Supporting information for

One-pot surface modification of magnetic nanoparticles using phase-transitioned lysozyme for robust immobilization of enzyme

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Preparation of DNS reagent.

After seignette salt (18.2 g) and 50 mL ultrapure water were put into a 100 mL flask, 3,5-dinitrosalicylic acid (0.63 g), NaOH (2.1 g) and phenol (0.5 g) were successively added, and the mixture solution was heated at lower than 45 ºC under magnetic stirring in order to dissolve completely. Then, the mixture solution was cooled to room temperature, and diluted to 100 mL in the brown volumetric flask.

Preparation of iodine solution.

Iodine (1.3 g) and potassium iodide (3.5 g) were dissolved into 100 mL ultrapure water, and then the solution was diluted to 1 L in the brown volumetric flask.

Cells culture and cytotoxicity assay

The L929 cells were purchased from Procell Life Science & Technology Co., Ltd. (Wnhan, China).

L929 cells were incubated with DMEM (containing 10% FBS) in tissue culture flask at 37 °C in an incubator (5% CO_2 gas). After the complete growth (80%), the culture medium was moved out followed by cleaning twice with PBS. Then trypsin (2 mL/dish) was added to the medium and cultured for 5 min for the sake of the desorption of the cells from the tissue culture flask. DMEM medium were added to stop the enzymolysis of trypsin, certain amounts of cells were dispensed to 96-well plate for cytotoxicity test. L929 cells lines were incubated in the medium for 24 h to ensure the cells were attached to the culture dish before further application.

MTT assay was used to evaluate the viability of L929 cells cultured on different substrates. L929 cells were seeded onto different samples (control DMEM, DMEM containing different concentrations leach liquor of Fe_3O_4 NPs or Fe_3O_4@PTL/enzyme) at a density of 3000 cells/cm^2. After culture for 24, 48 and 72 h, 90 μL of new medium and 10 μL of MTT solution were added to each well of a 96-well plate and then incubated for another 3 h. Finally, MTT solution was sucked and mixed with DMSO, which was detected with a spectrophotometric microplate reader at a wavelength of 490 nm.

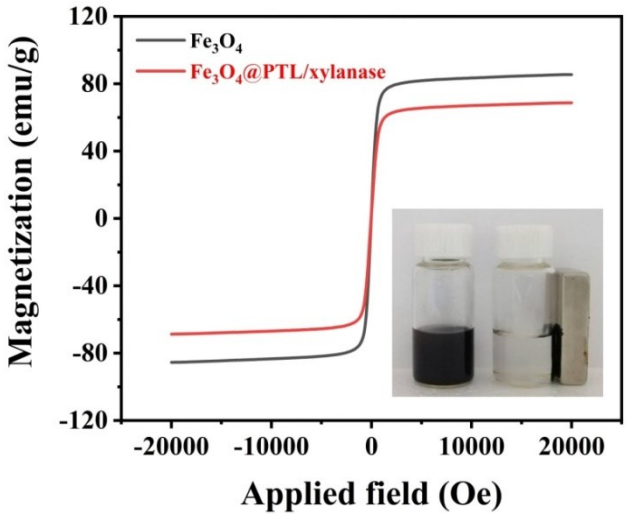

Figure S1. The magnetic hysteresis loops of Fe_3O_4 NPs (black line) and Fe_3O_4@PTL/xylanase nanocomposites (red line), and the inset shows the pictures of nanocomposite solution before and after magnetic separation.

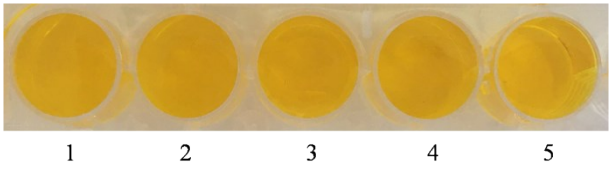

Figure S2. The chromogenic reactions between DNS and the supernatant of different mixture solutions: (1) xylan, (2-5) xylan and the supernatant of the immobilized xylanase after incubated with PBS buffer for 12, 24, 36, 48 h, respectively.

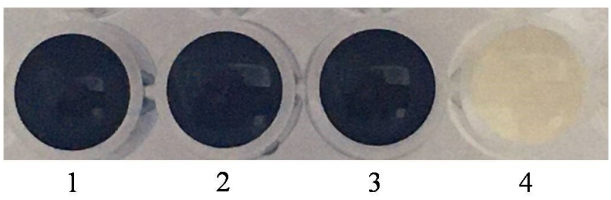

Figure S3. The chromogenic reactions between iodine solution and the supernatant of different mixture solutions: (1) starch, (2) Fe_3O_4 NPs + starch, (3) Fe_3O_4@PTL + starch, and (4) Fe_3O_4@PTL/amylase + starch.

