Supporting Information

Renewable boronic acid affiliated glycerol nano-adsorbents for recycling enzymatic catalyst in biodiesel fuel production

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1. SUPPORTING FIGURES

**Fig. S1** Schematic illustration to lipases catalyzed BDF production.

**Fig. S2** SEM images of (a) PVPBA@SiO$_2$ and (b) GL-PVPBA@SiO$_2$.

**Fig. S3** a) and b) TEM images c) the size change of SiO$_2$ (20 nm) before and after PVPBA coating, boron atomic content: 12.9wt%.
**Fig. S4** TEM images of SiO$_2$ (40 nm) before (a) and after PVPBA (b,c) coating; d) the size changes of SiO$_2$ (40 nm) before (a) and after PVPBA coating, boron atomic content: 9.0wt%.

**Fig. S5** Glycerol adsorption rate by PVPBA@SiO$_2$ with different size silica cores.
**Fig. S6** The size changes of PVPBA@SiO$_2$ (65 nm) formed at different time of precipitation polymerization reaction.

**Fig. S7** TEM images of PVPBA@SiO$_2$ (65 nm) formed at different time of precipitation polymerization reaction, boron atomic content: 6.8wt%.

**Fig. S8** FT-IR spectrum of PVPBA@SiO$_2$. 
Fig. S9 $^{11}\text{B}$ NMR chemical shift changes with different GL concentrations, (a) 0.2 mM, (b) 0.4 mM, (c) 0.6 Mm.

Fig. S10 Release of GL from GL-PVPBA@SiO$_2$ triggered by CO$_2$ acidolysis.
Fig. S11 Zeta Potential changes of GL-PVPBA@SiO$_2$ nanoparticles bubbled with CO$_2$ (15 mL/min), Concentration: 1.7 mg/mL in water.

Fig. S12 FAME yield catalysed by recycled NS81006 layer.
**Fig. S13** FAME yield produced from NS81006 layer after GL adsorption by renewable PVPBA@SiO$_2$.

**Fig. S14** The NS81006 content in lipase layer after glycerol was adsorbed by PVPBA@SiO$_2$ for 60 min.
2. EXPERIMENTAL SECTION

Materials and agents: The 4-vinylphenyl boronic acid (VPBA) and divinylbenzene (DVB) were purchased from J&K Company. Tetraethoxysilane (TEOS), azodiisobutyronitrile (AIBN) and 3-(trimethoxysilyl) methacrylate (TMSPMA) was purchased from TCI, Shanghai. Soybean oil was purchased from Jinlongyu Co. Ltd (Shenzhen, China). Free lipase NS81006 (activity 3300 U mL\(^{-1}\)) from the genetically modified Aspergillus niger microorganism, was donated by Novo Industries (Denmark). One unit (U) of free lipase activity was defined as the quantity of lipase that hydrolyzes butyrin to liberate the equivalent of 1 \(\mu\)mol of fatty acid per min at 45 °C with Glycine-NaOH buffer (pH = 9.4). In addition, all the solvents were used directly without any purification.

Characterization: \(^{11}\)B NMR spectra for the polymer structural analysis were obtained from JEOL JNM-ECA400 (400MHz) spectrometer with D\(_2\)O and 0.05% BF\(_3\).OEt\(_2\) as solvent. FT-IR spectrum were obtained using a PerkinElmer Spotlight 400. X-ray Photoelectron Spectroscopy (XPS) was performed using a PHI Quantera SKMTM. A Marven Zetasizer Nano ZS90 equipped with a 633 nm He-Ne laser and an avalanche photodiode detector (DLS) was used to characterize the hydrodynamic sizes and zeta potentials of the particles. The morphologies of the particles were characterized by an 80 kV Hitachi H-7650B transmission electron microscope and a 120 kV JEM-2010 microscope (TEM). The dispersions with proper concentration were dropped onto carbon coated copper grids and were blotted up after 1 min. This process was repeated for three times. The copper grids were air-dried before observation. Scanning electron microscopy (SEM) images were recorded on a Hitachi SU-8010 field emission scanning electron microscope at 5 kV. The boron content was measured using inductively coupled plasma mass spectrometry, the type is ThermoFisher X II. The hydrodynamic sizes and zeta potentials of the nanoparticles were measured with a Marven Zetasizer Nano ZS90. A 633 nm He-Ne laser was equipped as the laser source, and the scattering light at 90° angle was detected by an avalanche...
photodiode detector. The lipase concentration was measured using a Tecan GENios F129038 micro plate reader. X-ray Photoelectron Spectroscopy (XPS) was performed using a PHI Quantera SKMTM.

