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Improved Catalytic Performance of Lipase Accommodated in the Mesoporous Silicas with Polymer-Modified Microenvironment

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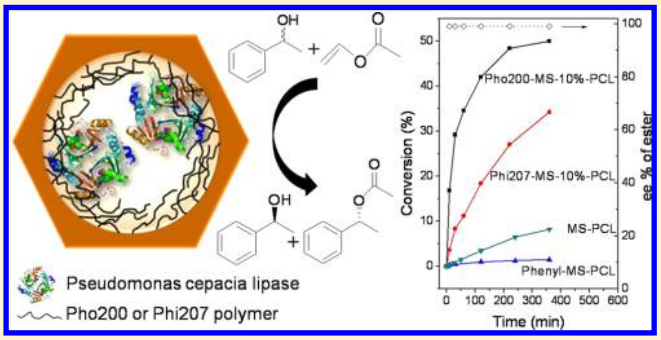
Supporting Information

ABSTRACT: The highly ordered mesoporous silicas with elaborately controlled microenvironment were synthesized via covalent incorporation of long-chain polymers (M_w = 2000 g mol^{-1}) bearing specific hydrophilic/hydrophobic balance. The microenvironment (hydrophilicity/hydrophobicity) of the mesoporous silicas was quantitatively determined by gas adsorption experiments and investigated by lysozyme (LYZ) adsorption. The relative activity of lipase from *Pseudomonas cepacia* (PCL) encapsulated in the mesoporous silica with moderate hydrophobic microenvironment (hereafter denoted as MHM) reaches up to 281% compared with the free PCL, notably higher than that of PCL accommodated in the mesoporous silicas with hydrophilic or strong hydrophobic microenvironment (20.7−26.2% relative to the free PCL). Moreover, PCL entrapped in the nanochannels with MHM affords the highest initial rate in the kinetic resolution of (*R*,*S*)-1-phenylethanol relative to other immobilized PCL. The above results suggest that the MHM could render the active center of PCL entirely exposed to the substrates without interrupting its native conformation in the "interfacial activation". In addition, the nanochannels with MHM could markedly improve the thermal stability of PCL (preserving nearly 60% of the initial activity after the incubation at 70 °C for 2 h) and facilitate the recycling of the immobilized PCL in both aqueous and organic media. Our work demonstrates that the subtle modulation of the microenvironment of mesoporous silicas for enzyme immobilization designates a very promising strategy to fabricate the highly active and stable heterogeneous biocatalysts for industrial application.



1. INTRODUCTION

The utilization of enzyme as biocatalyst has become an important avenue for chemical and pharmaceutical industries on account of the extraordinary catalytic efficiency as well as selectivity under mild and environmentally friendly conditions.[1] However, the native enzyme itself is not an ideal catalyst for commercial application owing to its proclivity to denaturation under harsh industrial conditions and the difficulty in separation and recovery.[2] Fortunately, the immobilization of native enzyme onto solid material has shown to be a promising protocol to overcome these limitations, which displays a number of advantages over the native enzyme such as the enhanced stability, simplicity in separation, and the capability in recovery and reuse.[3] A wide range of materials has been applied for enzyme immobilization, among which the ordered mesoporous silica-based materials have drawn a lot of attention since their distinct properties fulfill most of the requirements for enzyme immobilization, such as large surface area, ordered mesostructure, chemical and mechanical stability, and toxicological safety.[4,5] More importantly, the internal surface of mesoporous materials surrounding the immobilized enzymes could be facilely functionalized with various organic groups, offering a great opportunity to impart desired microenvironmental properties to the immobilized enzymes via a simple modification.[6,7]

When enzyme molecules are located inside the nanopores, the microenvironment surrounding the immobilized enzymes would determine the final performance of the heterogeneous biocatalyst. It has been confirmed that the hydrophilicity/hydrophobicity balance of the microenvironment is a key factor. It affects the interaction between enzyme and support, the configuration of immobilized enzyme, the diffusion rate of substrate, and the accessibility of active site, therefore dominating the eventual catalytic activity, stability, and reusability of the immobilized enzyme.[1,8] A representative example is the lipase, which has been widely utilized in both hydrolytic and synthetic reactions. Lipase is well-known for its sensitivity to the hydrophilicity/hydrophobicity of the support because of the "interfacial activation"—a term to describe the significant increment in lipase activity when adsorbed on a hydrophobic support.[9−11] Since the first publication in 1936 by Holwerda,[12] substantial works of lipase adsorption on various

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hydrophobic supports have been reported.[13−16] The generally accepted concept is that when entrapping lipase in hydrophobic support, the uptake capacity, catalytic active, and even stability of the immobilized lipase are entirely in line with the increment in support hydrophobicity. However, the different argument proposed that greater hydrophobic interface dose not always produce higher lipase activity. In the contrary, the moderate surface hydrophobicity of the support is preferable for elevated activity of the immobilized lipase.[17−19] Therefore, the materials with systematic modified surface hydrophilicity/hydrophobicity should be prepared to investigate the correlation between hydrophilicity/hydrophobicity of the support and the performance of the immobilized lipase.

