Supporting Information

Improving laccase activity and stability by HKUST-1 with cofactor via one-pot encapsulation and its application for degradation of bisphenol A

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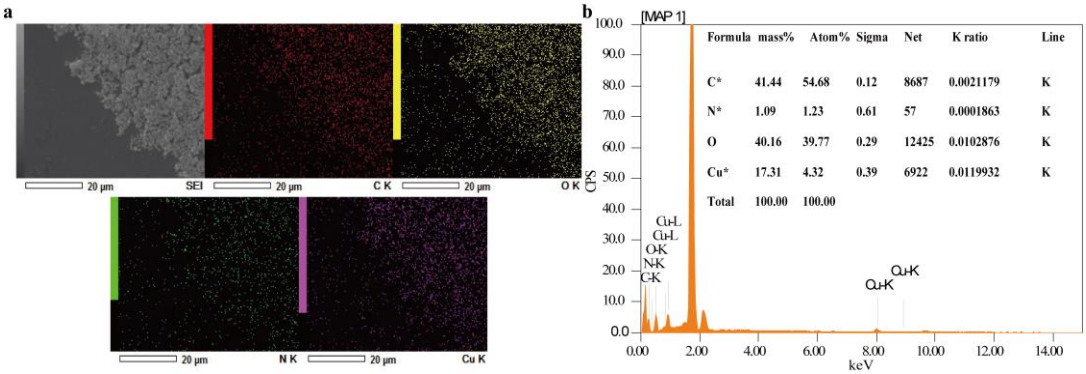

Fig. S1. (a) EDS picture and (b) EDS analysis of laccase@HKUST-1 from select inside SEM image.

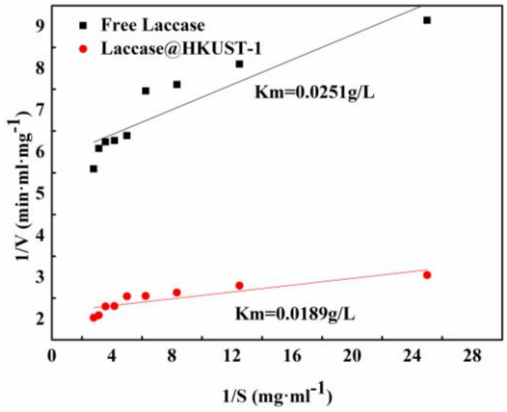

Fig. S2 Lineweaver-Burk plots of the free laccase and laccase@HKUST-1.

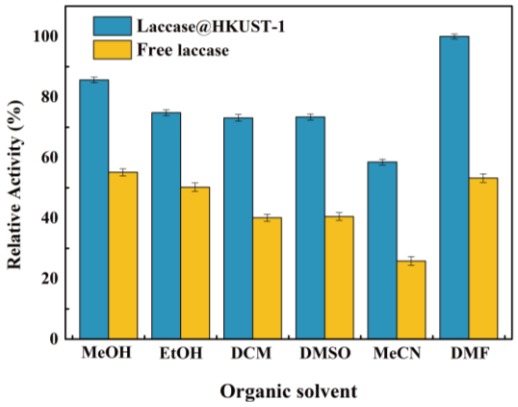

Fig. S3 Effects of organic solvent on the activity of free laccase and laccase@HKUST-1.

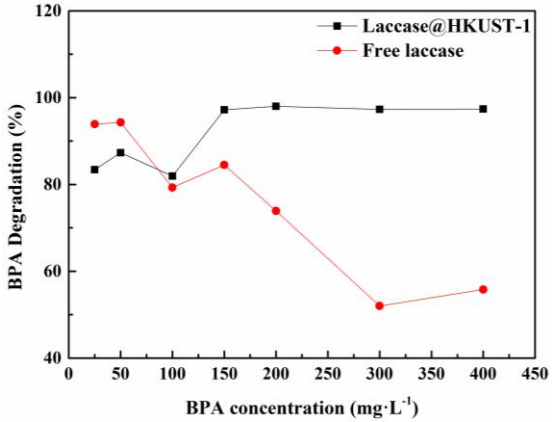

Fig. S4 The effects of BPA initial concentration on its degradation efficiency by free and immobilized laccase