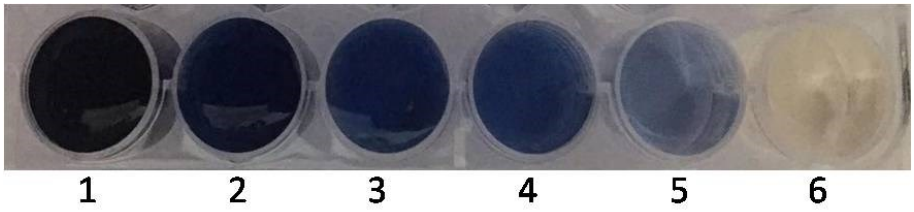

Figure S4. The chromogenic reactions between iodine solution and the supernatant of the incubation mixture of Fe_3O_4@PTL/amylase and starch solution. (1-6) shows the effect of the different incubation time at 1, 3, 5, 7, 9 and 11 min, respectively.

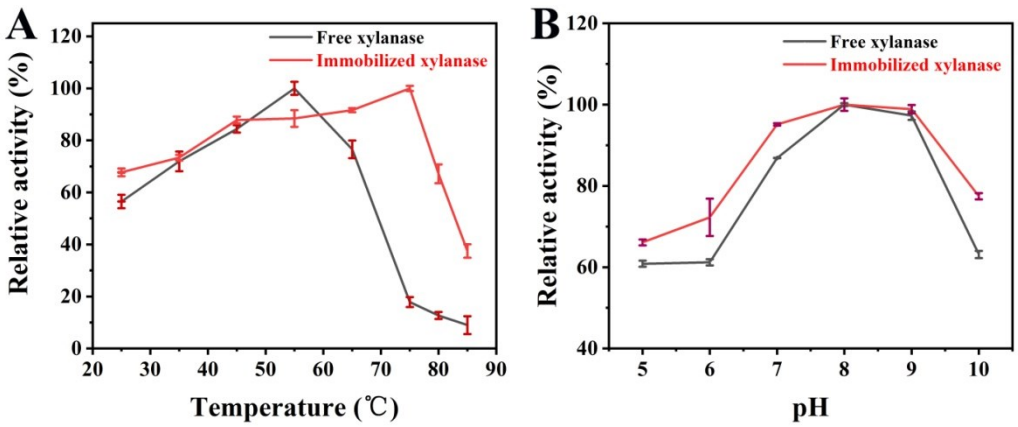

Figure S5. The optimization of enzymatic hydrolysis temperature (A) and pH (B) of free and immobilized xylanase.

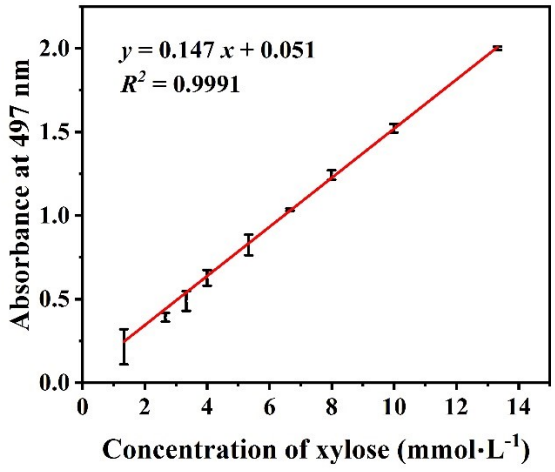

Figure S6. The linear relationship between the absorbance at 497 nm and xylose concentration.

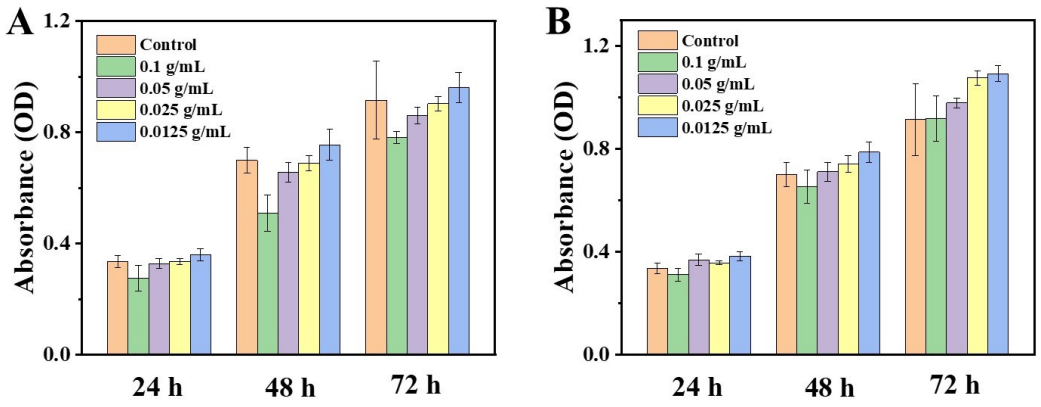

Figure S7. The cytotoxicity results of (A) Fe_3O_4 NPs and (B) Fe_3O_4@PTL/xylanase.