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Research Article

The Stability Improvement of α-Amylase Enzyme from *Aspergillus fumigatus* by Immobilization on a Bentonite Matrix

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The stability of the α-amylase enzyme has been improved from *Aspergillus fumigatus* using the immobilization method on a bentonite matrix. Therefore, this study aims to obtain the higher stability of α-amylase enzyme from *A. fumigatus*; hence, it is used repeatedly to reduce industrial costs. The procedures involved enzyme production, isolation, partial purification, immobilization, and characterization. Furthermore, the soluble enzyme was immobilized using 0.1 M phosphate buffer of pH 7.5 on a bentonite matrix, after which it was characterized with the following parameters such as optimum temperature, Michaelis constant (K_M), maximum velocity (V_{max}), thermal inactivation rate constant (k_i), half-life ($t_{1/2}$), and the change of energy due to denaturation (ΔG_i). The results showed that the soluble enzyme has an optimum temperature of 55°C, K_M of 3.04 mg mL^{-1} substrate, V_{max} of 10.90 μmole mL^{-1} min^{-1}, k_i of 0.0171 min^{-1}, $t_{1/2}$ of 40.53 min, and ΔG_i of 104.47 kJ mole^{-1}, while the immobilized enzyme has an optimum temperature of 70°C, K_M of 8.31 mg mL^{-1} substrate, V_{max} of 1.44 μmole mL^{-1} min^{-1}, k_i of 0.0060 min^{-1}, $t_{1/2}$ of 115.50 min, and ΔG_i of 107.37 kJ mole^{-1}. Considering the results, the immobilized enzyme retained 42% of its residual activity after six reuse cycles. Additionally, the stability improvement of the α-amylase enzyme by immobilization on a bentonite matrix, based on the increase in half-life, was three times greater than the soluble enzyme.

1. Introduction

Hitherto, microbial α-amylase is used commercially as biocatalysts in various industries, such as detergent, syrup, bread and cake, dairy products, starch processing, animal feed, textile and leather, pulp and paper, candy, sugar, bioethanol, pharmaceuticals, and waste treatment [1, 2]. Furthermore, it has a 25% demand in the global market [3]. The α-amylase enzyme (α-1,4-glucan-4-glucanohydrolase) breaks down the starch molecule by acting on the α-1,4-glucosidic bonds, thereby producing maltose, dextrin, or D-glucose [2]. However, despite its wide usage on the industrial scale, mostly enzymes are easily soluble in water when applied in batch processes, and this weakens the hydrogen bonds which contribute to stability [4]. The decrease in the stability of enzymes causes denaturation due to the loss of equilibrium in the noncovalent bonds [5], resulting in the once use of nonimmobilized enzymes. However, the price of commercial enzymes in the global market is quite expensive [6].

Immobilization is one of the simple ways to stabilize the enzyme structure, and it is the process of binding or retaining molecules to material or matrix which is insoluble in water [7]. Furthermore, one of the advantages of immobilized enzymes is that they are used repeatedly. In this study, the soluble α-amylase was immobilized on bentonite as an inorganic material and the smectite clay mineral with a structure consisting of two tetrahedral and one octahedral layer. The silicate of each tetrahedral layer interacts with the hydroxyl groups in the octahedral layer; hence, the

tetrahedral-octahedral-tetrahedral (TOT) layers are formed. Furthermore, there are cations between the octahedral and tetrahedral layers that are involved in enzyme immobilization [8]. The characteristics of bentonite include insoluble in water, has large particle surface area, chemically inert, and thermally stable, has a well-defined layered structure, has the ion-exchange ability, abundant raw material, inexpensive, and eco-friendly [9]. Based on the previous study [10], the immobilized α-amylase from *Bacillus subtilis* ITBCCB148 on the bentonite from Sigma Aldrich™ has two-fold higher thermal stability than nonimmobilized enzyme, with residual activity of 43% after five cycles of reuse. In the present study, *Aspergillus fumigatus* was chosen as the host enzyme because this fungus produces the α-amylase without any special nutritional needs [11].

