ABSTRACT

ENZYMATIC CONVERSION OF TAPIOKA SOLID WASTE STARCH TO BE GLUCOSA USING IMMOBILIZED α-AMILASE FROM Bacillus Subtilis ITBCCB148 FOR BIOETANOL PRODUCTION

By

Arum Widyasmara

In this study, the α-amylase enzyme from Bacillus subtilis ITBCCB148 was immobilized through crosslinking method on chitosan matrix. The aims of this study were to determine the effect of immobilization on the stability of α-amylase to temperature and pH, and to convert cassava solid waste starch by the immobilized α-amylase to be glucose which is used for bioethanol production. The steps of this study were done as following: production process, isolation, purification, immobilization, characterization of purification enzyme and immobilization, enzymatic conversion of cassava solid waste starch and bioethanol fermentation. The results showed that the specific activity of the purified α-amylase enzyme after the dialysis stage was 4504.15 U mg^{-1} and its purity increased 4.87 times than the crude extract enzyme. The purified α-amylase enzyme has an optimum temperature of 65°C, K_M = 1.63 mg mL^{-1} substrate, and Vmax = 39.68 μmol mL^{-1} min^{-1}. The immobilized α-amylase enzyme has an optimum temperature of 75^oC, K_M = 3.514 mg mL^{-1} substrate, and Vmax = 7,05 μmol mL^{-1} min^{-1}. The residual activity of the purified enzyme and immobilized enzyme in the study of thermal stability on 60^oC for 80 minutes were 58% and 86.15%, respectively. The immobilized enzyme can be used repeatedly up to 6 times. The kinetic studies of purified enzyme were obtained $t_{½}$ = 113.61 minutes, k_i = 0.0061 min^{-1}, and ΔGi = 107.34 kJ mol^{-1}. The kinetic studies of the immobilized enzyme were obtained $t_{½}$ = 433.13 minutes, k_i = 0.0016 min^{-1}, and ΔGi = 111.06 kJ mol^{-1}. Based on the decrease of k_i value, increase of ΔGi and half-life ($t_{½}$), the immobilization by chitosan can improve the stability of α-amylase from B. subtilis ITBCCB148. Furthermore, the *Saccharomyces cerevisiae* fermentation on cassava solid waste starch converted by the immobilized α-amylase obtained ethanol approximately 0.129%.

Keywords: α-amylase, *Bacillus subtilis* ITBCCB148, chitosan, immobilization, *Saccharomyces cerevisiae*

ABSTRAK

KONVERSI ENZIMATIS PATI ONGGOK MENJADI GLUKOSA MENGGUNAKAN α-AMILASE AMOBIL DARI *Bacillus Subtilis* ITBCCB148 UNTUK PRODUKSI BIOETANOL

Oleh
Arum Widyasmara

Pada penelitian ini telah dilakukan amobilisasi enzim α-amilase dari *Bacillus subtilis* ITBCCB148 melalui metode ikatan silang pada matriks kitosan. Penelitian ini bertujuan untuk mengetahui pengaruh amobilisasi pada kestabilan enzim α-amilase terhadap suhu dan pH serta konversi enzimatis pati onggok oleh enzim α-amilase amobil menjadi glukosa reduksi yang selanjutnya digunakan untuk produksi bioetanol. Tahap penelitian ini meliputi proses produksi, isolasi, pemurnian, amobilisasi, karakterisasi enzim hasil pemurnian dan amobilisasi serta konversi enzimatis pati onggok dan fermentasi bioetanol. Hasil penelitian menunjukkan bahwa aktivitas spesifik enzim α-amilase hasil pemurnian hingga tahap dialisis adalah 4.504,15 U mg^{-1} dan kemurniannya meningkat 4,87 kali dibandingkan dengan ekstrak kasar enzim. Enzim α-amilase hasil pemurnian memiliki suhu optimum 65^oC, K_M = 1,63 mg mL^{-1} substrat, dan V_{maks} = 39,68 µmol mL^{-1} $menit^{-1}$. Enzim α-amilase hasil amobilisasi memiliki suhu optimum 75^oC, K_M = 3,514 mg mL^{-1} substrat, dan V_{maks} = 7,05 µmol mL^{-1} $menit^{-1}$. Aktivitas sisa enzim hasil pemurnian dan hasil amobilisasi dalam uji stabilitas termal pada suhu 60^oC selama 80 menit berturut-turut sebesar 58% dan 86,15%. Enzim amobil dapat digunakan berulang hingga 6 kali. Data kinetika enzim hasil pemurnian diperoleh $t_{½}$ = 113,61 menit, k_i = 0,0061 $menit^{-1}$, dan ΔG_i = 107,34 kJ mol^{-1}. Data kinetika enzim hasil amobilisasi diperoleh $t_{½}$ = 433,13 menit, k_i = 0,0016 $menit^{-1}$, dan ΔG_i yaitu 111,06 kJ mol^{-1}. Berdasarkan penurunan nilai k_i, peningkatan nilai ΔG_i dan waktu paruh ($t_{½}$), diketahui bahwa amobilisasi menggunakan kitosan dapat meningkatkan stabilitas enzim α-amilase dari *B. subtilis* ITBCCB148. Selanjutnya, hasil fermentasi *Saccharomyces cerevisiae* pada pati onggok yang dikonversi dengan enzim α-amilase didapatkan kadar etanol berkisar 0,129%.

Kata kunci : α-amilase, amobilisasi, *Bacillus subtilis* ITBCCB148, kitosan, *Saccharomyces cerevisiae*