Fatty acid methyl esters (FAME) were analysed with a 7890 A gas chromatography system (Agilent Technologies, Santa Clara, California, USA) equipped with a CP-FFAP capillary column (0.32 mm× 25 m) (Agilent Technologies, USA). The initial column temperature was set at 180°C and maintained for 0.5 min, then heated to 250°C at a rate of 10°C per min and held for 6 min. Detector and injector were set at 250°C and 245°C, respectively. 6 mg of product was dissolved in 0.6 mL of ethanol solution containing 0.6 g L⁻¹ heptadecanoic acid methyl ester as an internal standard. After mixing thoroughly, 1μL of sample was injected for GC analysis. FAME yield was calculated as the percentage of the obtained mass of FAEE to the theoretical maximum mass of FAME.

The glycerol concentration was measured by the LCMS, which was an Agilent Technologies Model LC/MSD supplied with Chem Station 1100 software (Wilmington, DE). Nitrogen gas was supplied to the LC/MSD using, a Jun-Air Model 2000-25M air compressor (Buffalo Grove, IL) connected to a Waterman Model 75-72-K727 nitrogen generator (Haverhill, MA). The HPLC column used was a Shodex Asahipak NH2P-50 4E, 4.6 3 250 mm (Thompson Instrument Co., Clear Brook, VA).by LC—10ATVP from Shimadzu.

**Water contact angle test:** 2 g of PVPBA@SiO₂ were divided into three sets. Every set of sample was dispersed into 13 mL water/ethanol (1/1). Then, the pH values of two samples were adjusted to pH 10.0. One of the alkalized samples were added glycerol. After that, these samples were added into a groove template. The white film based on these samples were formed after 48 h. Further lyophilized overnight were performed for the water contact angle test.

**Synthesis of PVPBA@SiO₂ (65 nm):** To obtain the silica nanoparticles with different size, a classical Stöber method was used. Via changing the amount of TEOS, the size was modulated. For ~20nm silica NPs, the TEOS feed amount was 2.3 mL, the ammonium hydroxide was 1.6 mL; for ~40nm silica NPs, the TEOS feed amount was 4.5 mL, the ammonium hydroxide was 3.3 mL. A representative synthesis route for silica NPs (~65 nm) was as the follows.

7.0 mL of TEOS was added into the mixture of 100 mL of ethanol and 6.8 mL of ammonium hydroxide. Then the system was stirred for 24 h at room temperature. Followed, 1.4 mL of TMSPMA was added into the above system, and the reaction was continued overnight. The obtained SiO₂-MPS was separated by centrifugation at 5000 rpm, and the crude product was washed using ethanol and water for several times. The final product was obtained as white powder after lyophilized.

Second, 10 mg of SiO₂-MPS was added into the mixture of 25 mL n-butanol and 2.8 mL water. After intensively stirred for 10 min, 0.56 g of VPBA, 5 μL DVB and 0.2% AIBN were added into the above system. The mixture was slowly heated to 90 °C, and the reaction was processed for 24 h. Then, the shiny white powder was obtained after being washed
using water and ethanol for several times. The PVPBA@SiO$_2$ was saved in water with a concentration of 10 mg/mL. In this reaction, the sample was drawn at different time for TEM and DLS test.

