

## **Immobilization of partially purified alpha-amylase enzyme produced by a soil born *Bacillus* sp.**

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### **ABSTRACT**

*Alpha amylases have various applications in different industries. Immobilization of alpha -amylase produced by a strain of Bacillus sp isolated from soil of Dibrugarh District of Assam, India was studied. Partially purified enzyme with 4062.62U/ml activity was used for immobilization study. 4% Sodium alginate was used for immobilization of enzyme and the optimum temperature, pH and incubation period for immobilized alpha amylase enzyme were 60°C, 6.0 and 10minutes, respectively. The immobilized enzyme was very effective in reducing starch viscosity and the immobilized enzyme was found to be stable. Activity yield was 86.39%. This immobilized enzyme can be used commercially as a replacement of free enzyme because it has shown greater operational flexibility and higher enzymatic activity.*

**Key words:** *Bacillus, immobilization, starch*

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### **INTRODUCTION**

Among bacteria, *Bacillus* sp. is widely used for thermostable  $\alpha$ -amylase production to meet industrial needs. An immobilized enzyme is an enzyme that is attached to an inert, insoluble material. Enzymes can be immobilized to a multitude of different carriers by entrapment, adsorption, ionic binding and covalent binding. This is a the method for protecting and stabilizing the enzymes, thereby enhancing their properties and their repetitive utilization either in batch, or continuous mode. Immobilization of enzymes prevents their deactivation by various physical and chemical denaturing agents and thereby enhancing their operational stability. Microbial amylases are available commercially, and they have almost completely replaced chemical hydrolysis of starch in the starch processing industry [6]. In spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and ease of process modification and optimization [7], [3]. The main use of enzymes includes hydrolysis of starch to yield glucose syrup, amylase-rich flour and in the formation of dextrin during baking in food industries[12]. Amylases have been derived from several fungi, yeast, bacteria and actinomycetes but members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment[11].Entrapment requires that the immobilized enzyme should be a large enough molecule to be kept inside the gel matrix but the substrate and product should be small enough to pass through the pores of the gel Immobilized amylases offer several advantages : it can be reused, involved processes can be operated continuously with better controls, easy separation of the product(s), simpler handling of materials and effective reduction in process cost. Sodium alginate beads are widely used in enzyme immobilization because the gel formation occurs at mild conditions and poses no risk of harm to humans. Physical

entrapment of alpha-amylase in sodium alginate beads has shown to be a relatively easy, rapid and safe technique. Starch molecules are very large, often reaching a molecular weight of 80 million Daltons [5]. It is expected that starch hydrolysis reaction could occur more effectively if enzyme bound to surface. The use of enzymes in a soluble or free form must be considered as wasteful because the enzyme generally cannot be recovered at the end of the reaction. A new and valuable area of enzyme technology is that concerned with the immobilization of enzymes on insoluble polymers, such as membranes and particles that act as supports or carriers for the enzyme activity [10]. In the present study alpha amylase produced by *Bacillus sp* was used for immobilization with sodium alginate.

## MATERIALS AND METHODS

### Isolation and Identification of alpha amylase producing bacteria:

Isolation of bacterial strains from randomly collected soils of Dibrugarh districts were performed as per standard serial dilution technique and biochemical characterization of bacterial strains were done as reported by [2]. Their amylase activity was measured using agar cup method [7]. 5 ml of 24 hrs old culture broth containing soluble starch, yeast extract, peptone, MgSO<sub>4</sub>·7H<sub>2</sub>O, NaCl and CaCl<sub>2</sub> (HiMedia, India) was transferred to 45 ml of the same sterile medium and was incubated for 35°C. The culture was centrifuged at 10,000 rpm for 10 min at 0°C (Refrigerated Centrifuge, Sigma, Germany). The supernatant was used as crude enzyme.