2. Materials and Methods

The local fungal isolate of *A. fumigatus* was obtained from the Laboratory of Microbiology, Department of Biology, Lampung University. Also, bentonite was purchased from Sigma Aldrich™. All chemicals and reagents used in this study were of analytical grade.

2.1. Study Procedure. The study procedures were based on a previous study, comprising production, isolation, partial purification, immobilization, and characterization of the soluble and immobilized enzymes [12]. Furthermore, the crude enzyme was soluble by fractionation using ammonium sulphate and dialysis as described in [13], while the immobilization method was based on [10]. The crude enzyme was partly refined by ammonium sulphate in an ice bath. The precipitated protein was collected by centrifugation at 5000 rpm for 15 min at 4°C and dissolved in a minimum volume of phosphate buffer (0.025 M; pH 6.5). Then, the enzyme suspension was dialyzed at 4°C against phosphate buffer (0.01 M; pH 6.5) for 24 h at 4°C [13].

2.2. Analysis of α-Amylase Activity and Determination of Protein Content. The α-amylase enzyme activity in the partial purification steps was analyzed by the Fuwa method using iodine reagent [14]. Meanwhile, the activity has been analyzed in characterization steps by Mandel's method using dinitrosalicylic acid reagent [15], whereby the protein content was determined based on the Lowry method [16].

2.3. Determination of Initial Buffer pH. The soluble enzyme was immobilized on a bentonite matrix using 0.1 M phosphate buffer with variations in pH which includes 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. Furthermore, the bentonite matrices (0.20 g) were washed 2-3 times using each buffer by centrifugation at 5000 rpm for about 15 min until the pH was reached. 0.5 ml of the soluble enzyme and 0.5 mL of each buffer were then added to the matrices. Afterward, these samples were incubated at 4°C for 30 min and centrifuged for about 15 min. From these supernatants, 0.25 mL each was collected separately as "binding" and control samples. After which, the immobilized enzymes at pH 5.0–6.5 were then eluted from their matrix using a 1.0 mL mixture of 0.1 M phosphate buffer pH 8.0 and 1 M NaCl (1 : 1), while the immobilized enzymes at pH 7.0-8.0 were eluted from their matrix using 1.0 mL mixture of phosphate buffer 0.1 M pH 5.5 and 1 M NaCl (1 : 1). All samples were then centrifuged for up to 15 min, and from the supernatants, 0.25 mL was as the "elution" sample. The enzymatic activity was analyzed by the Fuwa method, whereby the pH that has both lower and higher activity in the binding and elution process was selected as the initial buffer pH in the immobilization procedure.

2.4. Immobilization of α-Amylase on a Bentonite Matrix. The bentonite (0.20 g) was washed 2-3 times using the initial buffer by centrifugation for up to 15 min until the pH was reached. 0.5 ml of the soluble enzyme and 0.5 mL of initial buffer were then added to the matrix. Afterward, it was incubated at 4°C for 30 min and centrifuged for about 15 min. From the supernatant, 0.25 mL was collected as a control. Subsequently, 0.75 ml of the starch substrate was added to the immobilized enzyme, which was incubated at its optimum temperature for 30 min, and centrifuged for about 15 min. The supernatant was assayed for α-amylase enzyme activity by Mandel's method.

2.5. Determination of Optimum Temperature. This was performed at different incubation temperatures in Mandel's assay which includes 50, 55, 60, 65, 70, and 75°C for 30 min. Therefore, the incubation temperature, which generates the highest enzyme activity, was used to determine the optimum temperature.

2.6. Steady-State Kinetics. Kinetic parameters, the Michaelis constant (K_M) and the maximum velocity (V_{max}), were calculated using the Lineweaver–Burk plot from experimental data on the effect of starch substrate concentration against the enzyme activity in the following concentration ranges: 0.2, 0.4, 0.6, 0.8, and 1.0%. The enzyme activity is proportional to the enzyme-catalyzed reaction rate [17]. Meanwhile, both were analyzed by Mandel's method at each optimum temperature, and the K_M values of soluble and immobilized enzymes were used as each optimum substrate concentration for subsequent procedures.

