Supporting Information for

A two-enzyme system in an amorphous metal−organic framework for the synthesis of D-phenyllactic acid

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Experimental Procedures

S1: Expression and purification of D-lactate dehydrogenase and glucose dehydrogenase

Based on the previous research[1], our laboratory (Taizhou Key Laboratory of Biomass Functional Materials Development and Application) preserved *E. coli*/D-lactate dehydrogenase(D-LDH, abbreviated as LDH) engineering bacteria and *E. coli*/glucose dehydrogenase (GDH) engineering bacteria. The steps for the expression and purification of two-enzyme are as follows.

The expression strain was grown in Luria–Bertani (LB) medium containing 50 $\mu g \cdot mL^{-1}$ kanamycin at 180 rpm and 37 ℃ for 10 h. Then, the pre-culture was transferred to a fresh LB/kanamycin medium for further growth at 37 ℃ and 180 rpm. After 2 h, lactose with a final concentration of 10 $g \cdot L^{-1}$ was added to induce expression, and the culture was continued for 12 h at 28 ℃ and 150 rpm. Cells were collected by centrifugation at 6300 ×*g* and 4 ℃ for 10 min, and crude enzyme solution was obtained from lysed cells.

The crude enzyme was loaded onto a Ni^{2+}-nitrilotriacetic acid column (1.6 cm×10 cm, BioRad, USA) pre-equilibrated with Buffer A (20 mM potassium phosphate buffer, pH 8.0, 500 mM NaCl and 20 mM imidazole). Buffer B (20 mM potassium phosphate buffer pH 8.0, 500 mM NaCl and 500 mM imidazole) was used to elute the enzyme at a flow rate of 1.0 $mL \cdot min^{-1}$. The purified enzyme was collected and dialyzed overnight against 20 mM potassium phosphate buffer (pH 8.0) to remove salt ions and imidazole.

S2: Characterization of LDH/GDH-ZIFs biocomposites

Powder X-ray Diffraction (XRD): XRD patterns were obtained using a Bruker D8 Advance X-ray diffractometer with Kα radiation (λ = 1.5405 Å) from a Cu anode. The scan speed was 10 °·min^{-1}, with a 0.02 ° step, and the diffraction data were collected over a range of 2θ = 5-40 °.

Thermogravimetric Analysis (TGA): TGA data were collected using a TA instruments synchronous thermal analyzer (STA) (TGA/DSC). A sample of approximately 5 mg was placed on a ceramic pan and heated from 30 to 800 ℃ at a rate of 5 ℃·min^{-1}. Each sample was heated in a constant flow of air.

Fourier transform infrared (FT-IR) spectroscopy: FT-IR spectra were recorded on a Nicolet 5700 FTIR spectrometer using samples of approximately 1 mg. Thirty-two scans were recorded over the range of 4000−650 cm^{-1}.

Scanning Electron Microscope (SEM)/Energy Dispersive Spectrometer (EDS): SEM images were recorded using a Hitachi S-4800 field-emission scanning electron microscope (FESEM). The elemental composition was determined using an energy-dispersive spectrometer (EDS).

Gas Sorption: Gas adsorption isotherms were recorded on an ASAP-2020-HD88 surface characterization analyzer. Samples comprising approximately 30 mg were placed into a glass analysis tube and degassed under a dynamic vacuum for 12 h at 105 °C before measurement. Nitrogen (N_2) adsorption and desorption isotherms were measured at 277 K.

X-Ray photoelectron spectroscopy (XPS): XPS analysis was performed using Thermo Fisher Scientific K-Alpha, Al Kα radiation (hv=1253.6 eV).

Circular dichroism spectra (CDs): CD data was obtained by Applied Photophysics Chirascan to evaluate the possible secondary structure changes of the enzyme. The LDH-aZIF-90, GDH-aZIF-90, LDH/GDH-aZIF-90, free LDH, free GDH, and free LDH/GDH were incubated in the same buffer. CD spectra were measured on a CD spectrophotometer at room temperature using a quartz cuvette with a path length of 1 mm and recorded in the range 190-250 nm in steps of 0.5 nm.

