



ISOLATION, CHARACTERIZATION AND OPTIMIZATION OF PROCESS VARIABLES FOR PARTIALLY PURIFIED IMMOBILIZED α -AMYLASE ISOLATED FROM INDIAN CHICKEN FEATHER

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ABSTRACT

Immobilization of α -Amylase produced by a *Pseudomonas* sp isolated from Indian Chicken feather was studied. Partially purified enzyme with 486.77 IU and specific activity of 811.28 units mg (protein)⁻¹ was used for immobilization study. An easily available and inexpensive 3% Sodium alginate matrix with easy immobilization gel entrapment procedure was used for trapping the enzyme showed 555.5 IU activity. The optimization was carried out to study the catalytic properties which showed the optimum pH, temperature and substrate concentration at pH 7, 45⁰C and 16mg, respectively. The reusability of the immobilized enzyme preparation showed its use in continuous starch hydrolysis for up to 10 cycles. This immobilized enzyme can be used as a replacement of commercial enzyme since it has shown same greater operational flexibility and enzymatic activity of the pure enzyme.

KEY WORDS: Chicken feather, Calcium alginate, Immobilization, *Pseudomonas* sp.



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INTRODUCTION

Enzymes are biocatalyst, whose utilization has emerged as chief strategies towards the eco-friendly and energy saving chemical processes¹. Amylase is ubiquitous enzymes, which are found in animals, plants, fungi and bacteria and have been used in food, paper, textile, baking and detergent industry^{2,3}. 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 optimization⁴. Immobilization is a process of attachment of enzyme on solid supports is a very effective way to increase enzyme stability. Among the various matrices available, frequently used is gel entrapment within porous matrices, such as alginate in the form of beads^{5,6}. This is a method for protecting and stabilizing the enzymes, thereby enhancing their properties and reasonably simple, cheap, safe, and offer high porosity, good mechanical strength, for substrate and product diffusion^{7, 8}. It is expected that starch hydrolysis reaction could occur more effectively if an enzyme bound to surface⁹. The use of enzymes in a soluble form must be considered as less economic, wasteful because the enzyme generally cannot be recovered at the end of the reaction. Feathers are produced in major bulk as a biological waste by poultry processing industries in India and cause serious environmental issue¹⁰. In order to solve the problem biodegradation of feathers are made by microorganisms and are used as rich meal for animals and nitrogenous fertilizers for plants¹¹. It has been also reported use of feathers as Low cost building materials¹² and for production biodiesel¹³. But feathers as a source of microorganisms and enzyme have not been studied. Hence, in the present study, new source of amylase producing microorganism was isolated from Indian chicken feather and its activity were studied and compared with commercial purified enzyme.

MATERIALS AND METHODS

Sodium alginate, CaCl₂ was purchased from Himedia. All other chemicals used in the assay were of analytical grade.

Enzyme

Amylase producing microorganism was isolated from Indian chicken feather collected from the local market and was partially purified by ammonium sulphate and dialysis, purified enzyme were procured from Himedia.

Screening and Identification of amylase producing bacteria

Several methods have been proposed for screening of amylase. These methods either directly use the microorganism under study or measure the enzyme activity in the crude or purified culture preparations. In the present study, the screening of bacterial strain from Indian chicken feather collected from Bangalore districts were performed as per standard serial dilution technique and plated on starch agar media. Plate detection method was used for the observation of hydrolysis of starch through the presence of clear zones around bacterial strain. Biochemical characterizations of bacterial strains were carried out as per Bergys Manual to identify the bacteria¹⁴.

Extracellular extraction and purification of amylase

Extraction of enzyme process was followed as per Devi¹⁵ the 24 hr old culture bacterial strain was inoculated into the broth containing soluble starch, yeast extract, peptone, MgSO₄.7 H₂O, NaCl and CaCl₂ and was incubated at 37⁰ C for 48 hrs. After 48hrs of incubation the broth is centrifuged at 10000 x g rpm for 10 min at 0°C. Supernatant is used as crude extract of amylase.