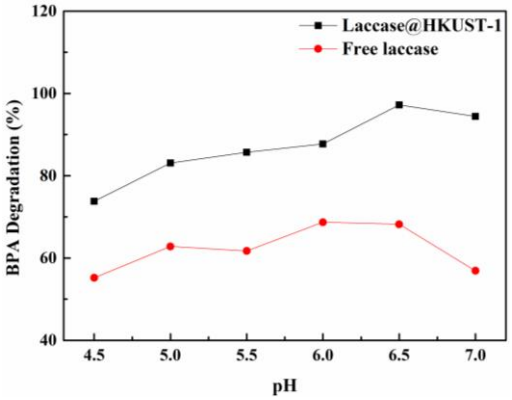

Fig. S5 The effects of solution pH on the degradation efficiency of free and immobilized laccase for BPA

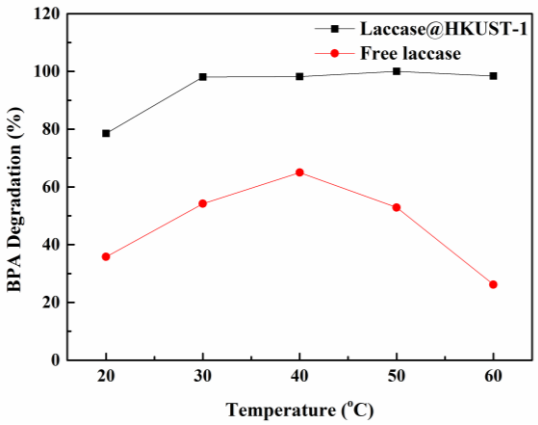

Fig. S6 The effects of reaction temperature on the degradation efficiency of free and immobilized laccase for BPA

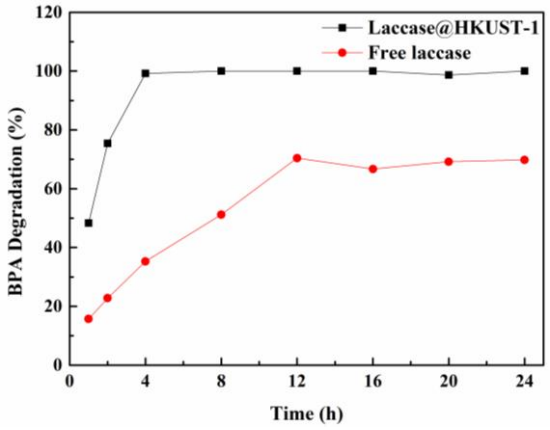

Fig. S7 The effects of reaction time on the degradation efficiency of free and immobilized laccase for BPA

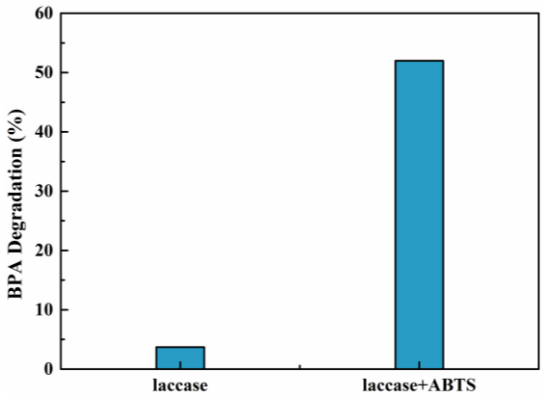

Fig. S8 The effect of ABTS on laccase degradation BPA system

Table S1. Effects of different concentrations of metal ions and organic acids on the encapsulation efficiency.

Cu^{2+}(mM) / BTC(mM)	62.5	75	87.5	100
25	49.4±0.9%	50±1.2%	54±1.1%	44.7±1.2%
12.5	50±1.1%	52.7±0.8%	49.9±1.2%	46.5±1.1%
6.25	52±1.3%	50±1.2%	50.5±0.8%	49.6±1.1%

Table S2. Effects of different concentrations of metal ions and organic acids on the retaining activity of immobilized enzyme.

Cu^{2+}(mM) / BTC(mM)	62.5	75	87.5	100
25	111.7±1.7%	128.9±1.6%	150.1±2.1%	103±1.8%
12.5	100.6±1.5%	128.6±1.9%	112±1.7%	107.8±2.0%
6.25	114±2.0%	110.2±1.8%	108±0.9%	106.7±1.7%

Preparation of HKUST-1

The preparation of the HKUT-1 was based on the method reported earlier with slight modification. Cupric acetate monohydrate (87.5 mmol) and 1,3,5-benzenetricarboxylicacid (BTC) (25 mmol) were dissolved in NaAc-HAc buffer solution (200 mM, pH 7，1 L), respectively. Then 0.25 ml BTC solution was added into 0.25 ml cupric acetate monohydrate solution, and incubated under 30 ^{o}C for 8 h. Subsequently, the product was obtained by centrifuging and washed three times with deionized water. Eventually, Dried in vacuum freeze dryer for 12 h and saved at 4 ^{o}C.

