

## ABSTRACT

### THE IMMOBILIZATION OF $\alpha$ -AMYLASE ENZYME FROM *Bacillus subtilis* ITBCCB148 USING CM-Sephadex C-50

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$\alpha$ -amylase is an enzyme that breaks  $\alpha$ -1,4 glycoside bond in amyllum. It has been widely used in a number of industrial processes such as food industry and non food industry. In industrial process, this enzyme must be able to work in an extreme pH and temperature. However, an enzyme is not normally stable in these conditions.

The aim of this research is to increase the stability of  $\alpha$ -amylase obtained from *Bacillus subtilis* ITBCCB148 with immobilization method using CM-Sephadex C-50. To achieve this aim, the steps done in this research were production, isolation, and purification of the enzyme which includes fractionation with ammonium sulphate, dialysis and chromatography with CM-cellulose. The pure enzyme was then immobilized using CM-Sephadex C-50. The activity of  $\alpha$ -amylase was determined by Fuwa and Mandels methods, while the protein concentration was determined by Lowry method.

The result showed that the specific activity of pure enzyme was 21.400 U/mg, an increase of 15 times compared to the enzyme raw extract with a recovery of 11%. The optimum temperature for pure enzyme was 60°C,  $K_M$  value of 6.32 mg mL<sup>-1</sup> substrate and  $V_{max}$  value of 138.89  $\mu$ mol mL<sup>-1</sup> min.<sup>-1</sup>. The thermal stability test at 60°C for 60 minutes showed the residual activity of 23%,  $t_{1/2}$  = 33.64 minutes,  $k_i$  = 0.0206 min.<sup>-1</sup>.  $\Delta G_i$  = 103.97 kJ mol<sup>-1</sup>. The immobilized enzyme has optimum temperature of 70°C,  $K_M$  value of 6.90 mg mL<sup>-1</sup> substrate and  $V_{max}$  value of 90.09  $\mu$ mol mL<sup>-1</sup> min.<sup>-1</sup>. The thermal stability test of immobilized enzyme at 60°C for 60 minutes showed the residual activity was 51%,  $k_i$  = 0.0136 min.<sup>-1</sup>,  $t_{1/2}$  = 50.96 minutes.  $\Delta G_i$  = 105.12 kJ mol<sup>-1</sup>.

Compared to the pure enzyme, the thermal stability of the immobilized enzyme was increased 1.51 times. The decrease of  $K_M$  value, the increase of  $V_{max}$  value, the decrease of  $k_i$ , the increase of  $t_{1/2}$  and  $\Delta G_i$  showed that the immobilization process has increased the rigidity of the enzyme, as a result the enzyme was more stable against pH and temperature.

**Key word** :  $\alpha$ -amylase, *Bacillus subtilis* ITBCCB148, immobilization process, CM-Sephadex C-50.