2.7. Determination of Thermal Stability. Thermal stability of the enzyme was determined from the residual activity after being inactivated at 60°C for the following variation of time (t_i): 0, 10, 20, 30, 40, 50, 60, 70, and 80 min [18–20]. Furthermore, the enzyme activity was analyzed using Mandel's method. The data were also used to determine the values of k_i, $t_{1/2}$, and ΔG_i.

2.8. Determination of $t_{1/2}$, k_i, and ΔG_i. The thermal inactivation rate constant (k_i) and half-life ($t_{1/2}$) were calculated using the following first-order enzyme inactivation rate equation.

$$\ln \frac{E_i}{E_o} = -k_i \times t_i, \quad (1)$$

where k_i is the thermal inactivation rate constant, E_o is the residual activity at t_o, E_i is the residual activity at t_i, and t_i is the inactivation time. The slope of a graph $\ln(E_i/E_o)$ against t_i was determined as the k_i value [21, 22].

The free energy due to denaturation (G_i) is the energy required for the denaturation of the enzyme from the initial state. The ΔG_i value was calculated from the following derivation of thermodynamic equation.

$$\Delta G_i = -RT \ln \frac{k_i \cdot h}{k_B \cdot T}, \quad (2)$$

where ΔG_i is the change of energy due to denaturation, R is the ideal gas constant, T is the thermal inactivation temperature (K), k_i is the thermal inactivation rate constant, h is the Planck constant, and k_B is the Boltzmann constant [21].

2.9. Reusability Study. The immobilized enzyme that reacted with the substrate was washed by centrifugation using the initial buffer, and from the supernatant, 0.25 mL was collected as a control sample. Furthermore, the immobilized enzyme was reacted with a new substrate (0.75 mL) [10, 23] and the activity was determined by Mandel's method. This procedure was performed repeatedly six times.

2.10. Statistical Analysis. All measurements were done in duplicate ($n = 2$), and data were reported as mean ± standard deviation (SD). Analysis of variance (ANOVA) accompanied by the student t-test (paired two samples for means) was conducted to identify the significant differences between two replicate samples. The level of significance was set at $p < 0.05$. The null hypothesis had been rejected, and there is no difference between the two replicate measurements.

3. Results and Discussion

3.1. Purification of α-Amylase. The crude enzyme from *A. fumigatus* was partially purified by ammonium sulphate precipitation and followed by dialysis. The relationship between percent saturation of ammonium sulphate against the enzyme specific activity is shown in Figure 1. Based on Figure 1, the α-amylase yield was partly refined by 20–85% ammonium sulphate, and its specific activity was 3970.08 U/mg.

The result of partial purification steps of the α-amylase enzyme is given in Table 1. The outcomes in Table 1 indicated that the α-amylase yield of 345.57 and 179.68 U/ml was obtained from 20% to 85% ammonium sulphate and dialysis, respectively, in comparison to crude amylase of 100.37 U/ml. In addition, we observed increasing the specific activity of the enzyme that indicated an increase of its purity from each purification step. The purity of the enzyme increased tenfold after dialysis. It was supported by the decrease in total protein and the yield (%) of the enzyme which indicated that the enzyme was free from impurities.

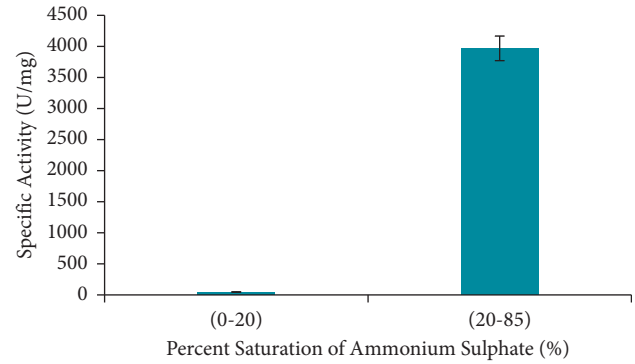

Figure 1: Ammonium sulphate fractionation scheme. The data are presented as mean ± SD; $n = 2$, significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

3.2. Determination of Initial Buffer pH. The α-amylase enzyme molecules were successfully bound to the bentonite matrix via physical adsorption at the alkaline pH range of 7.0–8.0. Furthermore, a pH 7.5 showed both the low and high enzyme activities in the binding and elution process, as shown in Figure 2. Hence, pH 7.5 was selected for the initial buffer.