The enzyme was partially purified by ammonium sulphate precipitation followed by dialysis using phosphate buffer (0.1M, pH 7.0). The Enzyme activity and specific activity of selected bacterial strain was determined by Dinitrosalicylic acid (DNSA) method and Lowry's method respectively¹⁶. The partially purified enzyme was used further for immobilization studies.

Entrapment of crude amylase extract on calcium alginate beads

The Partially purified enzyme was immobilized in Calcium alginate beads in 1:10 ratio of enzyme and 3% of Sodium alginate solution. The beads are formed by adding the sodium alginate solution into 0.2M CaCl₂ at height of approximately 4-5cm through the burette. Beads are left in the solution for 3 hrs time. The calcium alginate beads containing the enzyme were thoroughly washed with distilled water in order remove excess calcium chloride solution on the surface of the beads and later it is preserved in the phosphate buffer (0.1M, pH 7.0)². Same protocol was carried out for commercially available amylase enzyme procured from Himedia for the comparison.

Immobilized Enzyme Assay

Assay of immobilized α -Amylase was set up according to protocol described by Kumar¹⁷. For enzyme assay, four beads of calcium alginate of crude enzyme (2.4 μ g protein/bead) and commercial enzyme suspended in reaction mixture consist of 0.4 ml assay buffer (phosphate buffer, pH 7.0) was incubated with 1.6 ml of 1% starch solution at room temperature for 3 min and the reaction was stopped with 3 ml of 3, 5-dinitrosalicylic acid followed by heating the reactants in a boiling water bath for 10 min and then cooling down to room temperature. Solution was diluted by adding 9 ml of distilled and the amount of reducing sugar (maltose) produced was determined spectrophotometrically at 540 nm. Amylase activity was expressed in terms of IU. The amount of reducing sugar released during the reaction was estimated with the help of the maltose standard curve.

Optimization of temperature, pH and Substrate concentration

The optimum temperature for crude α -Amylase was determined by incubating the

reaction mixture of 14.5 ml containing 0.1 ml of soluble enzyme and 4 beads of calcium alginate at various temperature 0, 30, 40, 45, 50, 60 and 70 °C for 10 min. The enzyme assay reaction was stopped by removing the beads and incubating the reaction mixture in boiling water bath for 10 min. The activity of amylase was read at 540 nm spectrophotometrically. The Enzyme assay for determining the optimum pH was carried out by varying pH (4-10) at optimum temperature and for optimization of substrate (starch) concentration, varying concentrations (4-30mg) of substrate was used.

Reusability of calcium alginate amylase beads

Working efficiency of the beads with respect to the diffusion of substrate into the beads was studied by assaying the activity in the presence of beads several times for starch hydrolysis. The beads were reassayed for repeated cycles by washing with distilled water prior to the subsequent assay¹.

RESULTS AND DISCUSSION

α -Amylase, an enzyme which belongs to hydrolase family is produced by several bacteria and fungi. In our study amylase were extracted from the source Indian chicken feather using starch media as a substrate. The isolate which showed the maximum clear zone of hydrolysis around the bacterial colony when flooded with iodine solution on the starch plate at 37 °C incubated for 48 hr indicates the presences of amylase activity and was selected for further studies. The bacterial isolates were characterized on the basis of cultural characteristics, microscopic characteristics and biochemical tests as per Bergys manual and the isolate were identified has *Pseudomonas* sp. (Table 1).

Table 1
Characterization of Bacterial isolates from Indian chicken feather

| Mode of characterization | Characteristics of bacterial isolates | Result |
|------------------------------------|--|--|
| Cultural Characteristics | Colony morphology | Irregular, mucoid, creamy yellow and fast growing colonies |
| Microscopic Characteristics | Spore staining, Gram staining and Motility | Non spore forming, Gram negative, Non motile |
| Biochemical Characteristics | Indole test | Negative |
| | Methyl Red test | Positive |
| | Voges Proskauer test | Negative |
| | Citrate utilization test | Positive |
| | Catalase test | Positive |
| | Oxidase test | Positive |
| | Glucose fermentation | Negative |
| | Nitrate reduction test | Positive |
| | Starch hydrolysis test | Positive |

