

## Properties of commercial CGTase in enzymatic production of cyclodextrin from ungelatinized sago starch

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### Abstrak

Cyclodekstrin glukanotransferase (CGTase) merupakan enzim yang mengkatalis produksi cyclodekstrin (CD). Penelitian ini menggunakan satu enzim CGTase komersial (Toruzyme M) yang dihasilkan secara rekayasa genetika menggunakan bakteri *Bacillus* yang telah disisipkan gen GTase dari bakteri termofilik *Thermoanaerobacter*. Walaupun enzim ini dapat menghasilkan alfa, beta, dan gamma siklodekstrin ( $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD), namun beta siklodekstrin merupakan produk terbesar. Hasil penelitian memperlihatkan bahwa reaksi enzim CGTase ini dengan pati sago mentah sangat berbeda jika menggunakan pati sago tergelatinkan. Temperatur aneling dan temperatur reaksi enzimatis ditetapkan pada 65°C. Kondisi optimum diperoleh pada pH 9 (bufer glisin-NaOH 0.05M), 15% (b/v) pati sago dan 0.5% konsentrasi enzim. Total siklodekstrin maksimum (13.17 g/L) diperoleh selama 4 jam pada kelajuan agitasi 200 rpm. dengan perbandingan produk adalah 28%: 64%: 8% masing-masing untuk  $\alpha$ -CD:  $\beta$ -CD:  $\gamma$ -CD. Adanya  $\text{CuSO}_4$ ,  $\text{FeSO}_4$  and  $\text{Co}(\text{NO}_3)_2$  didalam substrat mampu menghambat aktivitas enzim secara keseluruhan sedangkan pemberian n-pentene dan etanol akan menghasilkan  $\alpha$ -CD sebagai produk utama. Nilai  $K_{max}$  dan  $K_m$  CGTase Toruzyme adalah 0.09 s  $\beta$ -CD/min and 16.695 % (w/v), secara berurutan.

Cyclodextrin glucanotransferase (CGTase) is the enzyme catalyzing the production of cyclodextrin (CD). This study was conducted using a commercial CGTase enzyme (Toruzyme T) produced from genetically modified strain of *Bacillus* carrying the CGTase gene of *Thermoanaerobacter*. Although this enzyme catalyses the formation of alpha, beta and gamma cyclodextrin from starch but beta-cyclodextrin is the major product. The result showed that the reaction behavior of the enzyme on ungelatinized sago starch was markedly different when compared with its reaction to gelatinized starch. Ungelatinised sago starch was annealed and reacted at 65°C. The optimum condition for the reaction occurred at pH 9 (0.05M Glycine-NaOH buffer) with concentration enzyme and sago starch was 0.5% (v/v) and 15 % (w/v), respectively. The highest amount of total cyclodextrin (13.17 g/L) was produced when the reaction mixture was agitated at 200 rpm for 4 hours consisting of  $\alpha$ -CD:  $\beta$ -CD:  $\gamma$ -CD at ratios of 28: 64: 8. The CGTase lost almost all its dextrinizing activity in the presence  $\text{CuSO}_4$ ,  $\text{FeSO}_4$  and  $\text{Co}(\text{NO}_3)_2$  in substrate. Addition of n-pentane and ethanol to the reaction mixture, shifted the reaction toward an increased yield of  $\alpha$ -cyclodextrin and eventually becoming the main product of the reaction. The  $K_{max}$  and  $K_m$  value of CGTase Toruzyme were 0.09 g  $\beta$ -CD/min and 16.695 % (w/v), respectively.