3.3. Determination of Optimum Temperature. The temperature profiles of soluble and immobilized enzymes are shown in Figure 3. As shown in Figure 3, the optimum temperature of soluble and immobilized enzymes was 55°C and 70°C, respectively. However, this changes position due to space obstruction by the bentonite matrix against enzyme molecules which prevents denaturation. Thus, the immobilized enzyme is more stable than the soluble.

3.4. Steady-State Kinetics. The K_M and V_{max} values were determined from the Lineweaver–Burk plot shown in Figure 4. The K_M and V_{max} values of soluble and immobilized enzymes are given in Table 2, whereby the Michaelis constant (K_M) shows the enzymatic affinity for its substrate, while the maximum velocity (V_{max}) measures the extent of the activity [17]. Based on the data in Table 2, the decreasing V_{max} and increasing K_M trends of the immobilized enzyme were observed. The results showed that a higher K_M of the immobilized enzyme leads to a lower enzyme affinity which is caused by structural changes and lower accessibility of the substrate to the active site of the enzyme, thereby reducing V_{max} of the reaction [17, 24]. The immobilized enzyme showed a significant ($p < 0.05$) decrease in its reaction rate compared to the soluble enzyme. Also, it showed that immobilized enzymes needed higher substrate concentration to achieve V_{max}. Furthermore, the bentonite structure exhibits an interlayer that entraps enzyme molecules and prevents the entrance of substrate. The hydroxyl groups (–OH) in the octahedral layer also strengthen noncovalent bonds between enzyme and bentonite [8, 25].

3.5. Reusability Study. One of the advantages of immobilized enzymes is that they are repeatedly used in new substrates. Furthermore, in the immobilization of enzyme molecules on

Table 1: Summary of partial purification procedures of α-amylase from *A. fumigatus* unit activity was determined with 0.1% soluble starch as a substrate in aquadest (pH ± 6.5) at 60°C for 10 min.

Step	V (mL)	Unit activity (U/mL)	Total activity (U)	Protein content (mg/mL)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purity
Crude enzyme	3500	100.37	351308.7	0.2123	742.91	472.88	100	1
Fraction $(NH_4)_2SO_4$ (20–85%)	140	345.57	48379.7	0.0870	12.19	3970.08	13.77	8
Dialysis	239	179.68	42993.4	0.0398	9.52	4514.67	12.24	10

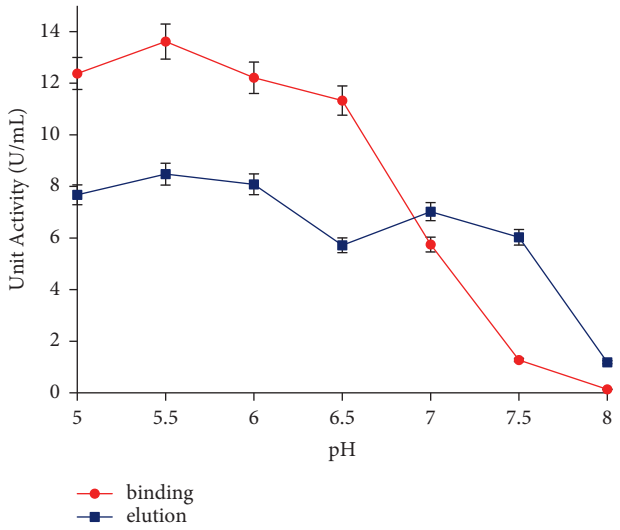

Figure 2: Binding buffer pH for enzyme immobilization. The data are presented as mean ± SD; $n = 2$; a significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