The crude α -Amylase enzyme was extracted, precipitated upto 70% using Ammonium sulphate solution and dialyzed in phosphate buffer (pH 7.0) showed the enzyme activity of 486.77 IU and specific activity of 811.28 units mg protein⁻¹. This partially purified enzyme was immobilized in calcium alginate through entrapment technique and it showed the enzyme activity of 555.5 IU. The commercial amylase showed 611.11 IU and 1018.5 units mg protein⁻¹ for soluble enzyme analysis and for immobilized 722.22 IU. The experimental results of optimization of crude *Pseudomonas*

amylase and commercial amylase has shown in fig 1, 2, 3. The soluble enzyme of pure and crude amylase showed maximum activity at 45 °C, 7 pH and 16mg protein concentration. The immobilized enzyme showed variation in optimum pH, there was a slight shift towards both acidic and alkaline direction. The kinetics of the enzyme varies based on the microenvironment has been cited in various reports³.¹ Reported the shift in the pH and temperature of immobilized amylase enzyme entrapped with agar and agrose matrix compare to soluble enzyme.

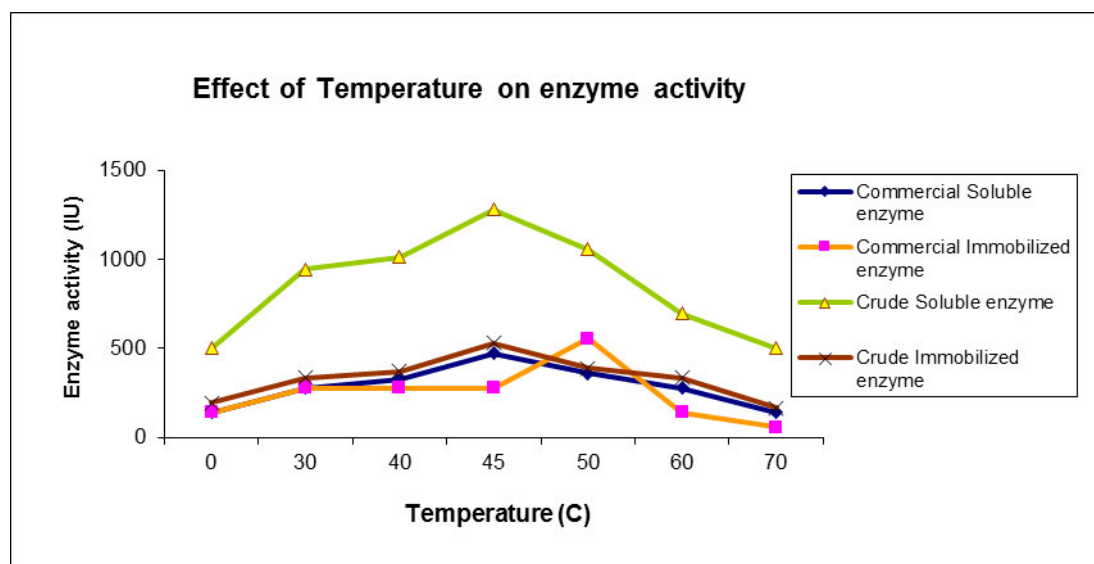


Figure 1
Variation in Enzyme activity due to Temperature

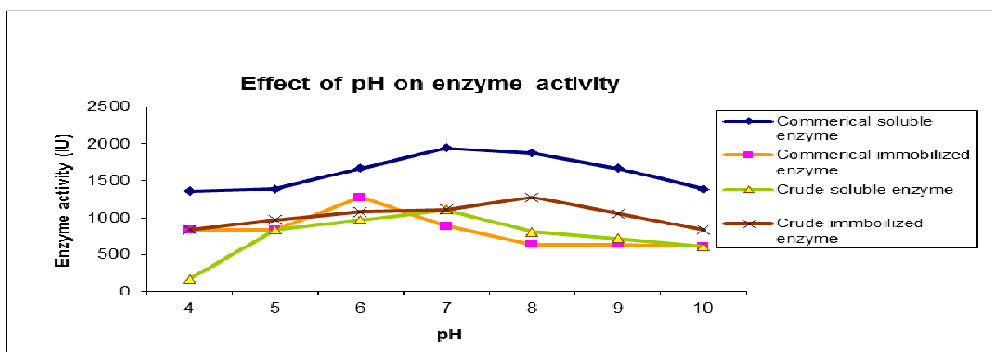


