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IMMOBILIZATION OF XYLANASE IN PVA-ALGINATE MATRIX AND ITS CHARACTERIZATION

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ABSTRACT

A cheap and non-toxic synthetic polymer, polyvinyl alcohol (PVA) has been used for enzyme immobilization. In this study immobilization of xylanase in PVA–alginate beads were developed and treated with sodium sulfate which prevented agglomeration and produced beads of high gel strength and conferred enzyme protection from inactivation by boric acid. Further immobilized Xylanase was characterized with respect to pH, temperature, incubation time and substrate concentration and compared with free Xylanase. The immobilized Xylanase activity was found to be lower than the free Xylanase activity. The activity was found to be highest at pH of 6, temperature of 55^{o}C, incubation time of 40 min and substrate concentration of 1.1mg/ml.

KEYWORDS: Xylanase, Immobilization, Polyvinyl alcohol, Alginate beads, Characterization.

INTRODUCTION

The use of enzyme immobilization lies in the economic application of enzymes in various industrial and technological processes. The yield and reusability of free enzymes as industrial catalysts are quite limited and, hence, attention has been paid to enzyme immobilization (Bajpai, 2003). Many researchers have studied the efficacy of entrapment of the Xylanase in different matrices. Each matrix has its own advantages and disadvantages. The reusability, thermal stability of the support at higher temperatures, and the cost effectiveness of the immobilization method is the best for polyvinyl alcohol (PVA)–alginate-based matrices. PVA is cheap, non-toxic, mechanically and chemically robust (Lozinsky, 1998; Durienx, 2000). Researchers have produced PVA beads using PVA cross-linked with boric acid, but the formed beads had a strong tendency to agglomerate into a mass of polymer, which is very difficult to break up (Yujian, 2007). To overcome this problem, sodium sulfate was introduced in the bead formation. The sulfate ions would probably form thiosulfate linkages among the cross-linked PVA, increasing the elasticity and the strength of the beads and thus improving their physical/mechanical properties (Long, 2004; Shet, 2018). The aim of this study was to immobilize Xylanase by PVA-alginate matrix and to study the characterization of the immobilized enzyme and compare it with free enzyme. The effect of temperature, pH, substrate concentration and incubation time on the performance of the enzyme was studied.

MATERIALS AND METHODS

Reagents and chemicals

NaOH, HCl, Polyvinyl alcohol, Sodium alginate, Sodium acetate, Boric acid, Calcium chloride, Potassium phosphate monobasic, Potassium phosphate dibasic, Sodium acetate, Acetic acid were procured from SRL private Ltd, India.

Immobilization of Xylanase

The enzyme xylanase was immobilized into PVA-alginate beads by dissolving 10g of PVA and 1% of sodium alginate in 120ml of distilled water at 80^{o}C, by continuously stirring using magnetic stirrer. When the temperature of the solution reached 40^{o}C, 10ml of enzyme was added to the solution. The following three different gelating solutions were used for the formation of gel beads. i. The mixture was dropped into saturated boric acid and 1% $CaCl_2$ solution, after 1h. Spherical beads were formed, then transferred to 20% sodium sulphate solution. ii. The mixture was dropped into saturated boric acid and 1% $CaCl_2$ solution and was left for 1h. to form gel beads, then transferred to 20% sodium phosphate solution. iii. The mixture was extruded as drops into a solution of sodium nitrate (50% w/v) and 1% $CaCl_2$, and then immersed in sodium nitrate for 1h. to form beads. The beads thus obtained were rinsed with distilled water and stored at 4^{o}C

Characterization of free and immobilized enzyme

Xylanase enzyme was characterized for different physico-chemical parameters. The physico-chemical

parameters considered for our study were pH, temperature, reaction time, and substrate concentration.

Effect of pH on enzyme activity

The optimum pH for the reaction was determined by varying pH from 3--8.The various buffers used were as follows, 50mM sodium acetate buffer (3-4), 50mM phosphate buffer (5-6) and 50mM tris HCl buffer (7-8), while all the other parameters were kept constant.1% Xylan was used as the substrate. It was incubated at 40^{o}C for 30 minutes. Enzyme activity was determined.