Confocal Laser Scanning Microscopy (CLSM): LDH and GDH were labeled with fluorescein isothiocyanate (FITC) and rhodamine B, respectively. The distribution of both enzymes in amorphous ZIF-90 was characterized by CLSM (Olympus, FV3000).

S3: Determination of enzyme kinetic parameters

The reaction mixture of 1.2 mL containing NAD^+, PPA, Glu and enzyme solution or NADH, PPA and enzyme solution was dissolved in 20 mM Tris-HCl buffer (pH=7). After incubation at 30 °C for 1 min, the amount of D-PLA was determined by HPLC. One enzyme activity unit (U) was defined as the amount of enzyme producing 1 μM D-PLA per minute. Enzyme activity was measured at different substrate concentrations (PPA, 2-10 mM;) and the data were fitted to the Michaelis-Menten equation.

Figures and Tables

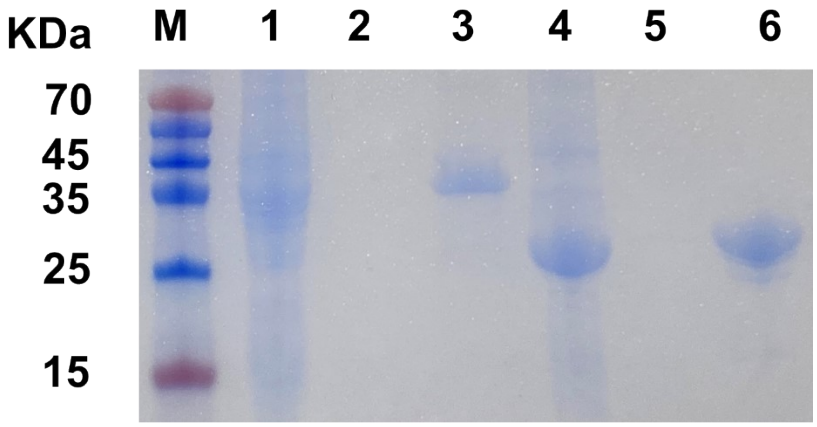

Fig. S1: SDS-PAGE analysis. M: protein molecular weight marker; lane 1 and 4: soluble fractions from lactose induced *E. coli*/LDH and *E.coli*/GDH, respectively; lane 2 and 5: precipitates from lactose induced *E. coli*/LDH and *E.coli*/GDH, respectively; lane 3 and 6: the purified LDH and GDH, respectively.

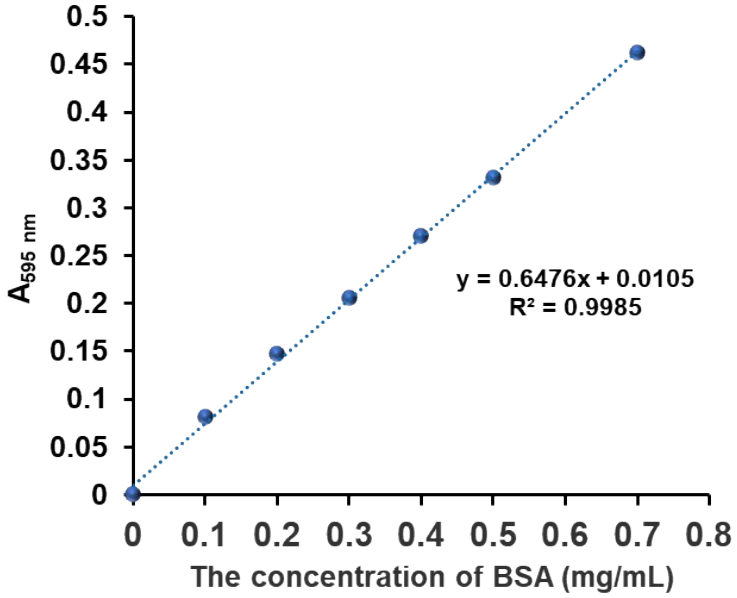

Fig. S2: The calibration curves of BSA for analyzing the encapsulation rate of protein.