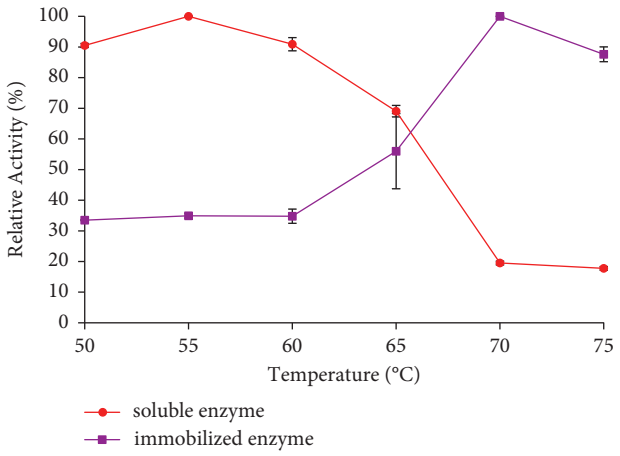

Figure 3: Optimum temperature of the soluble and immobilized enzymes. The data are presented as mean ± SD; $n = 2$; a significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

the bentonite matrix, physical adsorption occurs; hence, the enzyme molecules do not have direct interaction with the substrate [23, 26, 27]. The enzymatic molecules that were entrapped on the matrix react with the new substrate until exhausted. Therefore, the reuse of immobilized enzyme led

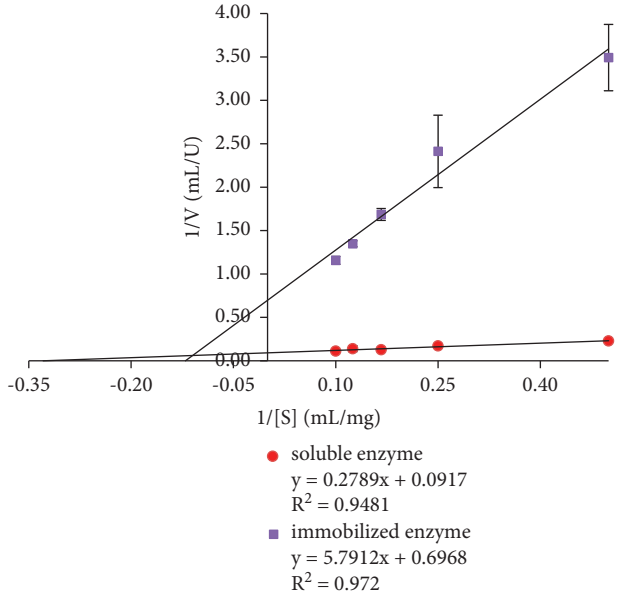

Figure 4: Lineweaver–Burk plot analysis of the enzymatic kinetics of soluble and immobilized enzyme. The values were presented as mean ± SD; $n = 2$; a significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates.

to the significant ($p < 0.05$) decrease of its activity due to repeated washing. The reuse cycle of immobilized enzyme is shown in Figure 5, and the result showed that the residual activity after six cycles of reuse was 42%.

3.6. Determination of Thermal Stability. The thermal stability of soluble and immobilized enzymes is shown in Figure 6 where the soluble enzyme retained 29% of the residual activity after inactivation at 60 C for 80 min. Nevertheless, the residual activity of the immobilized enzyme was higher than the soluble, which has a retainment of up to 56%. The immobilized enzyme molecules were protected by the matrix from the effect of extreme temperature. As a result of this, the immobilized enzyme has higher thermal stability than the soluble. After immobilization, the immobilized enzyme showed a significant ($p < 0.05$) increase in thermal stability compared to the soluble enzyme.

3.7. Determination of $t_{1/2}$, k_i, and ΔG_i. The residual activities of soluble and immobilized enzymes from the determination of thermal stability were plotted to the first-order enzyme inactivation rate graph, as shown in Figure 7. The slope of

Table 2: K_M and V_{max} values for soluble and immobilized enzymes. The kinetic parameters were determined at each optimum temperature and the starch concentrations from 2.0 to 10.0 mg/mL. The values were shown as mean ± SD, $n = 2$.

