ABSTRACT

ENZYMATIC CONVERSION OF ONGGOK STARCH INTO GLUCOSE USING α-AMILASE FROM *Bacillus subtilis* ITBCCB148 IMMOBILIZED BY NATURAL ZEOLITE FOR BIOETANOL PRODUCTION

By

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The demand of fuel energy are increasing time by time, but the stock of fossil fuel are decreasing. Nowadays renewable energy as an alternative sources are needed, such as bioethanol. Study about bioethanol production including the enzyme systems are required. The objective of this study is to determine the effect of immobilization on the stability of enzymes. The immobilized of α-amylase enzyme is used to convert onggok starch into glucose for bioethanol production. The steps of this study includes production process, isolation, purification, immobilization, characterization, enzymatic conversion, and fermentation. Our observation showed that the specific activity of purified enzyme by dialysis was 10,318.898 U/mg and its purity increased 13 times than the crude ones. The purified enzyme has an optimum temperature of 55^{o}C, K_M = 7.31 mg/mL substrate, V_{max} = 90.91 μmol/mL.min, moreover the immobilized enzyme has an optimum temperature of 70^{o}C, K_M = 14.78 mg/mL substrate, V_{max} = 36.9 μmol/mL.min. The residual activity of the purified and immobilized enzyme on thermal stability were 18 and 87% respectively. The kinetic study of the purified enzyme obtained k_i = 0.0226 min^{-1}, ΔG_i = 92.364 kJ/mol, and $t_{1/2}$ = 30.664 min, moreover the immobilized enzyme obtained k_i = 0.0013 min^{-1}, ΔG_i = 111.607 kJ/mol, and $t_{1/2}$ = 533.077 minutes. Our Investigation suggests that immobilization with natural zeolite can improve the stability of enzymes. The bioethanol that obtained from fermentation process using *Saccharomyces sereviciae* and yeast were 0.14 and 0.30% respectively.

Keywords : α-amylase, *Bacillus subtilis* ITBCCB148, immobilization, natural zeolite, onggok starch, bioethanol.

ABSTRAK

KONVERSI ENZIMATIS PATI ONGGOK MENJADI GLUKOSA MENGGUNAKAN ENZIM α-AMILASE DARI *Bacillus subtilis* ITBCCB148 YANG DIAMOBILISASI DENGAN ZEOLIT ALAM UNTUK PRODUKSI BIOETANOL

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Kebutuhan terhadap energi semakin meningkat namun ketersediaan bahan bakar fosil semakin menurun. Energi terbarukan dibutuhkan sebagai sumber alternatif, salah satunya adalah bioetanol. Penelitian mengenai produksi bioetanol oleh enzim sangatlah dibutuhkan. Penelitian ini bertujuan untuk mengetahui pengaruh amobilisasi terhadap kestabilan enzim. Enzim α-amilase hasil amobilisasi digunakan untuk mengonversi pati onggok menjadi glukosa untuk produksi bioetanol. Tahap penelitian ini meliputi proses produksi, isolasi, pemurnian, amobilisasi, karakterisasi, konversi enzimatis, dan fermentasi. Hasil penelitian menunjukkan aktivitas spesifik enzim hasil pemurnian sebesar 10.318,898 U/mg dan kemurniannya meningkat 13 kali dibandingkan ekstrak kasarnya. Enzim hasil pemurnian memiliki suhu optimum 55^{o}C, K_M = 7,31 mg/mL substrat, V_{maks} = 90,91 µmol/mL.menit, sedangkan enzim hasil amobilisasi memiliki suhu optimum 70^{o}C, K_M = 14,78 mg/mL substrat, V_{maks} = 36,9 µmol/mL.menit. Aktivitas sisa dari enzim hasil pemurnian dan hasil amobilisasi pada uji stabilitas termal berturut-turut sebesar 18 dan 87%. Data kinetika enzim hasil pemurnian diperoleh nilai k_i = 0,0226 menit^{-1}, ΔG_i = 92,364 kJ/mol, dan $t_{1/2}$ = 30,664 menit, sedangkan enzim hasil amobilisasi diperoleh nilai k_i = 0,0013 menit^{-1}, ΔG_i = 111,607 kJ/mol, dan $t_{1/2}$ = 533,077 menit. Data tersebut menunjukkan bahwa amobilisasi dengan zeolit alam dapat meningkatkan kestabilan enzim. Kadar bioetanol yang diperoleh dari proses fermentasi menggunakan *Saccharomyces sereviciae* dan ragi berturut-turut sebesar 0,14 dan 0,30%.

Kata kunci : α-amilase, *Bacillus subtilis* ITBCCB148, amobilisasi, zeolit alam, pati onggok, bioetanol.