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IMMOBILIZATION AND STUDIES ON SOME PROPERTIES OF B-GLUCOSIDASES PRODUCED BY *PENICILLIUM PURPUROGENUM*

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Abstract

Extracellular and intracellular β-glucosidases produced by *P. purpurogenum* were immobilized by standard method using Calcium alginate entrapment. The immobilized enzymes were active and retained all the basic properties similar to that of the native enzymes such as optimum pH, optimum temperature for activity. For reusability the native enzymes were less stable as compared to the immobilized enzymes. Km values for soluble and immobilized extracellular β- glucosidase were 0.74, 1.11, and for intracellularnative and immobilized β- glucosidase the Km were and 0.77, 1.13, respectively. V max changed from 505×10^2, to 532×10^2 µmol/min per mg protein for extracellular enzyme after immobilization and from 229×10^2 to 240×10^2 µmol/min per mg protein for intracellular enzyme after immobilization.

Key words: Immobilization, Enzymes, β-glucosidase, *Penicillium*.

INTRODUCTION

Applications of enzymes for commercial purpose were mainly restricted because of their high cost, limited availability and non reusability. Immobilized enzymes overcome all these draw backs because they can be readily separated from reaction media and reused over a long period of time [1]. The glucosidases have many applications including the production of fuel ethanol from cellulosic material, preparation of feed for live stock, etc. [2, 3]. β-glucosidase is also a key enzyme in flavor industries for debittering of fruit juices [4, 5].

Many reports have been published on immobilization of β-glucosidases with chitosan, cellulose carbonate; cyanogens activated cellulose, and microcrystalline cellulose [6-9]. The method of entrapment of enzyme in calcium alginate was most traditional and commonly used method [10, 11]. In the present paper immobilization of extracellular and intracellular β-glucosidases of *P. purpurogenum* in calcium alginate beads has been reported.

MATERIALS AND METHODS

Chemicals: Sodium alginate, Calcium chloride, were purchased from Qualigens, Mumbai. β- pNPG was obtained from SRL, Mumbai.

Microorganism: *P. purpurogenum* was isolated from the decaying woods in our laboratory and identified by IMT, MTCC, and Chandigarh, India. The isolate was maintained on PDA slants, pH 5.2 to 5.8, at 4^0 C, by periodic transfer. The culture was transferred to new slants at every two months to keep it viable.

Enzyme production and assay: The composition of medium (g/L) used for β-glucosidase production was as follows: Sucrose-10g, $NaNO_3$ -25, KH_2PO_4-1.5, $MgSO_4.7H_2O$-0.5, NaCl-6 , pH-5.5, Distilled water-1000 mL (Patil and Shastri, 1981) [12].

A series of 250 mL volumetric flasks each containing 50 mL sterilized liquid medium was inoculated separately with 1 mL spore suspension of *P. purpurogenum* (10^8 spores/mL in sterile distilled water). Inoculated flasks were incubated at 30^0C for 4 days for intracellular and 14 days for extracellular enzyme production. β-glucosidase activity was assayed using β- pNPG as substrate, by the method of Riou *et al*., 1998 [13]. One unit of β-glucosidase activity corresponded to the release of 1µmole of *p*-nitrophenol per min under assay conditions. Protein content was measured by the method of Lowry *et al*., (1951) using BSA as standard protein [14].

Immobilization: Sodium alginate solution 4% was prepared in 0.1 M sodium acetate buffer, pH 5.5. Enzyme was mixed with the cold sodium alginate and dropped in 500 ml chilled solution of calcium chloride as described by Keirstan and Bucke, 1977 and Mahmood *et al*.., 1990 [15, 16]. Stirring was continued for 5 hours and excess C was removed by washing with buffer. Finally the beads were stored in sodium acetate buffer at 4^0C. For immobilized enzyme the beads were incubated in 0.1 M sodium acetate buffer pH 5.5 at 50^0C using 50mM β-pNPG. After desired period of incubation the reaction was stopped by adding 1.5 mL of sodium carbonate solution. The yellow coloration was measured at 420 nm using suitable controls.

RESULTS AND DISCUSSION

Effect of pH: The pH optimum for both the immobilized and soluble form of β-glucosidase was approximately 5.5 (Fig.1).The immobilized enzymes showed more activity at pH 2.5. This increase in activity might be due to surrounding microenvironment. Increase in activity of immobilized of β-glucosidase was also reported by Bissett and Sternberg, (1978) for Aspergillus species. [6].

Effect of temperature: The optimum temperature for maximum activity of both the immobilized and soluble β-glucosidases was 50^0C. Immobilized enzymes have increased thermal stability as indicated by the enhanced half life at the same temperature and also at higher temperature where soluble enzyme does not show activity (Fig.2). The increased thermal stability was also reported by Bissett and Sternberg (1978) for β-glucosidase of *Aspergillus* species [6].

Reusability

The immobililized form could be recycled at least three times without any appreciable loss of activity (Fig. 3). There was slight of enzyme activity during recycle, beyond that activity decreases. The activity at the end 5^{th} cycle was 50 & 40 % for extracellular β- glucosidase preparation.

