

Method S1: Laccase Activity Data and Method Description

Method:

1 mg of Laccase F was dispersed in 1 mL of water. A 1 µL aliquot of the supernatant was added to 1.999 mL of an ammonium acetate-buffer solution (10 mM, pH 4) and measured as a blank at 420 nm using a V-750 UV/Vis Spectrometer (Jasco, Pfungstadt, Germany). For activity determination, 1.999 mL of ABTS solution (1 mM; in the same buffer) was treated similarly and continuously measured over the course of 3 minutes. The measurements were performed in triplicate, and the absorbance-to-time ratio (ΔA) was calculated by the acquisition software over a time window of 100-200 seconds. The molar extinction coefficient (ϵ) of $3.6 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$, as reported by Alam et al.¹, was used as an approximation, given the lack of data for ammonium acetate buffered solution at pH 4.

Calculation Formula:

The enzyme activity in a mL of enzyme solution (U/mL) was calculated using the formula (S1):

$$(S1) \quad \frac{U}{mL} = \Delta A \cdot \frac{V_t}{\epsilon \cdot d \cdot V_s} \cdot 10^6$$

with:

- ΔA : Change in absorbance per minute (A/min)
- V_t : Total reaction volume (L)
- ϵ : Molar extinction coefficient (L/mol/cm)
- d : Path length of the cuvette (cm)
- V_s : Enzyme solution volume (mL)
- 10^6 : Conversion factor for volumes

Experimental Parameters:

- Wavelength: 420 nm
- Molar Extinction Coefficient (ϵ): $3.60 \times 10^4 \text{ L/mol/cm}$
- Cuvette Width (d): 1 cm

Konzentration ABTS (mg/mL)	Enzyme Solution Volume (mL)	Total Reaction Volume (L)	ΔA (A/min)	Time Interval (s)	Initial Reaction Velocity (mol/L/s)	U/µL Enzyme Solution	U/mL Enzyme Solution
0.54	0.001	0.002	0.2936	100-200	8.15556×10^{-6}	0.0163	16.31
0.54	0.001	0.002	0.2805	100-200	7.79167×10^{-6}	0.0156	15.58
0.54	0.001	0.002	0.3698	100-200	1.02722×10^{-5}	0.0205	20.54
Mean		17.48 U/mL;					
Standard Dev.		2.68 U/mL					

¹ Alam, R.; Ardiati, F. C.; Solihat, N. N.; Alam, M. B.; Lee, S. H.; Yanto, D. H. Y.; Watanabe, T.; Kim, S. Biodegradation and Metabolic Pathway of Anthraquinone Dyes by *Trametes Hirsuta* D7 Immobilized in Light Expanded Clay Aggregate and Cytotoxicity Assessment. *J. Hazard. Mater.* **2021**, *405* (October 2020), 124176. <https://doi.org/10.1016/j.jhazmat.2020.124176>.