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SupplementaryInformation

Efficient and recyclable Pickering magnetic interface biocatalyst: application in biodiesel production

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Contents

I. Experimental Section

Reagents and materials

Styrene (St, > 99%), divinylbenzene (DVB, 80%), 4-(chloromethyl)styrene (CMS, > 90%), ammonium hydroxide ($NH_3 \cdot H_2O$, 28%), oleic acid (OA, > 93%), sodium dodecyl sulfate (SDS, > 99%), hexadecane (> 99%), octane (> 99%), potassium persulfate (KPS, > 99%), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, > 99%), iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, > 99%) and Candida rugosa lipase (CLAB) were all obtained from Sigma-Aldrich and used without further purification. Anhydrous sodium sulphate (Na_2SO_4, > 99%), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, 99%), potassium phosphate monobasic (KH_2PO_4, 99.5%), glutaryl chloride (50% in H_2O), dichloromethane (DCM, 99.5%), 1,3-Propanediamine (98%), albumin from bovine serum (96%), Brilliant Blue G (AR), ethanol (99.8%) and methanol (99.5%) were purchased from Aladdin Co. Ltd. Refined soybean oil (molecular weight of 882 $g \cdot mol^{-1}$, acid value of 0.12 mg KOH g^{-1}) was purchased from Jiali Cereal & Oil Co., Ltd. Standard undecanoic acid methyl ester ($C_{11:0}$) and other methyl esters (palmitate ($C_{16:0}$), linolenate ($C_{16:1}$), stearate ($C_{18:0}$), oleate ($C_{18:1}$), linoleate ($C_{18:2}$) and linolenate ($C_{18:3}$) were purchased from Sigma-Aldrich.

Preparation of N-p-formaldehyde benzyl-1,3-propanediamine [1]

1,3-propanediamine (0.15 mol in 125 mL DCM) was taken in a 250 mL four-neck round bottom flask. A 4-(chloromethyl)styrene solution (0.03 mol CMS in 225 mL DCM) was slowly added to the flask under ice bath and reacted for 24 h under nitrogen atmosphere. After finished, the mixture was washed several times with deionized water, and the organic phase was dried over Na_2SO_4 and rotary evaporated to give an orange-yellow liquid product. The 1H NMR spectrum of compound was given in Fig. S1.

Preparation of Fe_3O_4@PS-NH_2

Magnetic fluid (Fe_3O_4/HD) was prepared according to the previously reported procedures.[2] SDS (0.9 g) and H_2O (90 mL) were charged to reactor, and then add a dispersion of Fe_3O_4/HD magnetic fluid (5 g), styrene (4.55 g), divinylbenzene (0.455 g), N-p-vinylbenzyl-1,3-propanediamine (2.2 g) and hexadecane (0.2 g) to the aqueous phase, mechanically stirred for 2 h to obtain a brown-black oil drop suspension; It was finely emulsified in a 300 W cell pulverizer for 24 min in an ice bath to obtain a uniform and stable mini-emulsion. The obtained emulsion was transferred to a 250 mL four-necked flask, stirred under nitrogen for 1 h, then KPS (0.040 g) was added, and the mixture was heated to 70℃ for 17 h. Finally, the mixture was demulsified with methanol, washed with water, and lyophilized to obtain a dark orange solid Fe_3O_4@PS-NH_2.

Preparation of Fe_3O_4@PS-NH-lipase

Add a dispersion of Fe_3O_4@PS-NH_2 (100 mg Fe_3O_4@PS-NH_2 in 10 mL DCM) to a reaction flask. Then, 1 mL of glutaraldehyde solution and enzyme phosphate buffer solution (0.1 mol·L^{-1}, pH = 7.5) were charged to reactor and reacted at 25 ℃. After further stirring for 12 h, the catalyst was separated by a magnet and washed by phosphate buffer solution several times, and lyophilized to obtain an immobilized lipase catalyst, which was stored at 4 ℃.