The optimal experiments on designing core-shell PVPBA@SiO$_2$ nanoparticles: As is well known, the size of the cores and thickness of the polymeric shell generally affect the chemical and physical properties of core–shell NPs. Based on this, three types of silica core with different sizes were used to prepare the core–shell NPs. Unexpectedly, transmission electron microscopy (TEM) and dynamic light scattering (DLS, Fig. S3 and S4) results indicated an intensively aggregated state when the silica cores had diameters of ~20 and ~40 nm, despite these two NPs having been treated under ultrasonic for re-dispersion. The reason for this phenomenon might be that the PVPBA shells on smaller core–shell NPs could be crosslinking as the reaction proceeds. And inductively coupled plasma optical emission spectrometer (ICP-OES) results indicated that the high boron atomic amounts of these core–shell NPs were 12.9 wt% for 20 nm cores, 9.8 wt% for 40 nm cores and 6.8 wt% for 65 nm cores. To shorten the time for which the alkalized PVPBA@SiO$_2$ was exposed to free lipase catalyst, a rapid GL-adsorption rate of the core–shell NPs was necessary. Thus, these core–shell NPs with the same equivalent of boron were quantified for GL adsorption and the core–shell NPs containing SiO$_2$ cores (65 nm) showed the most rapid GL-adsorption rate within 5 min, measured by liquid chromatography (LC, Fig. S5). This result is because the mono-dispersing performance endowed the PVPBA@SiO$_2$ (65 nm) with optimized interfaces for GL capture (Fig. 1a–c). Additionally, the thickness of the PVPBA shell was modulated by changing the precipitation polymerization time. When the monomer concentration was fixed at ~1.5 mmol mL$^{-1}$, the thickness of PVPBA@SiO$_2$ gradually grew to 11 nm within 24 h. When the reaction was delayed for another 12 h, the thickness of the shell showed a slight change (Fig. S6 and S7). Taking these results together, the core–shell NPs made from silica cores (65 nm) showed optimized dispersing properties and the most rapid GL-adsorption rate, which enable them to be chosen for further research.

Lipase-mediated methanolyis of soybean oil: The methanolyis of soybean oil was catalysed by free lipase NS81006 in a 500 mL three-neck round bottom flask immersed in a 45 °C thermostat water bath. The mixture of soybean oil (100 g), methanol (ethanol/oil molar ratio 5:1), 2.0% lipase (w/w, based on oil weight), 10 % water (w/w, based on oil weight) was stirred by a mechanical stirrer at 500 rpm. To minimize the toxicity to the enzymes, methanol was added stepwise with 1/4 of the total ethanol being added every 1 h. Samples were taken at specified times and were centrifuged at 60 °C and 0.02 MPa to evaporate methanol. The oil phase was then analysed by a 7890A gas chromatography system (Agilent Technologies, USA) for methyl ester content.

Recycle of NS81006 for BDF production: For the recycle of liquid lipase NS81006, after the reaction, layer separation was achieved by centrifugation (5000 rpm, 25 °C) or standing at room temperature. The aqueous phase was then directly recycled for another
batch of reaction.

**Adsorption of residual glycerol in NS81006 layer:** For the adsorption of residual glycerol in lipase layer, after the original lipase was recycled for 7 times, PVPBA@SiO$_2$ was packaged in dialysis bag and incubated diluted ammonium hydroxide (pH = 10.0) for 5 mins. The $^{11}$B NMR was used to monitor the boronate formation process. Then, alkaliized nanoparticles were quickly transferred into the NS81006 layer. After incubation for 5-6 min, the dialysis bag were removed. The glycerol removed lipase layer was used for BDF production.

**Renewal of nano-adsorbent based on PVPBA@SiO$_2$:** The glycerol-PVPBA@SiO$_2$ packaged in dialysis bag was put in water, then CO$_2$ was bubbled at a rate of 15.0 mL/min. while CO$_2$ acidolysis, 1.0 mL aqueous solvent was drawn for LC analysis to monitor the release of glycerol. After 5 min, the glycerol was found to be completely released. Meanwhile, the pH value of the system was measured using pH vale meter. The final pH of the solvent was around 7.5.

**NS81006 adsorption test:** 1.0 g of adsorbents were added into a dialysis bag. Then, the nanoparticles were incubated in 0.1 g of NS81006 (dissolved in 5 mL water) under stirring for 60 min. The lipase solution was drawn at different time. After adsorption, the nanoparticles were removed. Then, the lipase concentration of the supernatant was measured using a micro plate reader.

First, the standard curve of BSA was obtained by testing the absorbance of several BSA solutions with concentration gradients. 5 mL of supernatant was added to the testing plate and 250 mL of Bradford reagent was added to dye the protein. After 15 min of deposition, the absorbance was measured at a wavelength of 595 nm. Finally, the concentration of protein was obtained by comparing the absorbance of the supernatant with the standard curve of the BSA solutions: $Y = 0.0003X+0.0376$, $R^2 = 0.9986$. $X$ is content of lipase. $Y$ is the absorbance.

**NS81006 stability at pH 8.5:** 1.0 g of NS81006 (dissolved in 50 mL water) was adjusted to pH 8.5 and standby for 60 min. At different time, the lipase were drew and the pH was adjusted to 7.4 by CO$_2$. Then they were used for BDF production to check the activity of the NS81006.