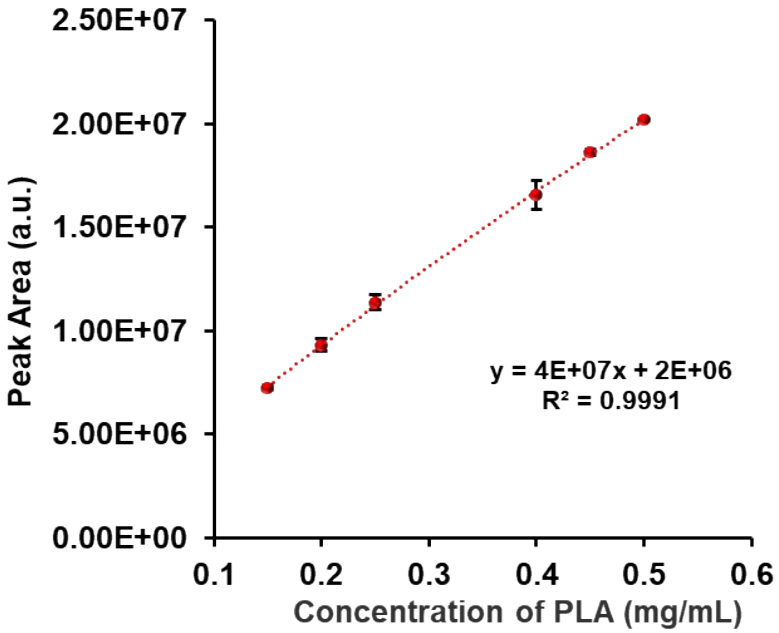

Fig. S3: Standard curve for determination of enzyme activity.

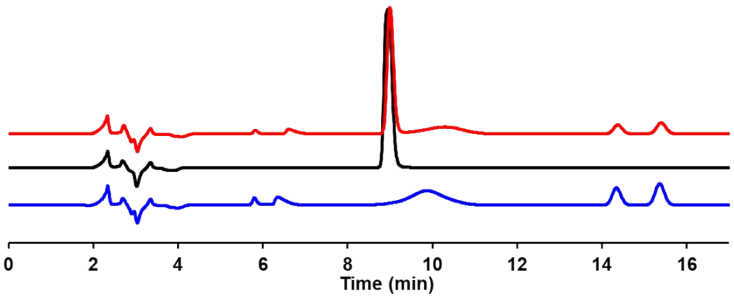

Fig. S4: Chromatographic peaks of the standards (Blue: 1 g/L PPA; Black: 1 g/L PLA; Red: 0.5 g/L PPA-PLA).

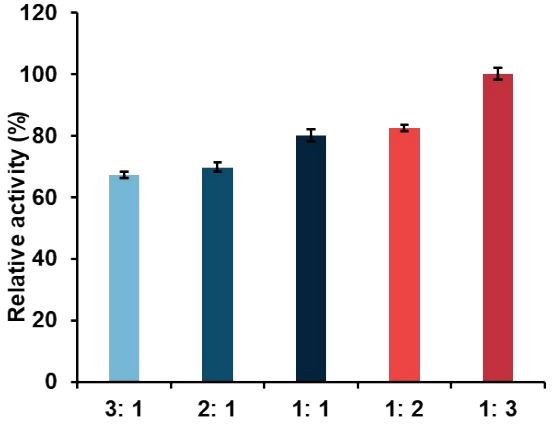

Fig. S5: Relative activity of different LDH: GDH ratios.