Fabrication of laccase@HKUST-1

Laccase@HKUST-1 biocomposite was fabricated by using the biomimetic mineralization method. The method, which was based on previously reported, was following. Mixture A was prepared by adding cupric acetate monohydrate solution (87.5 mM, 250 μL) into the solution of laccase (2 mg, 20 μl). Solution B was obtained by dissolving BTC (25 mM, 250 μL) in the acetate buffer saline solution (pH 7.0, 200 mM). Then solution A and solution B were mixed and incubated under 30 ^{o}C for 8 h for the formation of HKUST-1 protective coatings. Then the solids were separated by centrifugation at 6000 rpm for 10 min. The solids were washed three times by deionized water. Eventually, Dried in vacuum freeze dryer for 12 h and saved at 4 ^{o}C.

Activity assay

The activity of free laccase was determined by the principle of laccase oxidation ABTS. Formation of the cation radical was monitored at 420 nm (ε_{420} = 36.0 mM^{-1} cm^{-1}) at 40 ^{o}C. In terms of free laccase, the reaction mixture (0.5 ml) contained 0.005 mg of free laccase. So, for the immobilized laccase containing 0.005 mg free laccase was incubated in 0.5 ml ABTS solution at 40 ^{o}C. Prior to the UV test, the suspended solid was precipitated by centrifugation. In this work, one enzyme activity unit (U) was defined as the amount of enzyme needed to oxidize 1 μmol of ABTS per minute. The activity was calculated according to the following equation:

$$U = \frac{\Delta A \times V \times 10^{6}}{\varepsilon \times L \times t} \times 100\%$$

In this work, ΔA = the variation of absorbance before and after of the reaction, V (L)= the volume of liquid in cuvette , ε = the extinction coefficient, L (cm)= the length of cuvette's optical path and t (min)= the reaction time.

Characterization of laccase@HKUST-1

The morphologies and detailed structure of HKUST-1 and laccase@HKUST-1 biocomposite were recorded by JSM-6010 PLUS/LA scanning electron microscope (SEM) and FEI Tecnai G2 S-TWIN F20 transmission electron microscope (TEM). The powder X-ray diffraction (XRD) measurements were collected by using X-ray diffractometer (XRD-6100Lab, Japan) with scattering angles (2θ) of 10-80 ° at a scan

rate of 2 °/min. Fourier-transform infrared spectra (FT-IR) were obtained on a Nicolet Nexus 470 Spectrometer in the range of 400-4000 cm^{-1} by using KBr pellets. Thermogravimetric analysis (TGA) was carried out on a STA449C Thermal Analyzer.

Tolerance of organic solvents

Free laccase and laccase@HKUST-1 were incubated in 30 % (v/v) organic solvent and deionized water for 1 h, in which methyl cyanide (MeCN), methanol (MeOH), dichloromethane (DCM), dimethyl sulfoxide (DMSO), Dimethyl Formamide (DMF) and ethanol (EtOH) was used as organic solvent. The tolerance of laccase and laccase@HKUST-1 to organic solvents were tested by comparing their residual activity.

Storage stability

The storage stability of free and immobilized laccase at 4 ^{o}C were examined by comparing their residual activity. Finally, set the initial activity (first day activity) as 100% activity.

Operational stability

The reusability of the laccase@HKUST-1 was assessed by recycling 10 times. After reaction, the laccase@HKUST-1 was separated by centrifuging, washed with deionized water, and reused for next reaction. The reaction was repeated for 10 times and set the initial activity as 100 % activity.

BPA removal experiments and BPA detection

We investigated the effects of BPA concentrations (25 – 400 mg/L), reaction pH (4.5 – 7), reaction temperature (20 – 60 ^{o}C) and reaction time (1 – 24 h) on the degradation of BPA. The method as following, laccase and laccase@HKUAT-1 containing the same mass of enzyme (approximately contained 0.2 g laccase) were added to the BPA solution and the BPA was degraded according to the above variables. In order to speed up the reaction, we added ABTS to the reaction system.

The residual amounts of BPA in the samples were analyzed by using a high efficiency liquid chromatography system (Shimadzu, Japan) equipped with a quaternary pump , diode array detector (DAD) and an Ascentis® C18 column (4.6 mm×250 mm, 5 μm) and thermostatic at 40 ^{o}C. The mobile phase was water:

methanol (40 : 60, v/v) at a flow rate of 1 $mL \cdot min^{-1}$ for 16 min. Spectrophotometric detection was performed at 277 nm.