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The Effect of Cobalt on Immobilized D-Psicose 3-Epimerase Activity in Ca-Alginate Beads

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Abstract:*The enzyme, D-Psicose 3-Epimerase is animportant biocatalyst in the conversion of D-fructose into D-Psicose which possess high medical properties for treating obesity. This study aims to evaluate and differentiate the characteristics of immobilized and crude D-Psicose 3-Epimerase (DPEase) activity upon addition of Cobalt (Co) as cofactor. Crude DPEase was reacted with 1.5% Na-alginate solution and constructed into beads upon mixing with $CoCl_2$ solution. This study obtained 300 beads in which the activity from each immobilized and free enzyme bead is evaluated. The optimum condition for immobilized DPEase activity is 40^{o}C and pH 8.0. Meanwhile, the free DPEase also produced a similar trend with optimum activity at 40^{o}C but in different pH at 8.5. By knowing these characteristics on immobilized DPEase, further development in terms of industrial production may be achieved to yield D-Psicose in a more effective way.*

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I. Introduction

Cobalt (Co) is a transition metal between iron and nickel, with enormous function in biomolecules. Almost all three dimensional protein in the field of biochemistry and molecular biology contain cobalt. Classes of enzymes with non-corrincobalt have been characterized including isomerase, methionine aminopeptidase, prolidase, nitrate hydratase, glucose, methylmalonyl-CoA carboxy-transferase, aldehyde decarbonylase, and lysine-2,3 α-aminomutase[1]. The addition of metal ions to the enzymes acts to activate the substrate yet stabilizing the negative charge based on the properties of Lewis acid and base[2]. D-psicose 3-Epimerase (EC 5.1.3.30) or DPEase is an enzyme that converts fructose to D-psicose,with structural changes in atom C number 3 resulted in modification of final product[3]. DPEase has been developed molecularly using microorganisms by previous researchers considering its product, the D-psicose as an alternative sugar compound for diabetic and obese people[4]. D-psicose is a group of rare monosaccharide sugars which are not widely available in nature[5]. The magnitude of the efficacy of monosaccharide (D-psicose) as sugar derivatives has enforced researchers to continue developingeffective ways in producing D-Psicose. One strategy to improve the yield of D-Psicose is by using immobilized DPEase to achieve a high turn-rate of product synthesis. The immobilized DPEase when reacted with suitable substrate and metal ions may trigger the active side of the enzyme, leading to rapid conversion of product or D-Psicose. The matrices commonly used in immobilization are albumine, alginate, and cellulose[6]. In this study, we develop a Na-alginate beads containing crude DPEase in which the characteristics upon its activity are evaluated.

II. Material And Methods

D-Psicose 3-Epimerase (DPEase) is produced and isolated from recombinant *Escherichia coli* grown previously in Luria-Bertani (LB) medium.

Immobilization of DPEase: Na-alginate was used as matrice within final concentration at 1.5% (w/v). Ten mililitre of Na-alginate solution was mixed into 10 mL crude DPEase supplemented with 10 µL of $CoCl_2$ (1 mg/mL). The mixture was stirred upon addition of Na-alginate solution. The mixture was pipetted and structured into beads by injecting gel-like solution into 50 mL $CaCl_2$ 0.2 M. The beads were washed by sterile distilled water prior experimentation.

Stability test of Immobilized DPEase: Crude and immobilized DPEase were characterized for its enzyme activity under different temperature and pH treatment. A 50 µL of crude DPEase and 6 beads were reacted with substrate solution containing 500 µL fructose solution (100 g/mL in 50 µL Tris-HCl 1 M and 400 µL distilled water). Reaction mixture was incubated for 10 minute under five different temperatures, ranged between 30-70^{o}C and pH range between 6-10. Remaining D-fructose in reaction mixture was estimated quantitatively by

adding 1 mL of yeast suspension (*Saccharomyces cerevisiae*). The yield of D-Psicose was measured according to Cystein-Carbazole method[7].

III. Results

Immobilization of D-Psicose 3-Epimerase

D-Psicose 3-Epimerase used in this study has a molecular weight of 32 kDa based on previous report. Immobilization of DPEase within mixture of 1.5% Na-alginate and $CoCl_2$ yielded 300 beads. By applying immobilization technique to crude of free enzyme, stability of its activity may be improved under environmental condition, such as temperature and pH. The beads are characterized as round and smooth surface.

Stability of D-Psicose 3-Epimerase

The effect of temperature to DPEase activity resulted in a more improved stability of immobilized DPEase than free enzyme. Both enzymes showed an optimum temperature at 40^{o}C (**Figure 1**). However, the addition of cobalt ($CoCl_2$) yield a higher activity of free enzyme (0.897 U/mL) than immobilized enzyme (0.415 U/mL).