Effect of temperature on enzyme activity

In order to determine the effect of temperature, reaction mixture was incubated at different temperature ranging between 25^{o}C-65^{o}C. 1% Xylan was used as the substrate. The amount of xylose units produced was estimated using the xylose standard plot. Enzyme activity was determined.

Effect of incubation time on enzyme activity

In order to determine the effect of incubation time on enzyme activity, xylanase enzyme was incubated at different reaction time between 10 to 60 min. at 50^{o}C, while keeping all the other parameters constant. 1% xylan was used as the substrate. After incubation at 50^{o}C for different reaction time, Xylanase activity was determined.

Effect of substrate concentration on enzyme activity

In order to determine the effect of substrate concentration, xylan ranging between 0.1-1.5 mg/ml in 50mM sodium acetate buffer was added to the reaction media, while all other parameters were kept constant. Enzyme activity was determined.

RESULTS AND DISCUSSION

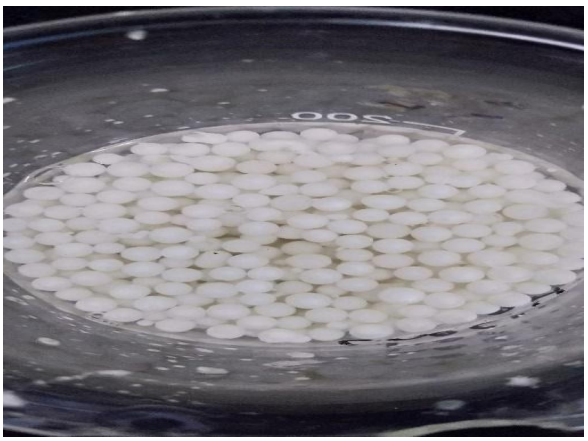

Figure 1: Shows immobilization of Xylanase in PVA-alginate matrix.

Effect of pH on Xylanase activity

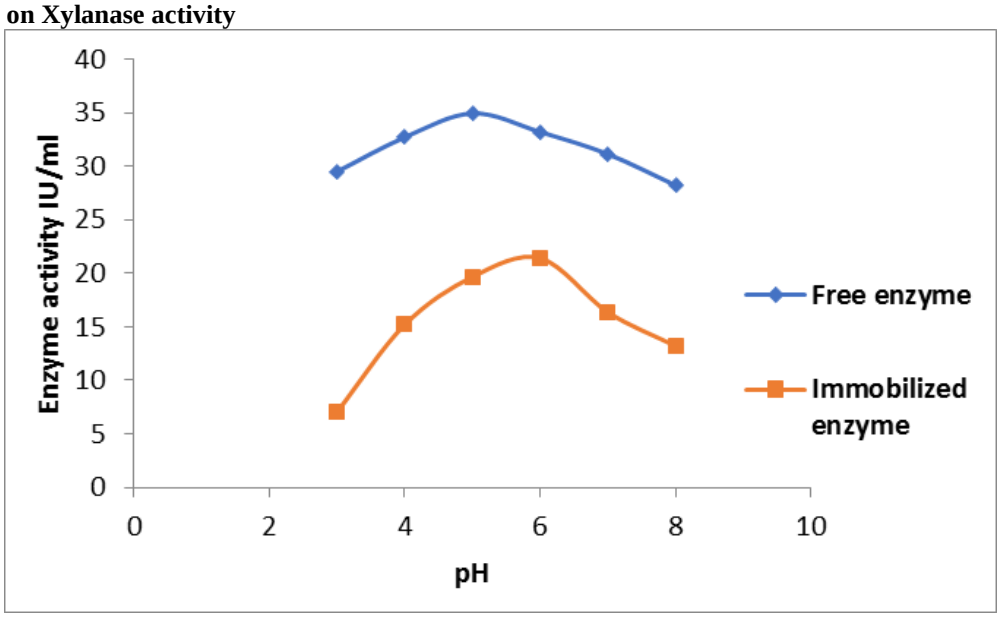

Figure 2: Indicates the effect of pH on free and immobilized Xylanase.

The effect of pH on the activity of free and immobilized xylanase was examined between pH 3.0 to 8.0. The activities obtained are presented in Figure 2. The maximum activity was observed at pH of 6 for both free and immobilized xylanase. The pH profile of the immobilized enzyme reveals a broader profile of enzyme activity, which is suggestive of a protective role played by the beads, more noticeable at extreme pH values tested. The activity of immobilized xylanase is lower than free xylanase. The lower activity of the immobilized xylanase may be attributed to alteration of enzyme structure during entrapment into the acid boric solution or to diffusion limitations (Dave, 2006; Tennalli, 2019).