In this work, we report the synthesis of a series of mesoporous silicas possessing a wide range of hydrophilicity/hydrophobicity balance via the modification with hydrophilic polymer SURFONAMINE-B200 or hydrophobic polymer SURFONAMINE-L207 by the co-condensation method under a mild acidic condition. The introduction of the two special polymers enables an elaborate modulation of the hydrophilicity/hydrophobicity of the microenvironment in the resulting mesoporous silicas, which could be difficult to be accomplished with simple organic molecules. The influence of the support hydrophilicity/hydrophobicity on enzyme immobilization was systematically investigated employing lysozyme (LYZ) and lipase from *Pseudomonas cepacia* (PCL) as the model biomolecules. The catalytic results of the immobilized PCL on mesoporous materials with varied hydrophilicity/hydrophobicity strongly suggest that there is a transition point relating to the hydrophobicity of the support which favors an excellent performance of the immobilized PCL.

2. EXPERIMENTAL SECTION

2.1. Materials. Amano lipase PS from *Pseudomonas cepacia* (PCL, 30 U mg^{-1}) and lysozyme (LYZ) both stored at 4 °C were purchased from Sigma-Aldrich and Dalian Chenyu Biochemical Reagents Co., respectively. SURFONAMINE-B200 ($CH_3[OCH_2CH_2]_6[OCH_2CH(CH_3)]_{29}NH_2$, M_w = 2000 g mol^{-1}, HLB = 2.8) and SURFONAMINE-L207 ($CH_3[OCH_2CH_2]_{33}[OCH_2CH(CH_3)]_{10}NH_2$, M_w = 2000 g mol^{-1}, HLB = 13.8) were kindly provided by Huntsman International LLC. P123 (poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), $EO_{20}−PO_{70}−EO_{20}$, M_w = 5800) and phenyltrimethoxysilane were purchased from Sigma-Aldrich. Triacetin and (*R*,*S*)-1-phenylethanol were commercially available from Alfa Aesar. Tetraethoxysilane (TEOS), (3-chloropropyl)-trimethoxysilane, sodium silicate solution (20% of SiO_2, 6% of Na_2O), triethylamine, vinyl acetate, and other reagents were obtained from Shanghai Chemical Reagent. Inc. of Chinese Medicine Group. All materials were of analytical grade and used as received.

2.2. Synthesis of Pho200-Organosilane and Phi207-Organosilane. To synthesis Pho200-organosilane, a two-neck round flask containing SURFONAMINE-B200 (5 mL, 2.5 mmol) was evacuated and filled thrice with argon, followed by the injection of (3-chloropropyl)trimethoxysilane (0.6 mL, 3 mmol, dehydrated) and triethylamine (0.35 mL, dehydrated). Then the reaction mixture was heated to 85 °C and refluxed for 24 h with continuously purged argon stream. After the reaction, triethylamine and residual (3-chloropropyl)-trimethoxysilane were removed through evaporation under reduced pressure. The resultant silane was denoted as Pho200-organosilane where Pho means hydrophobic and 200 refers to SURFONAMINE-B200. To acquire Phi207-organosilane, where Phi and 207 respectively denote hydrophilic and SURFONAMINE-L207, the same procedure was performed except that SURFONAMINE-L207 was used.