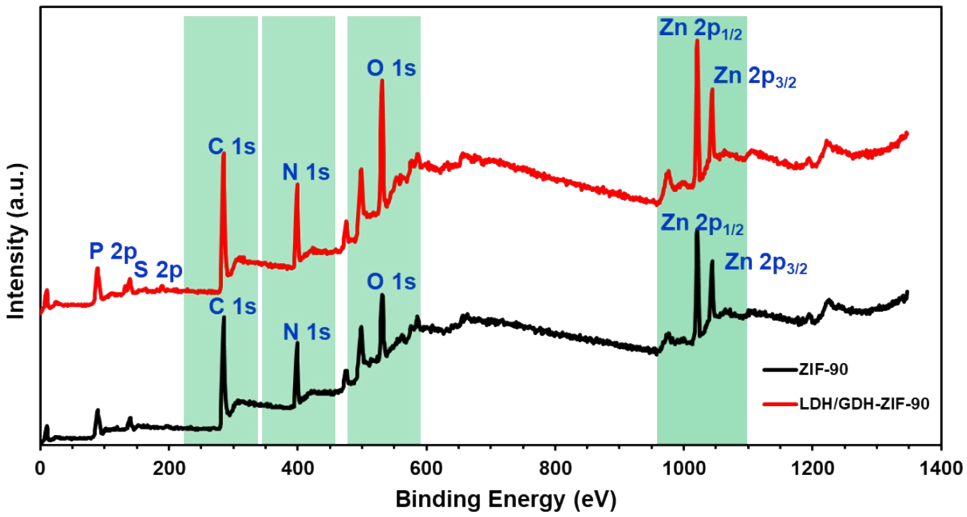

Fig. S6: XPS spectra of ZIF-90 and LDH/GDH-ZIF-90.

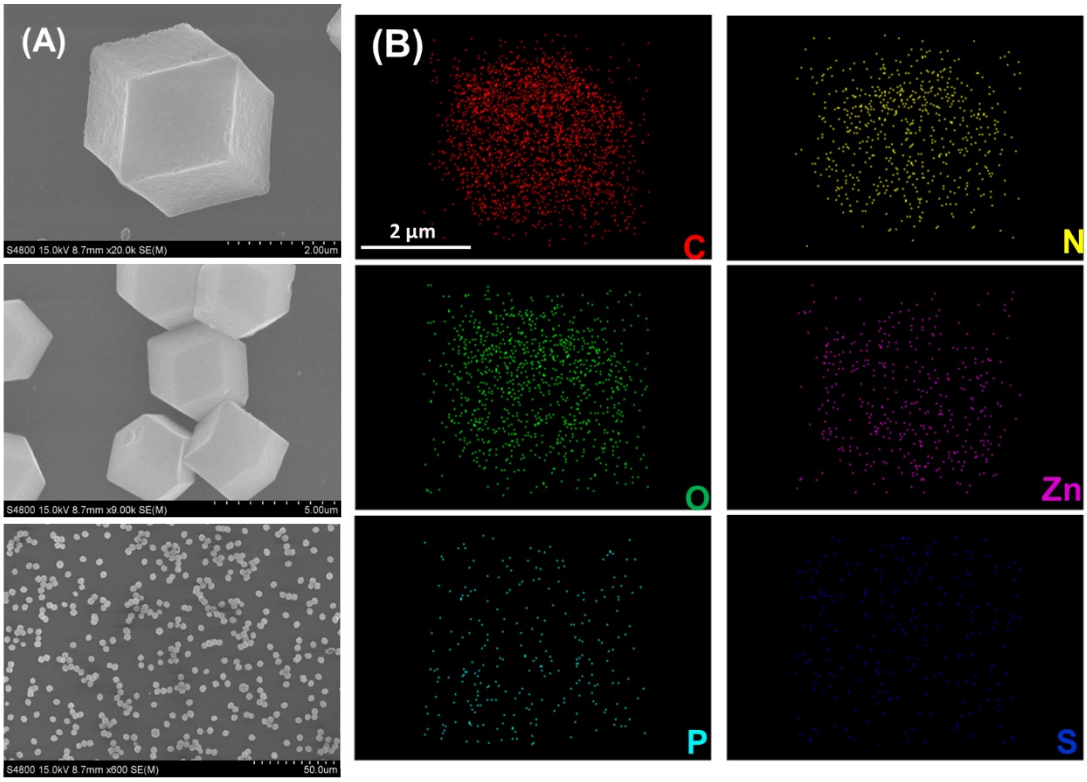

Fig. S7: (A) SEM image of LDH/GDH-ZIF-90 biocatalysts; (B) Elemental Mappings of LDH/GDH-ZIF-90 biocatalysts show the presence of C, N, O, Zn, S and P atoms.