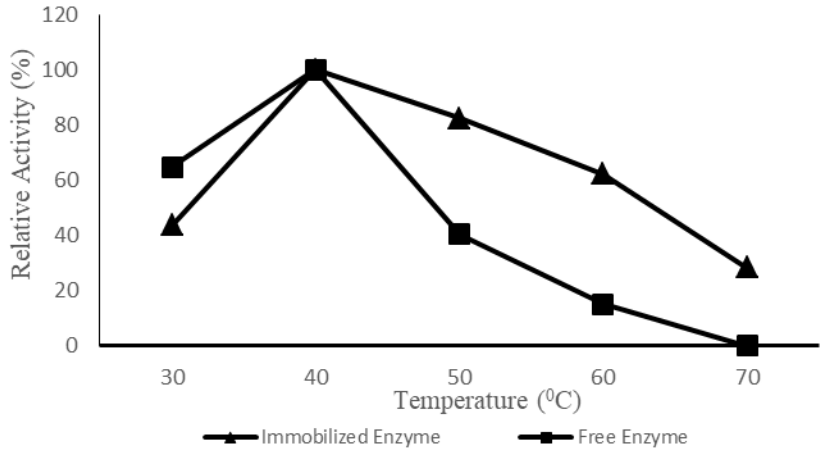

Figure 1. Free and immobilized D-Psicose 3-Epimerase activity under different temperature condition

The effect of pH on the activity of free DPEase with the addition of cobalt ($CoCl_2$) is optimal at pH 8.0, while the immobilized DPEase enzyme at pH 8.5 (**Figure 2**). The free enzyme is relatively stable and has a high activity compared to immobilized decreased significantly at pH 9.0 (no activity) while the immobilized DPEase still produced anenzyme activity.

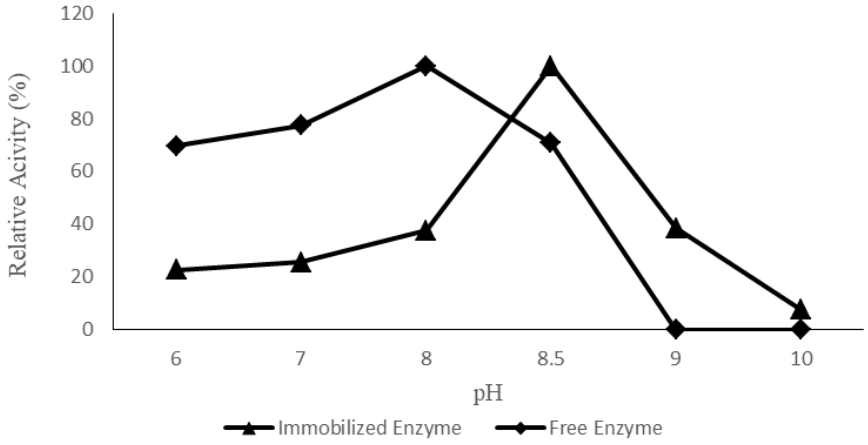

Figure 2. Free and immobilized D-Psicose 3-Epimerase activity under different pH condition

IV.Discussion

Enzyme DPEase activity in converting D-fructose into D-Psicose as final product is efficiently separated from its substrate in larger reaction mixture solution. The production of D-Psicose by using chitosan-immobilized DPEase was achieved optimally at 45^{o}C, pH 7.0 for 60 days in bioreactors resulting in an increase of 25% of total yield productivity [9].

Crude enzyme of DPEase decreased significantly at 50°C in contrary to immobilized DPEasewhich remained stable up to 60°C. The limited space for enzyme may lower the incidence of dentaruration by increasing the bond between substrate even under higher temperature.

Previous study reported that addition of cobalt improved the DPEase activity from being stable at 35^{o}C to 50^{o}C at pH 8.0[10]. Selection of metal ion as cofactor in DPEase enzymatic reaction is crucial and may be differed if tested against different ions. The addition of cobalt is better than mangan or Mn by improving the stability of DPEase to react in higher temperature. Based on this evidence, cobalt is not needed in catalytic reaction, but rather stabilizing the enzyme structure.

IV. Conclusion

D-Psicose 3-Epimerase isolated from recombinant *Escherichia coli* was immobilized into Ca-alginate beads yielding 300 beads for experimentation. The immobilized DPEase showed an improved stability upon addition of cobalt with optimum activity at 45^{o}C and pH 8.5 compared to crude or free DPEase.

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