Effect of temperature on Xylanase activity

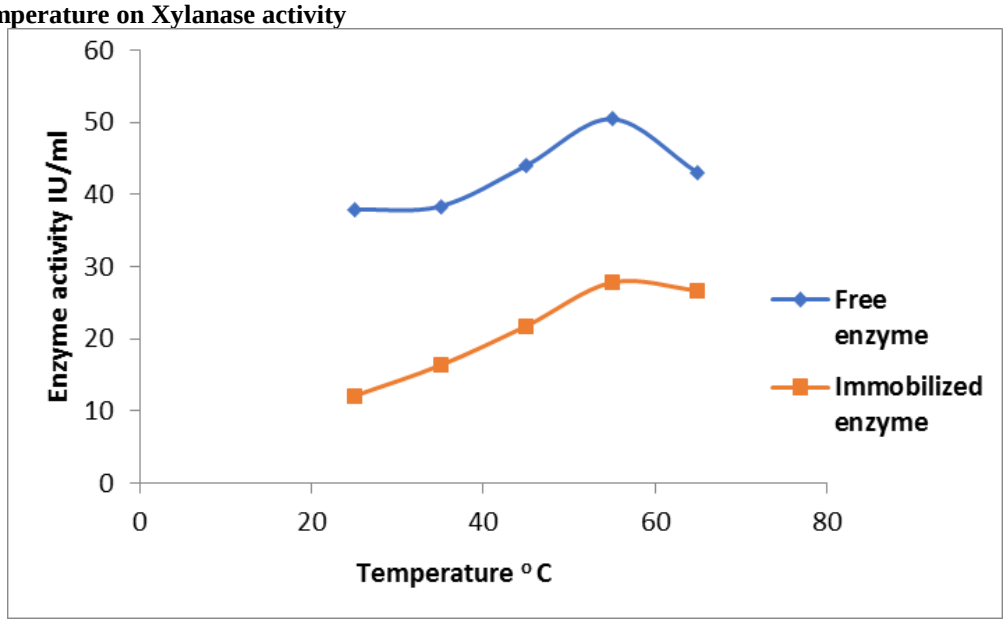

Figure 3: Indicates the effect of temperature on free and immobilized Xylanase.

The use of enzymes in bioprocess often encounters the problem of its thermal inactivation. Under high temperature, enzymes may undergo partial unfolding by heat induced destruction of non covalent interactions (Dave, 2006; Hombalimath, 2012).The temperature dependence of the free and immobilized xylanase activity was studied in the temperature range between 25–65°C. The results obtained are depicted in Figure 3. Maximum enzyme activity was observed at 55°C, for both free and immobilized xylanase. The free enzyme had a higher activity than the immobilized one. This may be due to decreased affinity of the enzyme for the substrate caused by internal diffusion restriction of the immobilized xylanase. The immobilized xylanase exhibited a broader profile at the optimum temperature, showing a better thermal stability, whereas the free enzyme was less stable towards heat (Buchholz, 1987).