Characterization

^{1}H NMR spectra were measured on Bruker Avance III spectrometer ($CDCl_3$-d_6). The morphology of Fe_3O_4@PS-NH_2 and Fe_3O_4@PS-NH-lipase were visualized by using a JEOL JEM-1010 TEM operating at 120 kV, and the samples (dispersed in methanol) were prepared by placing a drop of dispersion liquid on a carbon-coated copper grid and the solvent was evaporated at room temperature. The functional groups of samples were determined by Thermo Fisher Nicolet iS10 FTIR by using a KBr tablet. The particle size of Fe_3O_4@PS-NH_2 and Fe_3O_4@PS-NH-lipase dispersed in methanol were measured by Malvern ZS90 DLS. The static CA of water, methanol and soybean oil on

the surface of Fe_3O_4@PS-NH_2 and Fe_3O_4@PS-NH-lipase were determined by Dataphysics OCA-20 sessile drop method. The optical microscopy images of Pickering emulsion were obtained by Olympus BX41 optical microscope. The Pickering emulsion were obtained by T18 digital ULTRA TURRAX.

Quantitative analysis of immobilized lipase

Brandford method can be used to quantitatively determine the load of lipase. Coomassie Brilliant Blue G250 can combine with proteins to form blue compounds of varying degrees. The compound has a maximum light attraction at 595 nm which is directly proportional to protein concentration. A suitable amount of the reaction supernatant was added to a dry test tube. The configured Coomassie Brilliant Blue G250 (0.1 $g \cdot L^{-1}$) was added to the supernatant containing a trace amount of enzyme, and measure the absorbance after mixing. Calculate the lipase content in the supernatant of ten years by using the standard curve eqn (1):

$$Y = 1646.98278X - 28.40697 \qquad R = 0.99141 \qquad (1)$$

Where X is Protein concentration; Y is Absorbance.

The quantitative analysis of immobilization efficiency is based on eqn (2):

$$Loading\ capacity\ \left(mg \cdot 100mg^{-1}\ particles\right) = \frac{m-n}{w} \qquad (2)$$

Where m is total lipase; n is supernatant lipase amount; w is magnetic nanoparticle mass.

Preparation of Pickering emulsions

In a typical procedure for producing Pickering emulsions, Fe_3O_4@PS-NH-lipase-n (n = 1-3) (69 mg, 5 wt%) were dispersed in soybean oil (1.38 g) with ultra-sonication for 5 min (Fig. S2). Methanol (0.76 g) was added to the above suspension, and the mixture was emulsified by using T18 digital ULTRA TURRAX at 8000 rpm for 5 min.

Transesterification procedures and analysis

Typically, the emulsion obtained above was used for the transesterification reaction at 30 ℃. After the reaction was completed, Fe_3O_4@PS-NH-lipase-n (n = 1-3) were recycled by magnet, and the biodiesel was analyzed by GC-2014C. The recovered catalyst was repeatedly washed 3 times with phosphate buffer, and then directly recycled for the next time. The transesterification catalyzed by free lipase was similar with above procedures except that the free lipase was dispersed in soybean oil. Biodiesel yields were calculated by Equation S1, and undecanoic acid methyl ester (C11:0) used as internal standard and n-heptane was used as a solvent. One unit of lipase activity (U) is defined as 1 μmol of soybean oil consumed within 1 min. All reactions are repeated at least three times.

$$Yield = \frac{\sum f_{ester} \times A_{ester}}{A_{internal}} \times \frac{m_{internal}}{m_{esters}} \times 100\% \quad \text{(S1)}$$

Where A_{ester} is the peak area of fatty acid methyl esters, $A_{internal}$ is the peak area of internal standard (UAME, $C_{11:0}$), $m_{internal}$ is the mass of $C_{11:0}$, m_{esters} is the mass of fatty acid methyl esters (FAME) and f_{ester} is the correction factor of fatty acid methyl esters. The relative response factors of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ to that of $C_{11:0}$, were calibrated as 1.1147, 1.1312, 1.1040, 1.1066, 1.1168 and 1.154, respectively.