### Partial Purification of the Enzyme

Alpha amylase was partially purified by ammonium sulphate fractionation followed by dialysis and gel filtration chromatography. The crude enzyme was brought to 50% ammonium sulphate saturation at 4°C in an ice bath. The precipitated protein was collected by centrifugation at 10,000 rpm for 10 min at 0°C (Refrigerated Centrifuge, Sigma, Germany) and dissolved in a minimum volume of phosphate buffer (0.1M; pH, 7.0). The enzyme solution was dialyzed at 4°C against the same buffer for 24 h at 4°C with continuous stirring and three changes of the same buffer. The DEAE cellulose-ion exchange column was pre-equilibrated with the same buffer. The dialysate was concentrated through a freeze dryer Freeze dryer (Eyela) and dissolved in a minimum volume of phosphate buffer (0.1M; pH, 7.0) and applied to the DEAE cellulose column at a flow rate of 0.6 ml min<sup>-1</sup> with 50 mL linear NaCl gradient (0 to 1.0M). Fractions of 10 mL were collected and each fraction was analysed for protein concentration and α-amylase activity. The active fractions were pooled and concentrated through freeze dryer Freeze dryer (Eyela). The final concentrated enzyme solution was taken for comparative enzyme entrapment study. Enzyme activity of selected bacterial strains was assayed by DNSA method. Blank contained 2.5ml of buffer and 0.5ml of starch. Standard contained 0.5 ml of Glucose (1mg/ml in Phosphate buffer, 0.1 M and pH 7). Protein was estimated as per the method of Lowery *et al.* 1951.

### Enzyme immobilization:

Enzyme was prepared as per above said method. Preparation of alginate beads: 30% of Sodium alginate (Hi Media) was prepared with phosphate buffer (0.1M; pH, 7.0). A volume of enzyme solution and sodium alginate solution was mixed to give a 4% (w/v) final concentration. The beads are formed by chipping the polymer solution from a height of approximate 20 cm into an excess of (100 ml) of stirred 0.2 M CaCl<sub>2</sub> (HiMedia) solution with a syringe and a needle at room temperature. Left the beads in the solution for 3 hrs. The calcium alginate beads containing the enzyme were thoroughly washed with distilled water and used for further studies.

### Immobilized enzyme assay

Enzyme activity of selected bacterial strains was assayed by DNSA method.

### Determination of activity yield

Immobilization yield (%) =  $(I / A - B) \times 100$

where

A = Activity of added enzyme;

B = Activity of unbound enzyme

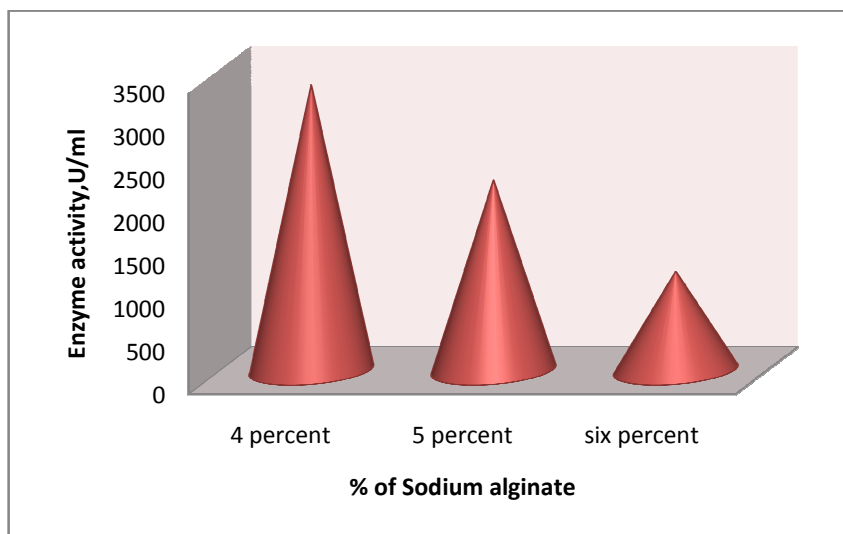
I = Activity of immobilized enzyme

**RESULTS AND DISCUSSION**

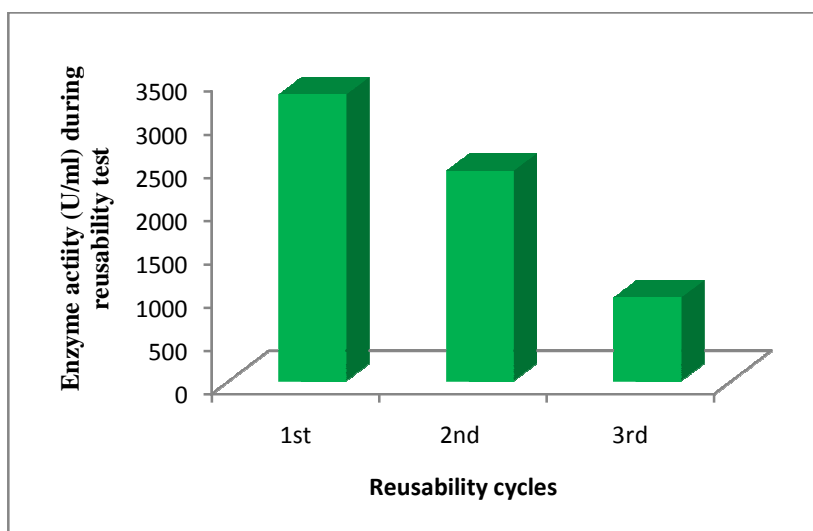
As in the agar cup method the one bacteria showed highest activity with 2.36 was identified as *Bacillus sp* (MTCC 9402) ,by IMTECH (Chandigarh, India). Specific activity of crude enzyme, Ammonium sulphate precipitation (50%) and After dialysis were 362.80, 7317.76 and 12310.97 units mg protein<sup>-1</sup> respectively . The optimum temperature, pH and incubation period for purified alpha amylase enzyme were 60°C, 6.0 and 10 minutes; which were similar with the crude enzyme (Data not shown)