Kinetic constants: Km values for soluble and immobilized extracellular enzyme produced by *P. purpurogenum* were 0.74, 1.11, and for intracellular β- glucosidase the Km were 0.77, 1.13, respectively (Table 1). V max changed from 505×10^2, to 532×10^2 µmol/min per mg protein for extracellular enzyme and from 229×10^2 to 240×10^2µmol/min per mg protein for intracellular enzyme after immobilization. The higher V max obtained for immobilized may reflect efficient catalysis. The ratios of Vmax/Km for soluble and immobilized extracellular and intracellular β--glucosidase were 682×10^2 and 479×10^2; 298×10^2 and 212×10^2, respectively. Thus the immobilized enzymes show comparable efficient catalysis. The change in Vmax was also reported by Dumitriu *et al*., (1989); Suh *et al*., (1987) for immobilized tannase produced by *Aspergillus* species. [17,18]. The data was analysed by using ANOVA technique as suggested by Ronald Walpole (1982) [19].

Table 1. Properties of native and immobilized extracellular and intracellular β- glucosidases of *P. purpurogenum*

Property	**Extracellular β-glucosidase native**	**Extracellular β- glucosidase Immobilized**	**Intracellular β- glucosidase native**	**Intracellular immobilized β- glucosidase**
Opt. pH	5.5	5.5	5.5	5.5
pH stability	4 – 6.5	4 – 6.5	4 – 6.5	4 – 6.5
Opt. Temp.	50°C	50°C	50°C	50°C
Temp. stability	40 - 60°C	40 - 60°C	40 - 60°C	40 - 60°C
Reusability	1 time	3 times	1 time	3 times
Km	0.74	1.11	0.77	1.13
Vmax µmol/min per mg protein	505×10^2	532×10^2	229×10^2	240×10^2
Vmax/Km ratio	682×10^2	479×10^2	298×10^2	212×10^2

Data is mean of three replicates. ($P< 0.05$)

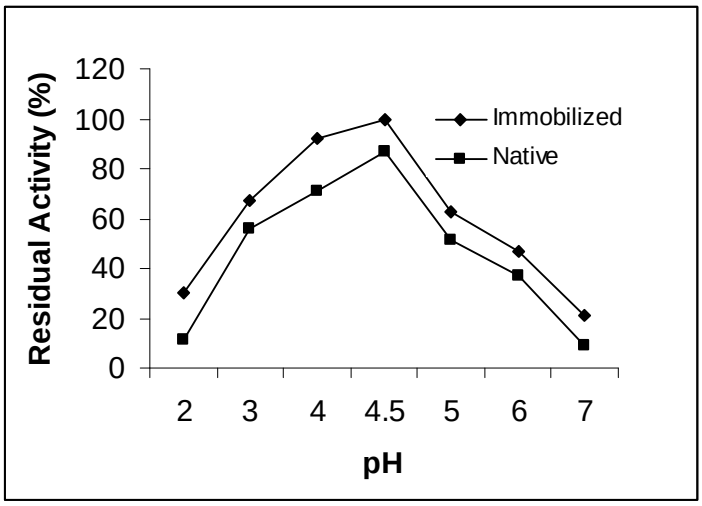

Figure 1. Effect of pH on immobilized and native B glucosidases of *Penicillium purpurogenum*.

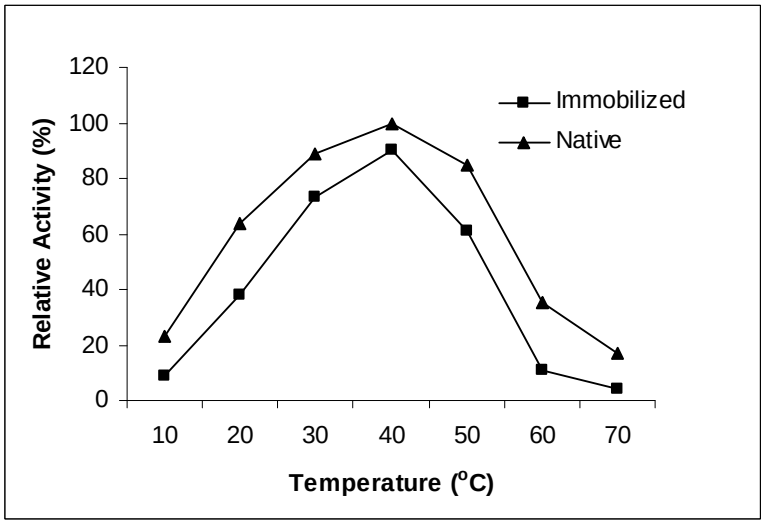

Figure 2. Effect of temperature on immobilized and native B glucosidases of *Penicillium purpurogenum*.

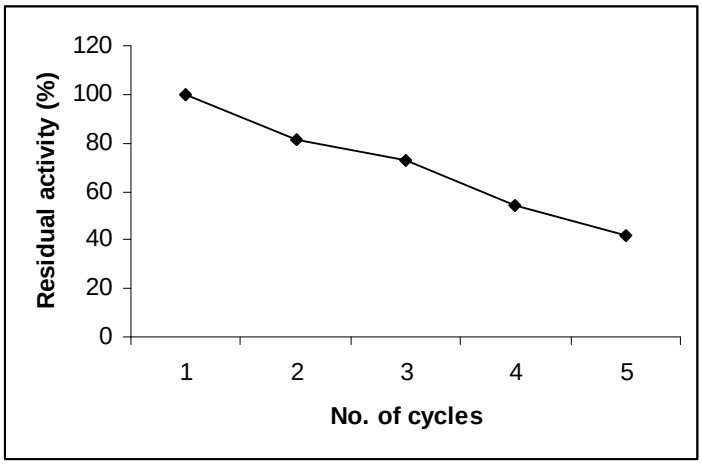

Figure 3. Reusability of the immobilized B glucosidases produced by *Penicillium purpurogenum*.

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