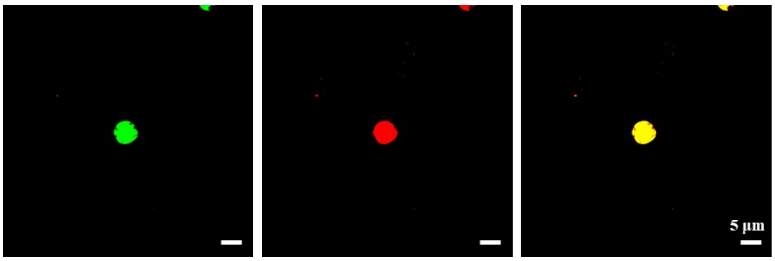

Fig. S8: Images of confocal laser scanning microscope. (A) Distribution of LDH labeled with fluorescein isothiocyanate (FITC) in LDH/GDH-aZIF-90 composites; (B) Distribution of GDH labeled with Rhodamine B in LDH/GDH-aZIF-90 composites; (C) Distribution of two-enzyme in LDH/GDH-aZIF-90 composites.

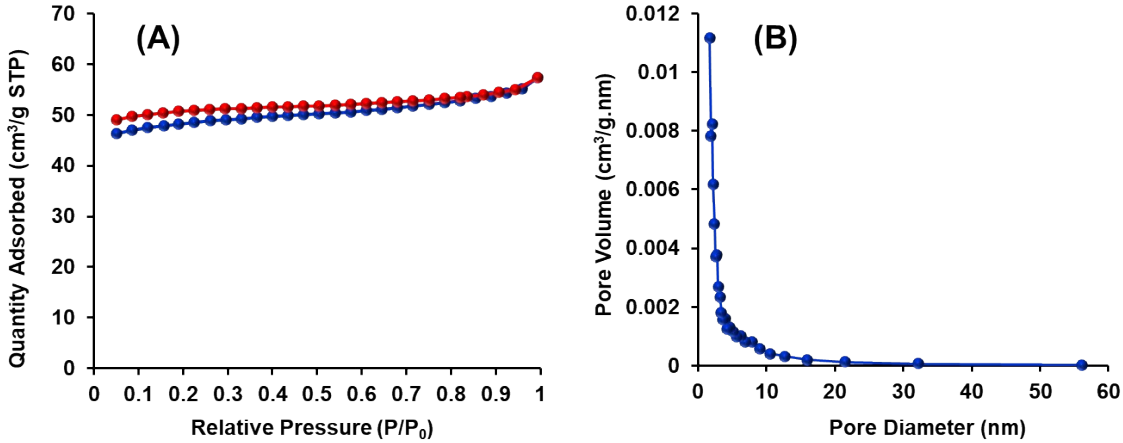

Fig. S9: (A) N_2 adsorption and desorption curves of LDH/GDH-ZIF-90 (Blue: adsorption; Red: Desorption); (D) pore size distribution of LDH/GDH-ZIF-90.

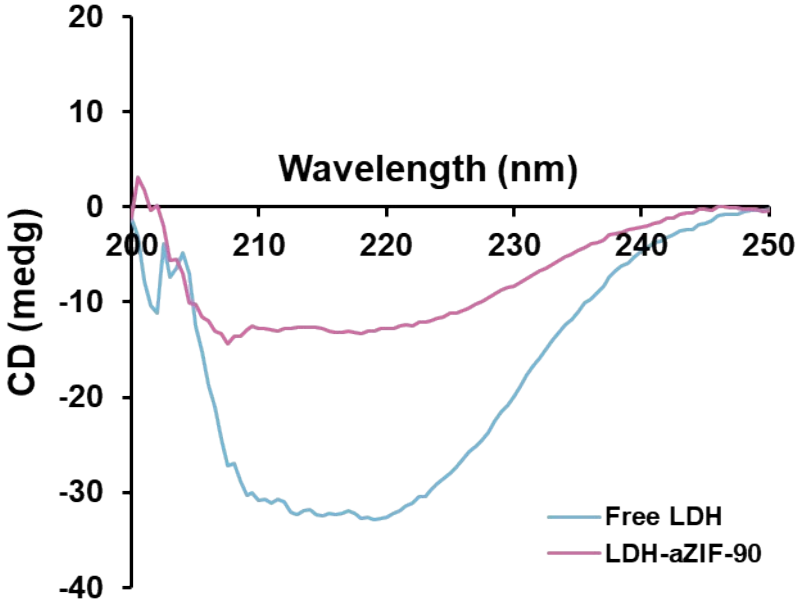

Fig. S10: Circular dichroism (CD) spectra of Free LDH and LDH-aZIF-90.