Purification steps	Enzyme activity (unit/ml)	Protein (mg/ml)
Crude enzyme	1414.91	3.9
Ammonium sulphate precipitation (50%)	3439.35	0.47
After dialysis	4062.62	0.33

**Table 1: Purification of alpha -amylase produced by *Bacillus sp***



**Fig 1: Activity in immobilized enzyme for *Bacillus sp* at different concentration of Sodium alginate.**



**Fig 2: Reusability of immobilized alpha amylase from *Bacillus sp***

Immobilization of the enzyme with 4% alginate beads gave a yield of 86.39% with 3312.12 U/ml enzyme activity, which was a very satisfactory result against 5% and 6 % sodium alginate concentration (Fig 1). Pore size of the beads should be such that substrate and product can easily diffuse in and out of the alginate gel matrix but the enzyme should retain in the micro environment of beads. Lower the concentration of sodium alginate solution, greater will be the pore size of the beads and consequently leakage of enzyme from the beads will increase. Similarly, the pore size of beads will decrease with the increase in concentration of sodium alginate solution. Lower the concentration of sodium alginate solution, greater will be the pore size of the beads and consequently leakage of enzyme from the beads will increase [1].

Increased sodium alginate concentration interfered the entry of substrate into the beads; that led to the lower immobilization efficiency [4].

In the reusability study the immobilized enzyme activity (Fig: 2) was found to be 965.16U/ml with the immobilization yield of 25.17%. With increase in cycle number, enzymatic activity decreases steadily indicating enzyme leakage from beads.

The optimum temperature for immobilized  $\alpha$  amylase enzyme was 60°C. The activity was determined at different incubation time with 1.5 % starch. Experiment was performed upto 80°C (Fig 3) where a decreased activity of immobilized enzyme was observed.

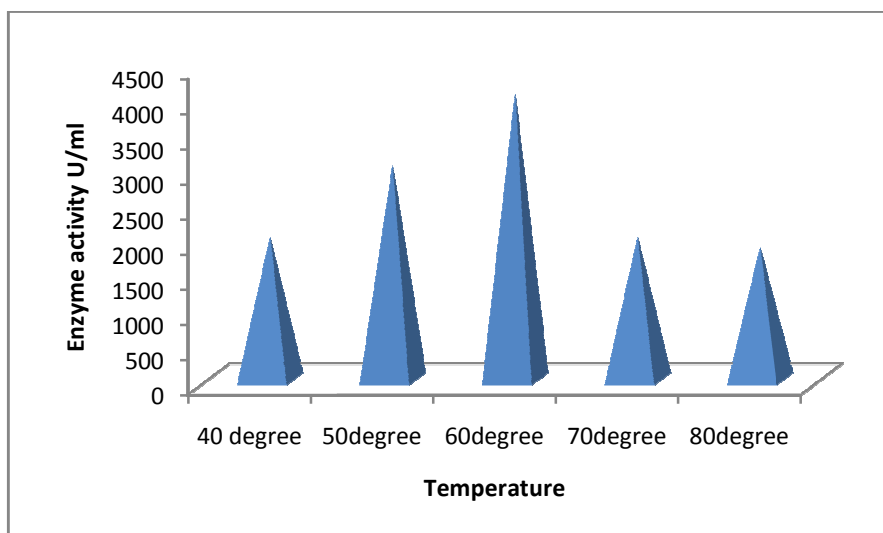


Fig.3: Activity of immobilized alpha amylase from *Bacillus sp* at different temperature

The optimum pH for immobilized  $\alpha$  amylase enzyme was 7.0. The activity was determined at different pH (Fig.4) with 1.5 % starch. In acidic and alkaline condition the immobilized enzyme could not show satisfactory activity.

