Supporting Information (BCREC-20874)

Immobilization of Purified α-Amylase from *Aspergillus fumigatus* on a Chitosan–Silica Gel 60 Hybrid: Enhanced Thermal Stability and Reusability

Santalum Alba, Yandri Yandri*, Tati Suhartati, Bambang Irawan, Stevenson Waruwu
*Corresponding author. E-mail: yandri.as@fmipa.unila.ac.id

DOI: https://doi.org/10.9767/bcrec.20874

This file provides the calculation of thermal inactivation parameters, the FT-IR band shifts on hybrid formation, and a summary of the assay conditions that support the main manuscript. All values are taken from, or recalculated from, the data reported in the main manuscript.

S1. Calculation of Thermal Inactivation Parameters

The first-order inactivation rate constant (k_i) was obtained from the slope of $\ln(E_i/E_0)$ versus time at 60 °C. The half-life and the free energy change of inactivation were then calculated using Equations (S1) and (S2).

$$t_{1/2} = \ln 2 / k_i \quad \text{(S1)}$$

$$\Delta G_i = -RT \ln(k_i h / k_B T) \quad \text{(S2)}$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the inactivation temperature (333 K), h is Planck's constant (6.626×10^{-34} J s), k_B is the Boltzmann constant (1.381×10^{-23} J K^{-1}), and k_i is expressed in s^{-1} in Equation (S2). For the enzyme–hybrid, $k_i = 2.657 \times 10^{-3}$ min^{-1} = 4.428×10^{-5} s^{-1}, which gives $t_{1/2}$ = 260.9 min and ΔG_i = 109.62 kJ mol^{-1}. Table S1 compares the values reported in Table 4 of the main manuscript with those recalculated from k_i; the small differences arise from rounding of k_i.

Table S1. Reported and recalculated values of $t_{1/2}$, ΔG_i, and stability increase.

Sample	k_i ($\times 10^{-3}$ min^{-1})	$t_{1/2}$ reported (min)	$t_{1/2}$ recalc. (min)	ΔG_i reported (kJ mol^{-1})	ΔG_i recalc. (kJ mol^{-1})	Stability increase, reported / recalc. (fold)
Purified enzyme	17.36	39.93	39.93	104.43	104.42	1 / 1.00
Enzyme–silica gel 60	3.153	219.81	219.84	109.15	109.14	5.5 / 5.51
Enzyme–chitosan	7.060	98.18	98.18	106.92	106.91	2.5 / 2.46
Enzyme–hybrid	2.657	260.85	260.88	109.62	109.62	6.5 / 6.53

S2. FT-IR Band Shifts on Hybrid Formation

Table S2. Shifts of selected FT-IR bands between the individual components and the chitosan–silica gel 60 hybrid (values from Table 2 of the main manuscript; ≈ marks bands not labeled by the instrument software and read from the spectrum).

Band assignment	Component	Individual component (cm^{-1})	Hybrid (cm^{-1})	Shift (cm^{-1})
Amide I (C=O); C=N in hybrid	Chitosan	1649.27	1632.04	-17.23

Band assignment	Component	Individual component (cm^{-1})	Hybrid (cm^{-1})	Shift (cm^{-1})
N–H bending; $–NH_3^+$ in hybrid	Chitosan	1587.54	1538.74	-48.80
CH_2 bending	Chitosan	1418.17	1426.78	+8.61
Si–O–Si asymmetric stretching	Silica gel 60	1066.50	1056.45	-10.05
Si–OH stretching	Silica gel 60	963.15	971.76	+8.61
Si–O–Si symmetric stretching	Silica gel 60	≈ 799	795.21	≈ −4
O–H and N–H stretching (broad)	Chitosan	≈ 3330	≈ 3320	≈ −10

S3. Summary of Enzyme and Protein Assays

Table S3. Assay conditions used in this study (compiled from Sections 2.4 and 2.6 of the main manuscript).

Assay	Purpose	Conditions	Detection
Fuwa (iodine–starch)	Unit activity during production and purification	0.25 mL enzyme + 0.25 mL 0.1% starch, 60 °C, 10 min; stopped with 1 N HCl	600 nm
Mandels (DNS)	Unit activity during characterization (reducing sugar)	0.25 mL enzyme + 0.25 mL 0.1% starch, 60 °C, 30 min; immobilized enzyme: 0.75 mL substrate at the optimum temperature, 30 min	510 nm; glucose standard curve
Lowry	Protein content	BSA standard 20–140 ppm; alkaline copper reagent, then Folin–Ciocalteu reagent	750 nm