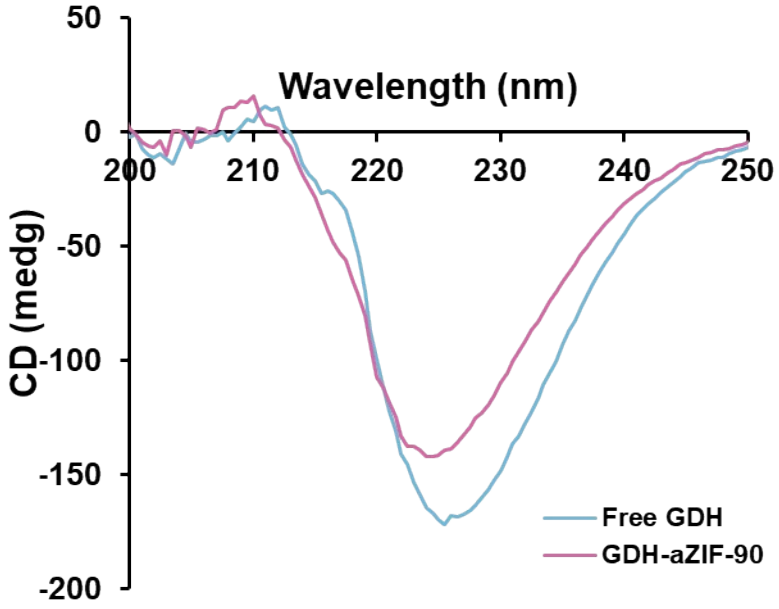

Fig. S11: Circular dichroism (CD) spectra of Free GDH and GDH-aZIF-90.

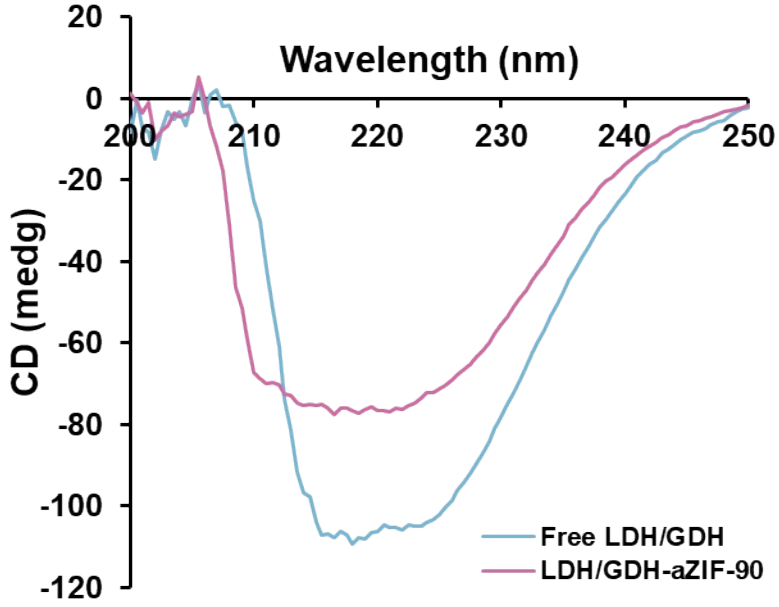

Fig. S12: Circular dichroism (CD) spectra of Free LDH/GDH and LDH/GDH-aZIF-90.

