

ABSTRACT

ENHANCEMENT OF α -AMYLASE STABILITY FROM *Aspergillus* sp. THROUGH IMMOBILIZATION USING SILICA GEL 60, CHITIN, AND SILICA-CHITIN HYBRID

By

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Enzymes are widely used as industrial biocatalysts; however, their application is limited by insufficient stability under extreme conditions. This study aimed to enhance enzyme stability and enable repeated use through immobilization using silica gel, chitin, and a silica–chitin hybrid. The research procedure consisted of enzyme production, isolation, purification by fractionation and dialysis, immobilization, and enzyme characterization. Enzyme activity was analyzed using the Fuwa and Mandels method, while protein content was determined by the Lowry method.

The purified enzyme exhibited a 12-fold increase in purity compared to the crude extract, with an optimum temperature of 50 °C and 59.9 % residual activity after 80 min of incubation. Thermal stability analysis of the purified enzyme showed a half-life ($t_{1/2}$) of 97.6 min and a ΔG_i value of 103.616 kJ mol⁻¹. The enzyme immobilized on silica gel showed an optimum temperature of 60 °C, a $t_{1/2}$ of 231.05 min, and a ΔG_i of 109.294 kJ mol⁻¹. The enzyme immobilized on chitin exhibited an optimum temperature of 55 °C, a $t_{1/2}$ of 150.68 min, and a ΔG_i of 106.446 kJ mol⁻¹. The silica–chitin hybrid–immobilized enzyme showed the best performance, with an optimum temperature of 55 °C, a $t_{1/2}$ of 301.37 min, and a ΔG_i of 108.336 kJ mol⁻¹. Residual activities of the immobilized enzymes on silica gel, chitin, and silica–chitin hybrid were 78.3, 68.6, and 81.1 %, respectively, after 80 min of incubation.

Based on the increase in half-life values, enzyme stability was enhanced by 2.37-, 1.54-, and 3.09-fold for silica gel, chitin, and silica-chitin hybrid immobilization, respectively. The enzyme immobilized on the silica-chitin hybrid retained 61.2% residual activity after 10 reuse cycles, indicating that immobilization significantly improves thermal stability and reuse potential of α -amylase as a more efficient biocatalyst.

Keywords: α -amylase, immobilization, silica gel, chitin, thermal stability, enzyme kinetics

ABSTRAK

PENINGKATAN KESTABILAN ENZIM α -AMILASE DARI *Aspergillus* sp. DENGAN METODE IMOBILISASI MENGGUNAKAN SILIKA GEL 60, KITIN, DAN HIBRIDA SILIKA-KITIN

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Enzim banyak dimanfaatkan sebagai biokatalis industri, namun penggunaannya terbatas oleh kestabilan terhadap kondisi ekstrem. Penelitian ini bertujuan meningkatkan kestabilan dan memungkinkan pemakaian berulang enzim melalui imobilisasi menggunakan silika gel, kitin, dan hibrida silika-kitin. Prosedur penelitian dilakukan melalui tahapan berikut : produksi, isolasi, pemurnian dengan fraksinasi dan dialisis, imobilisasi, serta karakterisasi enzim. Aktivitas enzim dianalisis menggunakan metode Fuwa dan Mandels, sedangkan kadar protein ditentukan dengan metode Lowry.

Enzim hasil pemurnian mengalami peningkatan kemurnian 12 kali dibandingkan ekstrak kasar, dengan suhu optimum 50 °C dan aktivitas sisa 59,9 % setelah inkubasi 80 menit. Uji stabilitas enzim hasil pemurnian menunjukkan nilai $t_{(1/2)}$ sebesar 97,6 menit dan ΔG_i 103,616 kJ mol⁻¹. Enzim hasil imobilisasi dengan silika optimum pada suhu 60 °C, nilai $t_{(1/2)}$ sebesar 231,05 menit dan ΔG_i 109,294 kJ mol⁻¹. Enzim hasil imobilisasi dengan kitin optimum pada suhu 55 °C, nilai $t_{(1/2)}$ 150,68 menit, dan ΔG_i 106,446 kJ mol⁻¹. Enzim hasil imobilisasi dengan hibrida silika-kitin optimum pada suhu 55 °C, nilai $t_{(1/2)}$ 301,37 menit, dan ΔG_i 108,336 kJ mol⁻¹. Aktivitas sisa enzim hasil imobilisasi dengan silika, kitin, dan hibrida silika-kitin sebesar 78,3; 68,6; dan 81,1 % setelah inkubasi selama 80 menit.

Berdasarkan peningkatan nilai $t_{(1/2)}$, kestabilan enzim hasil imobilisasi menggunakan silika, kitin, dan hibrida silika-kitin meningkat masing-masing sebesar 2,37; 1,54; dan 3,09 kali. Enzim terimobilisasi pada hibrida silika–kitin dapat digunakan hingga 10 kali dengan aktivitas sisa 61,2 %, menunjukkan bahwa imobilisasi secara signifikan meningkatkan kestabilan termal dan potensi penggunaan ulang enzim α -amilase sebagai biokatalis yang lebih efisien.

Kata kunci: α -amilase, imobilisasi, silika gel, kitin, kestabilan termal, kinetika enzim