Effect of incubation time on Xylanase activity

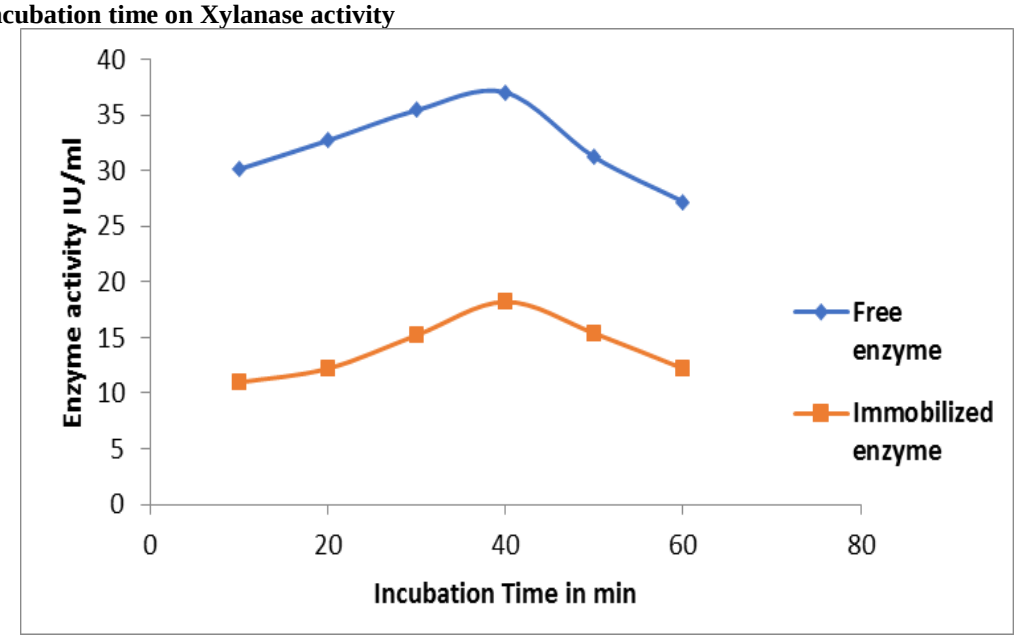

Figure 4: Indicates the effect of incubation time on free and immobilized Xylanase.

The effect of incubation time on the activity of free and immobilized xylanase was examined between 10 to 60 min. The activities obtained are presented in Figure 4. The maximum activity was observed at around 40 minutes for both free and immobilized xylanase. The immobilized enzyme had a lower activity than the free one. The incubation time profile of the immobilized enzyme revealed a broader profile of enzyme activity, which is suggestive of a protective role played by the beads.

Effect of substrate concentration on Xylanase activity

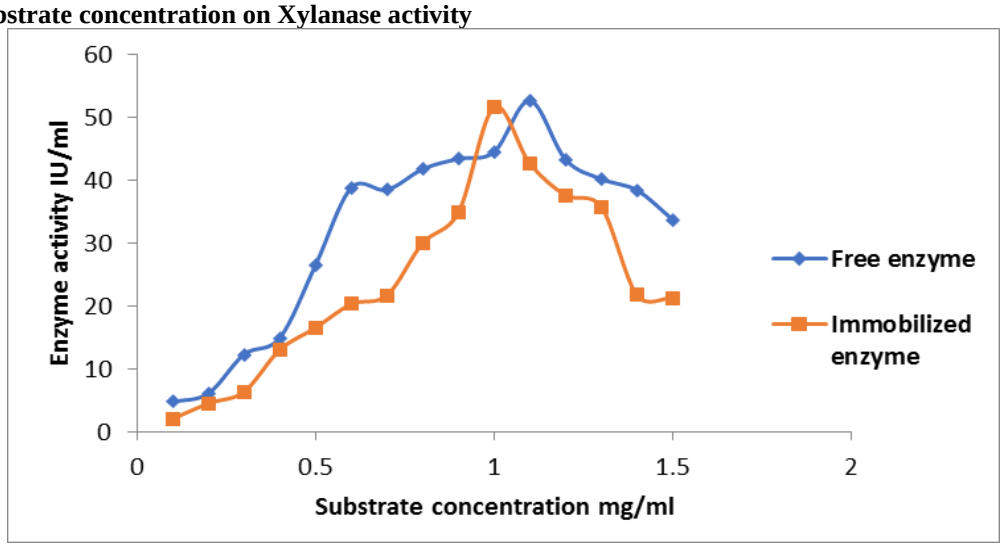

Figure 5: Indicates the effect of substrate concentration on free and immobilized Xylanase.

The substrate concentration dependence of the free and immobilized xylanase activity was studied in the concentration range between 0.1 to 1.5mg/ml. The results obtained are depicted in Figure 5. Optimum substrate concentration was observed at around 1.1mg/ml for free xylanase and 1.0 mg/ml for immobilized xylanase.

CONCLUSION

Xylanase was immobilized in PVA-Alginate Matrix successfully. The immobilized enzyme was characterized and its performance was compared with the free enzyme. The effect of different parameters like pH, temperature, substrate concentration and incubation time on free and immobilized enzyme was studied. It was found that the immobilized enzyme had a lower activity than the free one.

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