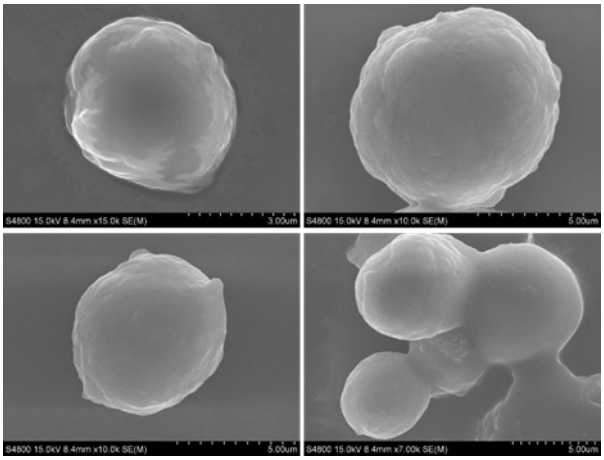

Fig. S13: SEM image of LDH/GDH-aZIF-90 biocatalysts after reusability experiments.

Table S1 LDH/GDH-ZIFs composites formed at different precursor concentrations

Sample	Concentrations of precursors $Zn(NO_3)_2.6H_2O$ (mM)	Concentrations of precursors HICA (mM)
1	40	160
2	40	120
3	40	80
4	40	40
5	40	20
6	30	150
7	30	120
8	30	90
9	30	60
10	30	30
11	30	15

Table S2 HPLC detection parameters

Parameters	Detecting Condition
Chromatographic Column	Hypersil ODS C_{18} (250 mm×4.6 mm, 5 μm)
Mobile Phase	0.1% HCOOH: CH_3CN=3: 1
Flow Rate	1.0 mL/min
Wavelength	210 nm
Injection Volume	20 μL
Column Temperature	40 ℃

Table S3 LDH/GDH-ZIFs composites formed at different ratios of LDH: GDH

Ratio of LDH: GDH	Volume of LDH	Volume of GDH
3: 1	150	50
2: 1	133	67
1: 1	100	100
1: 2	67	133
1: 3	50	150

Table S4 Adsorption average pore width (4V/A by BET) of the LDH/GDH-ZIF-90, aZIF-90 and LDH/GDH-aZIF-90

Sample	Adsorption average pore width (4V/A by BET)
LDH/GDH-ZIF-90	2.2 nm
aZIF-90	18.1 nm
LDH/GDH-aZIF-90	22.3 nm

Table S5 Comparison with other reported LDH/GDH system

strategies	reaction condition	reaction system	specific enzyme activity ($U \cdot mg^{-1}$)	productivity (%)	reference
two-enzyme expression	pH=7, 35 ℃	50 g/L PPA, 6.1 g/L Glu	/	87.6	[1]
two-enzyme expression	pH=7, 42 ℃	50 mM PPA, 100 mM Glu	9.48 ± 0.91	97.83	[2]
two-enzyme expression	pH=5, 40 ℃	100 mM PPA, 120 mM Glu, 0.1mM NAD^+	447.6	90.0	[3]
Two-enzyme immobilization	pH=7, 30 ℃	10 mM PPA, 10 mM Glu,	1.77±0.19	99.0%±5.6%	This work

10 mM

NAD^+

Table S6 Enzyme kinetic parameter

parameters	**K_m(mM)**	**V_{max}(U·mg^{-1})**	**k_{cat}(s^{-1})**	**k_{cat}/K_m($mM^{-1}·s^{-1}$)**
LDH	3.85	2.15	1.67	0.43
LDH-aZIF-90	4.12	2.07	1.66	0.40
LDH/GDH	4.70	2.60	2.08	0.44
LDH/GDH-aZIF-90	4.59	2.57	2.06	0.45

References

1 X. Luo, Y. Zhang, L. Yin, W. Zheng and Y. Fu, *3 Biotech*, 2020, **10**, 14.

2 Y. Zhu, Y. Wang, J. Xu, J. Chen, L. Wang and B. Qi, *Molecules*, 2017, **22**, 1966.

3 D. Zhang, T. Zhang, Y. Lei, W. Lin, X. Chen and M. Wu, *Front. Bioeng. Biotechnol.*, 2022, **10**, 846489.