alginate beads has shown to a relatively easy, rapid and safe technique (Dey *et al*., 2003) in comparison with other immobilization methods. In present work, we described the immobilization of *Phaseolus vulgaris* amylase onto chemically charged woven *Bombyx mori* silk fabric through covalent coupling with glutraraldehyde method which was given by Rani, 2011.

MATERIALS AND METHODS

Bombyx mori silk fabric was washed with double distilled water and then chlorinated with NaCl (3% content) along with $NaNo_3$. Amylase was extracted from sprouted *Phaseolus vulgaris* seeds.

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Extraction of crude enzyme

50 gm of sprouted seeds of *Phaseolus vulgaris* were homogenized at 0-4°C in 0.05M potassium phosphate buffer (pH-7) and centrifuged at 8000 rpm for 15 minutes. Supernatant was collected as crude amylase and stored at 4°C.

Amylase assay

Amylase was assayed according to the procedure followed by Bernfeld, 1955. The amount of reducing sugars in the final mixture was determined spectrophotometrically at 570 nm. One unit of enzymatic activity is defined as the amount of enzyme that produces 1 µmol of maltose per minute (Robert & Evan, 2003, Garen & Levinthal, 1960)

Chlorination and $NaNO_3$ treatment of woven *Bombyx mori* silk fabric

Silk fabric (B*ombyx mori*) was cut into 2 x2 cm long pieces and dipped in 2ml of 3% NaCl along with 10mg of $NaNO_3$ for half an hour for incubation according to the procedure reported previously (Rani, 2012). Enzyme activity of the immobilized and free enzyme on to chemically charged silk fabric was done by dinitrosalicylic acid method.

Immobilization of *Phaseolus vulgaris* amylase onto chemically charged woven *Bombyx mori* silk fabric

Pieces of treated fabrics (10-15mg) were put into a flask and 5 ml solution of an amylase (1mg/ml) was added. The flask was

kept at 37°C for 24 hours with occasional stirring. The amount of amylase immobilized onto the fabrics was calculated from decrease in enzyme concentration. The treated fabric pieces were washed several times with 1 M KCL for 2 hours at 30°C under shaking in incubator. These were stored in a refrigerator below 5°C by immersing them in 0.1 M KCL solution.

% Retention of enzyme activity

The enzyme bound to chemically charged woven silk fabric was estimated by determining the residual specific activity from solution of enzyme during immobilization.

Study of Kinetic parameters

Kinetic parameters were studied such as time of incubation (5, 10, 15, 25 and 30 minutes), pH in the range 2.5 – 10.2, temperature (20, 30, 40, 50, 60, 70ºC), starch concentration (2-6%) and effect of $CaCl_2$ concentration 2%-8%).

RESULTS AND DISCUSSION

82 % of immobilization was calculated which showed good and stable binding of *Phaseolus vulgaris* amylase on to chemically charged *Bombyx mori* silk fabric. The optimum incubation time for immobilized and free enzyme was found 15 minutes which was comparable to earlier reports (Lopez *et al*., 1997). The highest activity of enzyme was found at pH 7.5 for both crude and immobilized enzyme (Quinn *et al*., 2001).

Table 1: Kinetic parameters for free and immobilized *Phaseolus vulgaris* onto chemically charged charged *Bombyx mori* woven silk fabric

Kinetic Parameters	Free amylase	Immobilized amylase
pH	5.5	7.5
Temperature	40°C	60°C
Time of Incubation	15 minutes	15 minutes
Starch Concentration	1.0%	1.25%
$CaCl_2$ Concentration	1%	6.0%

The present study was showed maximal thermal stability at 60°C as compared to free enzyme (40°C) and comparable to earlier reports (Lopez *et al*., 1997 and Rani, 2012). There was no change in substrate concentration in free and immobilized enzyme activity which was in the range of 1-1.25%. The present study was showed that at 6% $CaCl_2$ concentration, the immobilized enzyme was showed maximum activity which was pretty similar to free enzyme (Rodriguez *et al*., 1993). Thermostable amylase is one of the most important enzymes in food, paper and detergent industries (Nigam and Singh, 1995). Industry also demands an immobilized amylase with increased stability, durability, reusability embodying the functions of all amylases with maximum activity. Thus, the thermal stability and storage stability of *Phaseolus vulgaris* immobilized amylase on to chemically charged woven *Bombyx mori* silk fabric were maximum as compared to free enzyme (Table 1).

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