Sample	V_{max} (μmole mL^{-1} min^{-1})	K_M (mg mL^{-1} substrate)
Soluble enzyme	10.90 ± 1.894	3.04 ± 1.043
Immobilized enzyme	1.44 ± 0.299	8.31 ± 2.666

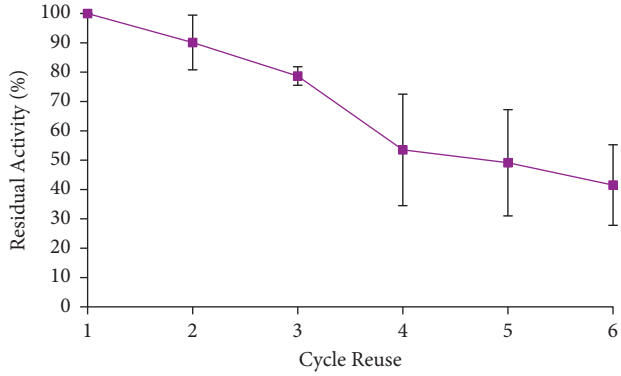

Figure 5: Reuse cycle of the immobilized enzyme. Assume the initial enzyme activity was 100%. The data are presented as mean ± SD; $n = 2$; a significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

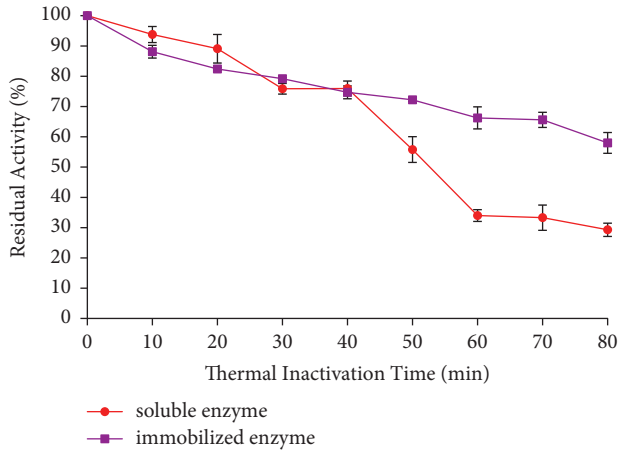

Figure 6: Thermal stability of the soluble and immobilized enzymes. The data were determined at each optimum temperature and each optimum starch concentration. Assume the initial enzyme activity was 100%. The data are presented as mean ± SD; $n = 2$, a significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

graph $\ln(E_i/E_o)$ against t_i is expressed as the value of k_i. The results in Figure 7 show that the k_i values of soluble and immobilized enzymes were 0.0171 and 0.0060 min^{-1}, respectively. The decrease in the k_i value of immobilized enzyme leads to the decrease of denaturation rate which is due to the flexibility of the enzyme in water. Hence, the folding conformation in the immobilized enzyme structure increase for higher stability [21]. The thermal inactivation rate constant (k_i) was used to calculate $t_{1/2}$ and ΔG_i values, which are given in Table 3.

From Table 3, there is an observed significant ($p < 0.05$) increase in the half-life ($t_{1/2}$) and ΔG_i of immobilized enzyme compared to the soluble enzyme. The half-life ($t_{1/2}$) is the time required for the enzyme to break down the substrate until the enzyme loses half of its activity [23]. These results showed that the immobilized enzyme takes a longer time to be converted into the enzyme-substrate complex after which it loses its activity. In addition, the immobilized enzyme has higher ΔG_i which showed the increase of folding conformation in the enzyme structure. The increase in ΔG_i caused a more rigid and less flexible enzyme conformation in water. Hence, the energy required for denaturation becomes higher [21]. Based on the increase of half-life, the stability of the immobilized enzyme also increased approximately three-fold compared to the soluble. This study showed better results than the previous [10].

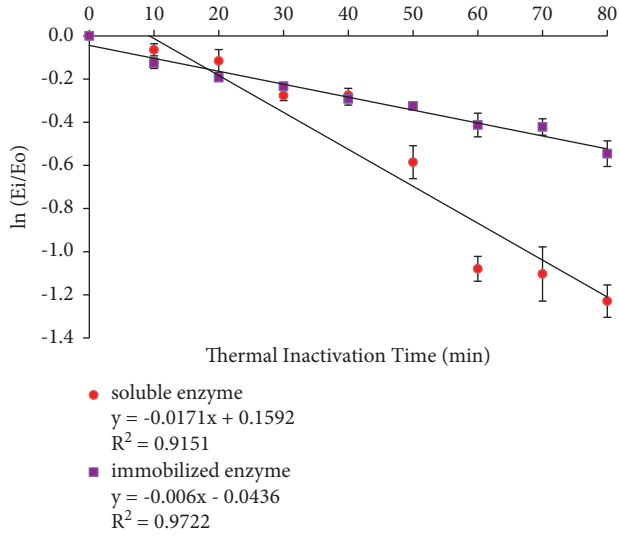

Figure 7: First-order inactivation rate plot of soluble and immobilized enzymes. The data are presented as mean ± SD; $n = 2$, significant difference = $p < 0.05$. The error bars represent standard deviations from two replicates measurements.