II. Supplementary Figures and tables

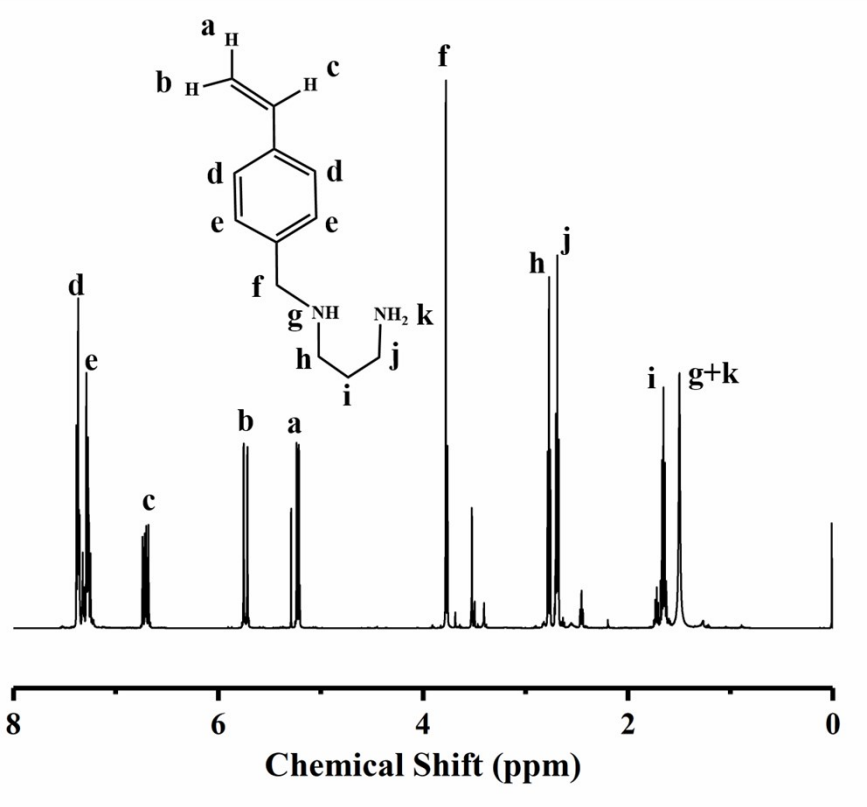

Fig. S1 ^{1}H NMR spectrum of N-p-formaldehyde benzyl-1,3-propanediamine (^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.27-7.38 (dd, 4H, Ar-H), 6.68-6.74 (q, J = 10.9 Hz, 1H; CH), 5.71 (d, J = 17.5 Hz, 1H; CH_2=), 5.21 (d, J = 10.7 Hz, 1H; CH_2=), 3.78 (s, 2H; Ar-CH_2), 2.78 (t, J = 6.7 Hz, 2H; $NHCH_2$), 2.70 (t, J = 6.8 Hz, 2H; CH_2NH_2), 1.66 (m, J = 6.8, 6.9 Hz, 2H; CCH_2C), 1.50 (s, 3H; NH, NH_2).)

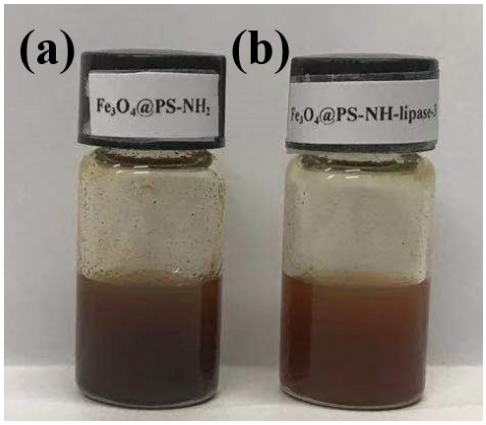

Fig. S2 Images of 69 mg (a) Fe_3O_4@PS-NH_2 and (b) Fe_3O_4@PS-NH-lipase-3 dispersed in 1.38 g soybean oil.

Table S1. The preparation conditions of Fe_3O_4@PS-NH-lipase and loading of enzyme

Sample	Lipase solution	Supporter	Loading(mg·100 mg^{-1})
Fe_3O_4@PS-NH-lipase-1	0.5 mL	Fe_3O_4@PS-NH_2	0.805
Fe_3O_4@PS-NH-lipase-2	1 mL	Fe_3O_4@PS-NH_2	1.660
Fe_3O_4@PS-NH-lipase-3	2 mL	Fe_3O_4@PS-NH_2	3.550

[a] The concentration of lipase is 2 mg/mL.

[b] The loading of lipase on Fe_3O_4@PS-NH_2 nanoparticles was analysed by the Bradford method using BSA as a standard.

Reference

1 E. Takeshi, N. Daisuke, M. Tomohiro, Y. Hiroshi and O. Bungo, *Macromolecules*, 2004, **37**, 2007–2009.

2 J. Tang, Q. Zhang, K. C. Hu, S. X. Cao, S. H. Zhang and J. L. Wang, *J. Catal.*, 2018, **368**, 190-196.