Figure 2
Variation in Enzyme activity due to pH

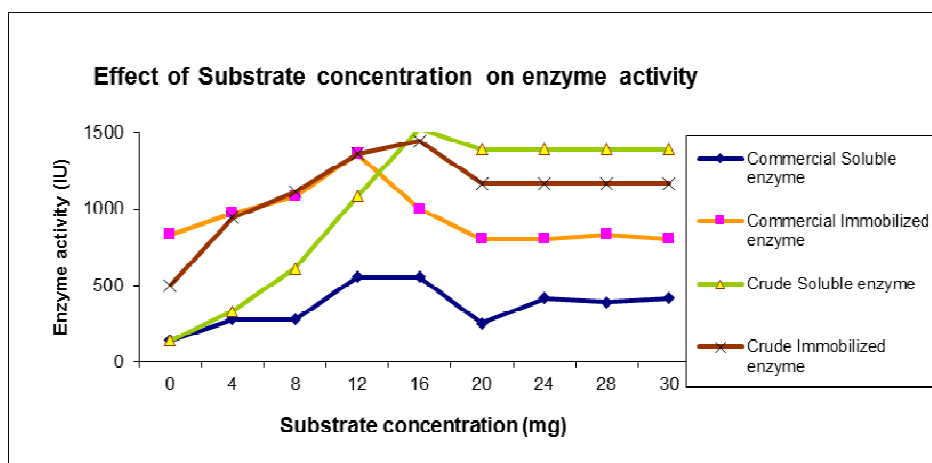


Figure 3
Effect of substrate concentration on α -amylase activity

One of the important characteristics of an immobilized enzyme is its stability and reusability over an extended period of time. The repeated use of the immobilized *amylase* for hydrolysis of starch was studied. The activity was decreased with increase in

number of cycles and activity stopped after 10 cycles (Fig 4). It has been reported that the loss of activity of entrapped enzyme was due to leakage of enzyme from the beads during washing of beads at end of each cycle¹⁸.

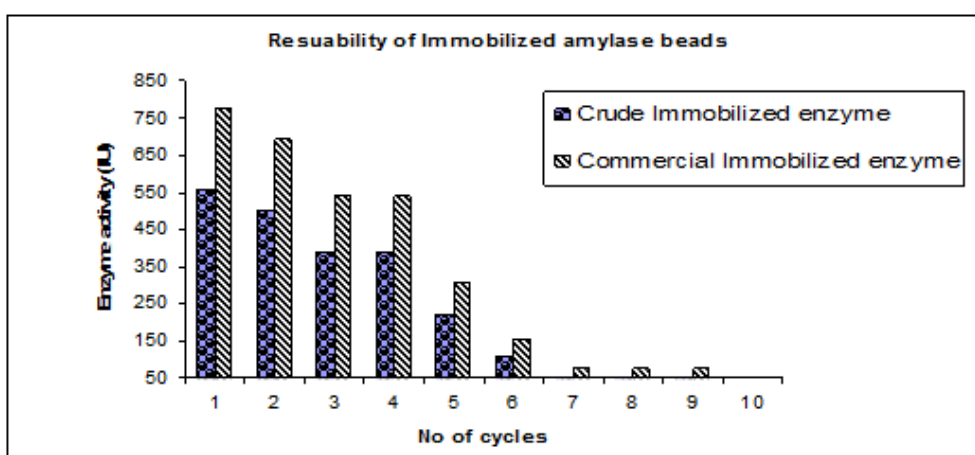


Figure 4
Reusability of immobilized α -amylase

CONCLUSION

The immobilized crude α -Amylase from *Pseudomonas* sp can be used as a replacement to commercial enzyme, since it showed activity very near to the commercial enzyme. The operating parameter were same

as commercial enzyme, it showed the greater satiability in high temperature and pH. The use of crude enzyme and reusability of enzyme is the one of the cost cutting strategies which can be implemented for industrial use.

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