2.3. Synthesis of Pho200-Functionalized Mesoporous Silicas (Pho200-MS) and Phi207-Functionalized Mesoporous Silicas (Phi207-MS). Pho200-MS and Phi207-MS were synthesized in a mild acidic condition.[20,21] For Pho200-MS, P123 (1.0 g) and ethanol (0.69 g) were dissolved in HAc-NaAc buffer solution (28 mL, pH = 4.4, HAc: 0.52 M, NaAc: 0.27 M) at 25 °C under vigorous stirring, followed by the addition of sodium silicate (8.62 mmol, 2 mL). The resulting mixture was stirred at 25 °C for another 20 min, and then the desired amount of TEOS and Pho200-organosilane in ethanol (1.0 g) was added (Table S1). The molar ratio of the synthetic recipe was sodium silicate:TEOS:Pho200-organosilane:P123:acetic acid:sodium acetate:ethanol:H_2O = 50:50−*x*:*x*:1.02:85.2:44.5:367.5:8921 (*x* = 5, 10). The reaction mixture was stirred at 40 °C for 20 h and aged at 100 °C under static conditions for an additional 24 h. After that, the white precipitate was filtered, washed with water as well as ethanol, and dried at ambient temperature. The templates were removed by refluxing the as-synthesized sample (1.0 g) in ethanol (200 mL) for 24 h. The extracted materials were denoted as Pho200-MS-*x*%, where *x* (*x* = 5, 10) is the molar ratio of Pho200-organosilane in the silane precursor mixture. Parallel procedures but substituting Phi207-organosilane for Pho200-organosilane were carried out to synthesize Phi207-MS-*x*%, where *x* (*x* = 5, 10) refers to the molar ratio of Phi207-organosilane in silane precursor mixture. The phenyl-functionalized mesoporous silica (Phenyl-MS) was also prepared according to the same procedure but replacing Pho200-organosilane with phenyltrimethoxysilane (the molar ratio of sodium silicate:TEOS:phenyltrimethoxysilane was 50:45:5). Pure mesoporous silica (MS) was synthesized using similar method without the addition of organosilane (the molar ratio of sodium silicate: TEOS was 50:50).

2.4. Lysozyme (LYZ) Adsorption. A series of LYZ solution with a concentration ranging from 30 to 350 μmol L^{-1} were prepared by dissolving a calculated amount of LYZ in sodium carbonate buffer solution (10 mM, pH = 10.8). In a typical adsorption process, the solid material (40 mg) was suspended in the LYZ solution (8 mL) with different concentration. The resulting mixture was continuously shaken at 20 °C with a speed of 160 rpm for 96 h. Thereafter, the supernatant and solid material were separated by centrifugation. The concentration of LYZ was measured by UV spectrophotometer at a wavelength of 280 nm, and the amount of LYZ loaded onto the solid sample was calculated by subtracting LYZ in the supernatant from the total LYZ amount. As for the experiments dealing with the adsorption kinetics of LYZ onto the mesoporous silicas with different microenvironment, the solid material (200 mg) was suspended in LYZ solution (40 mL, pH = 10.8) with a concentration of 300 μmol L^{-1}. During the adsorption process, samples (2 mL) were withdrawn periodically, centrifuged, immediately analyzed, and then returned to the mixture.

2.5. Lipase Adsorption and Activity Assay. Prior to immobilization, the crude lipase from *Pseudomonas cepacia* (PCL) was purified according to the method reported by Zhang:[22] crude PCL (1 g) was dissolved in phosphate buffer solution (PBS, 20 mL, pH = 8.0, 50 mM) and shaken (160 rpm) at 4 °C for 3 h. The mixture was centrifuged, and insoluble components were removed. For immobilization process, the suspension of solid material (30 mg) in purified PCL solution (6 mL) was shaken (160 rpm) at 4 °C for 12 h. The resulting product was centrifuged, washed several times with PBS, and then dried at 20 °C under vacuum. The protein concentration of PCL was determined by the Bradford method, and the amount of the immobilized PCL was calculated by subtracting the amount of PCL in supernatant and washings from the initial PCL content. The lipase activity was measured in the hydrolysis reaction of triacetin: triacetin (250 μL) and PBS (10 mL, pH = 8.0, 50 mM) were mixed at 30 °C under a vigorous stirring for 5 min to prepare a uniform emulsion; free lipase solution (100 μL) or lipase-loaded solid material (10 mg) was added and mildly stirred for another 10 min. After that, the mixture was titrated with sodium hydroxide solution (0.1 M) with phenolphthalein as the indicator. A blank experiment without adding enzyme was carried out following the same procedure. Based on the consumption amount of sodium hydroxide, the amount of acetic acid produced in the hydrolysis was acquired, and the activity of free lipase and immobilized lipase was calculated.

2.6. Determination of the Thermal Stability and Reusability of Lipase. To test the thermal stability of lipase, free lipase solution or

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lipase-loaded solid sample was placed in a screw-capped vial and then incubated in a thermostatically controlled water bath for 2 h. The temperature of the water bath was carefully controlled and varied in a temperature range of 40−70 °C. The residual activity of this thermal treated PCL was assayed at 30 °C via the hydrolysis of triacetin as described above. For each sample, its initial activity before undergoing the thermal incubation was taken as 100%. With regard to the reusability test, the PCL-loaded Pho200-MS-10% was recovered from the reaction system by centrifugation after each batch. Then the sample was washed several times with PBS and dried at ambient temperature for the next batch. The initial activity prior to the first recovery was taken as 100%.