Table 3: k_i, ΔG_i, and $t_{1/2}$ values for soluble and immobilized enzymes. The values are shown as mean ± SD, $n = 2$.

Sample	k_i (min^{-1})	$t_{1/2}$ (min)	ΔG_i (kJ $mole^{-1}$)	Stability improvement
Soluble enzyme	0.0171 ± 0.0008	40.53 ± 1.9001	104.47 ± 0.1296	1
Immobilized enzyme	0.0060 ± 0.0003	115.50 ± 5.7895	107.37 ± 0.1386	2.9

4. Conclusions

The stability of α-amylase enzyme from *A. fumigatus* had been improved by the immobilization method on a bentonite matrix. The increase in $t_{1/2}$ and ΔG_i showed that the immobilized enzyme was more stable than the soluble. Furthermore, the improvement in the stability of α-amylase enzyme by immobilization on a bentonite matrix based on increasing of its half-life, was three-fold higher than the soluble enzyme, while the residual activity of the immobilized enzyme after six cycles of reuse was 42%.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] B. Elyasi Far, Y. Ahmadi, A. Yari Khosroshahi, and A. Dilmaghani, "Microbial alpha-amylase production: progress, challenges and perspectives," *Advanced Pharmaceutical Bulletin*, vol. 10, no. 3, pp. 350–358, 2020.

[2] A. Vogel and O. May, *Industrial Enzyme Applications*, Wiley-VCH Verlag GmbH and Co., Weinheim, Germany, 2019.

[3] B. Parameswaran, S. Varjani, and S. Raveendran, *Green Bio-Processes, Energy, Environment, and Sustainability*, Springer Nature Singapore Pvt Ltd, Singapore, 2019.

[4] B. A. Shirley, P. Stanssens, U. Hahn, and C. N. Pace, "Contribution of hydrogen bonding to the conformational stability of ribonuclease T1," *Biochemistry*, vol. 31, no. 3, pp. 725–732, 1992.

[5] J. Fitter, "Structural and dynamical features contributing to thermostability in α-amylases," *Cellular and Molecular Life Sciences*, vol. 62, no. 17, pp. 1925–1937, 2005.

[6] G. Brahmachari, A. L. Demain, and J. L. Adrio, *Biotechnology of Microbial Enzymes*, Elsevier, London, UK, 2017.

[7] M. D. Trevan, *Immobilized Enzymes, an Introduction and Application in Biotechnology*, John Wiley and Sons, Inc., New York, NY, USA, 1980.

[8] M. Asgari and U. Sundararaj, "Silane functionalization of sodium montmorillonite nanoclay: the effect of dispersing media on intercalation and chemical grafting," *Applied Clay Science*, vol. 153, pp. 228–238, 2018.

[9] M. Ghiaci, H. Aghaei, S. Soleimanian, and M. E. Sedaghat, "Enzyme immobilization Part 1. Modified bentonite as a new and efficient support for immobilization of Candida rugosa lipase," *Applied Clay Science*, vol. 43, no. 3-4, pp. 289–295, 2009.

[10] Yandri, T. Suhartati, S. D. Yuwono, H. I. Qudus, E. R. Tiarsa, and S. Hadi, "Immobilization of α-amylase from *Bacillus subtilis* ITBCCB148 using bentonite," *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, vol. 20, no. 2, pp. 487–492, 2018.

[11] F. L. van de Veerdonk, M. S. Gresnigt, L. Romani, M. G. Netea, and J.-P. Latgé, "*Aspergillus fumigatus* morphology and dynamic host interactions," *Nature Reviews Microbiology*, vol. 15, no. 11, pp. 661–674, 2017.