The optimum incubation period for immobilized  $\alpha$  amylase enzyme was 10 minutes.

The activity was determined at different incubation time (Fig.5) with 1.5 % starch. Incubation time was continued upto 60 minutes. Ten minutes incubation time was sufficient for required enzyme activity.

In the immobilization process, the activity yield should be as high as possible. Very low activity yield indicated a worthless immobilization process. The 86.39% activity yield for this study should be acceptable. Immobilized enzyme has shown greater stability in high temperature i.e. 60°C, pH 7 and 10 minutes incubation time. Such greater operational stability has been beneficial from industrial point of view as it will maximize its potential to use in textile and detergent industry. The enzyme entrapped in alginate beads could be reused.

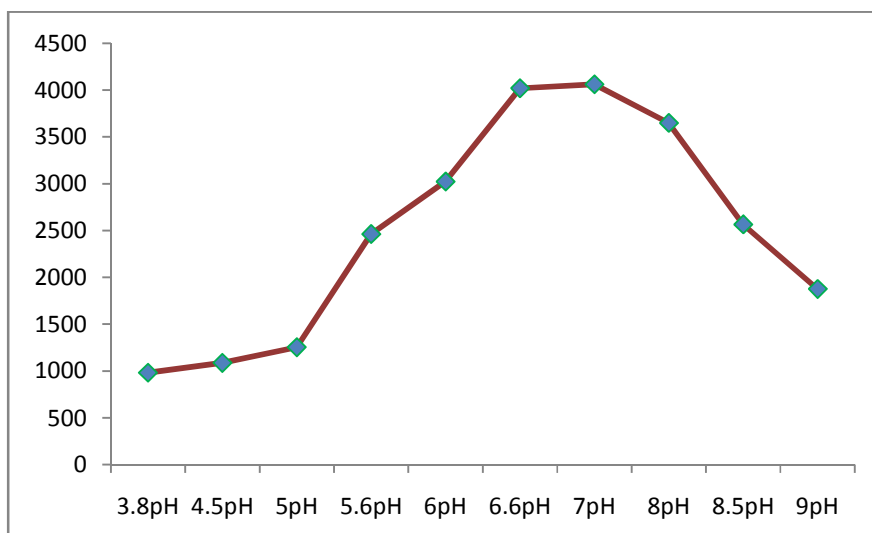


Fig 4: Activity of immobilized alpha amylase from *Bacillus sp* at different pH

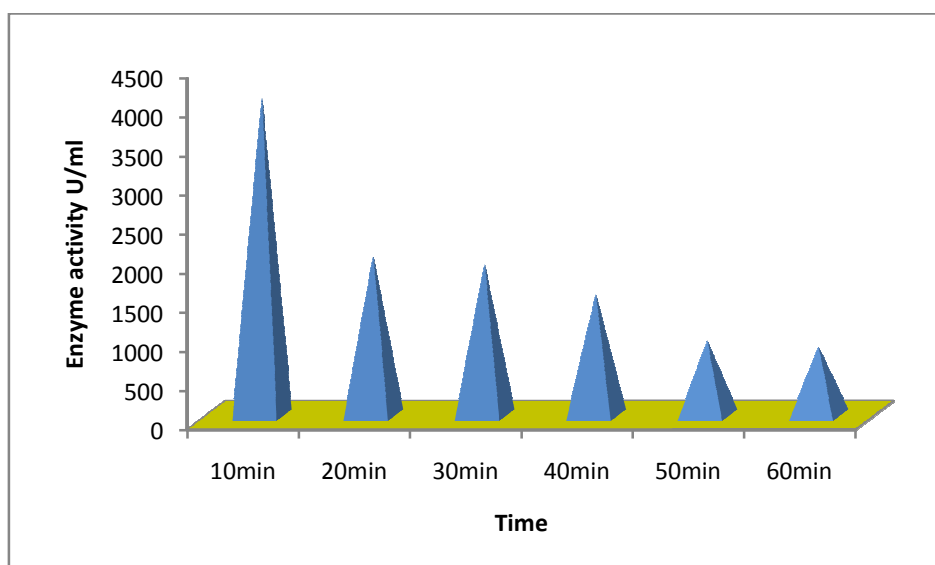


Fig 5: Activity of immobilized alpha amylase from *Bacillus sp* at different pH

### CONCLUSION

The immobilized alpha amylase from *Bacillus sp* can be successfully utilized in practical application and this can be used commercially as a replacement of free enzyme system as immobilized system has shown greater operational flexibility and higher enzymatic activity.

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