[12] T. Yandri and S. H. Suhartati, "Purification and characterization of the extracellular α-amylase enzyme from locale bacteria isolate *Bacillus subtilis* ITBCCB148," *European Journal of Scientific Research*, vol. 39, pp. 64–74, 2010.

[13] D. M. Bollag, M. D. Rozycki, and S. J. Edelstein, *Protein Methods*, John Wiley and Sons, Inc., New York, NY, USA, 2nd edition, 1996.

[14] H. Fuwa, "A new method for microdetermination of amylase activity by the use of amylose as the substrate," *The Journal of Biochemistry*, vol. 41, no. 5, pp. 583–603, 1954.

[15] D. E. Eveleigh, M. Mandels, R. Andreotti, and C. Roche, "Measurement of saccharifying cellulose," *Biotechnology for Biofuels*, vol. 2, no. 21, pp. 1–8, 2009.

[16] O. Lowry, N. Rosebrough, A. L. Farr, and R. Randall, "Protein measurement with the folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[17] P. K. Robinson, "Enzymes: principles and biotechnological applications," *Essays in Biochemistry*, vol. 59, pp. 1–41, 2015.

[18] N. E. Ahmed, A. R. El Shamy, and H. M. Awad, "Optimization and immobilization of amylase produced by *Aspergillus terreus* using pomegranate peel waste," *Bulletin of the National Research Centre*, vol. 44, no. 109, pp. 1–12, 2020.

[19] A. S. Santos, N. Rosa, M. Souza, H. K. Philippsen, and E. Medeiros, "Thermal-stability of enzyme activity and its application in the hydrolysis of starchy residue from *Mandioca* processing," *International Journal of Science and Research*, vol. 4, no. 10, pp. 2277–8179, 2015.

[20] Z. Yang, M. Domach, R. Auger, F. X. Yang, and A. J. Russell, "Polyethylene glycol-induced stabilization of subtilisin," *Enzyme and Microbial Technology*, vol. 18, no. 2, pp. 82–89, 1996.

[21] D. Kazan, H. Ertan, and A. Erarslan, "Stabilization of *Escherichia coli* Penicillin G Acylase against thermal Inactivation by cross-linking with dextran dialdehyde polymers," *Applied Microbiology and Biotechnology*, vol. 48, no. 2, pp. 191–197, 1997.

[22] S. Stahl, *Thermophilic Microorganisms: The Biological Background for Thermophily and Thermoresistance of Enzymes in Thermostability of Enzymes*, Springer, New York, NY, USA, 1999.

[23] Z. Baysal, Y. Bulut, M. Yavuz, and Ç. Aytekin, "Immobilization of α-amylase via adsorption onto bentonite/chitosan composite: determination of equilibrium, kinetics, and thermodynamic parameters," *Starch - Stärke*, vol. 66, no. 5-6, pp. 484–490, 2014.

[24] H. M. Abdel-Mageed, H. M. El-Laithy, L. G. Mahran, A. S. Fahmy, K. Mader, and S. A. Mohamed, "Development of novel flexible sugar ester vesicles as carrier systems for the antioxidant enzyme catalase for wound healing applications," *Process Biochemistry*, vol. 47, pp. 1152–1162, 2012.

[25] M. E. Sedaghat, M. Ghiaci, H. Aghaei, and S. Soleimanian, "Enzyme immobilization. Part 3: immobilization of α-amylase on Na-bentonite and modified bentonite," *Applied Clay Science*, vol. 46, no. 1, pp. 125–130, 2009.

[26] J. Zdarta, A. S. Meyer, T. Jesionowski, and M. Pinelo, "A general overview of support materials for enzyme immobilization: characteristics, properties, practical utility," *Catalyst*, vol. 8, no. 92, pp. 1–27, 2018.

[27] Y. Q. Almulaiky, R. M. El-Shishtawy, M. Aldhahri et al., "Amidrazone modified acrylic fabric activated with cyanuric chloride: a novel and efficient support for horseradish peroxidase immobilization and phenol removal," *International Journal of Biological Macromolecules*, vol. 140, pp. 949–958, 2019.

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