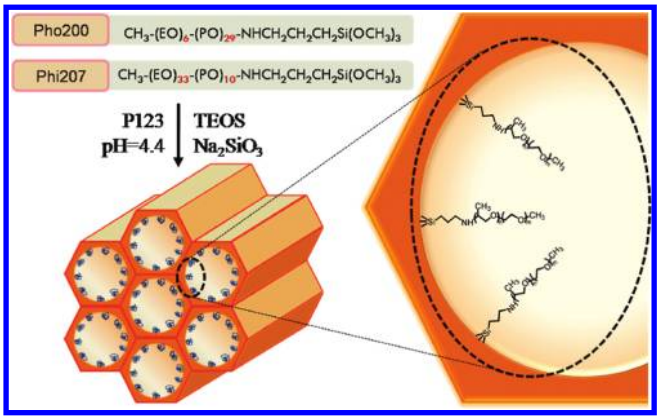
2.7. Kinetic Resolution of (*R*,*S*)-1-Phenylethanol. In a typical experiment, (*R*,*S*)-1-phenylethanol (0.5 mmol) and vinyl acetate (2.0 mmol) were dispersed in the dry hexane (5 mL) and stirred at 30 °C. The desired amount of lipase-loaded solid material containing identical protein content was added to the mixture to start the reaction. Aliquots of the samples were periodically withdrawn from the reaction system at fixed time intervals, then centrifuged to remove the precipitate, and analyzed by the gas chromatograph with a chiral column (Agilent HP-19091G-B233, 30 m × 250 μm × 0.25 μm). As for the reusability, the solid sample was collected from the reaction system by centrifugation after each batch. Then the sample was washed with dry hexane and dried at ambient temperature for the next cycle. For each immobilized PCL, the initial activity prior to the first recovery was taken as 100%.

2.8. Characterization Methods. The nitrogen sorption experiment was performed at 77 K on a Micromeritics ASAP 2020 system. Prior to the measurement, the sample was outgassed at 90 °C for at least 6 h. The Brunauer−Emmett−Teller (BET) specific surface area was calculated based on the adsorption isotherm. The pore size distribution was calculated from the adsorption branch using the BJH (Barrett−Joyner−Halenda) method. The total pore volume was estimated from the amounts adsorbed at a relative pressure (P/P_0) of 0.99. The adsorption of water and benzene vapors was measured at 273 K on a Hiden Isochema Intelligent Gravimetric Analyzer after the sample was degassed for 6 h at 353 K. The ultrapure water and benzene used in the vapor adsorption experiments were both treated by freeze−pump−thaw technique for three cycles prior to the adsorption. FT-IR spectra were recorded on a Thermo Nicolet Nexus 470 Fourier transform infrared (FT-IR) spectrometer using KBr pellets. UV−vis spectra were collected on a Shimadzu UV-2550 double-beam spectrophotometer using 1 cm quartz cell. X-ray diffraction (XRD) patterns were recorded on a Rigaku RINT D/Max-2500 powder diffraction system using Cu Kα radiation of 0.15406 nm wavelength. Transmission electron microscopy (TEM) was performed at the voltage of 120 kV. Thermogravimetric (TG) analysis was carried out under air flow on a Perkin-Elmer Pyris Diamond TG instrument in a temperature range of 20−900 °C with a heating rate of 5 °C min^{-1}.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Pho200-MS and Phi207-MS. SURFONAMINE-B200 and SURFONAMINE-L207 are two models chosen from a series of polyether monoamines produced by Huntsman International LLC. These polyether monoamines contain one primary amine group attached to the ends of a polyether backbone, thus benefiting the chemical modification. The polyether backbone constitutes poly(propylene oxide) and poly(ethylene oxide). The ratio of ethylene oxide (EO) to propylene oxide (PO) which determines hydrophilicity or hydrophobicity of the polymer could be altered freely in an extensive range. These features allow an accurate control of hydrophilicity/hydrophobicity balance of the internal surface of the mesoporous silicas (the microenvironment surrounding enzyme molecules) via introducing a certain polyether monoamine into the nanochannels. In this work, SURFONAMINE-B200 (the ratio of EO/PO is 6/29, HLB = 2.8) and SURFONAMINE-L207 (the ratio of EO/PO is 33/10, HLB = 13.8) were exploited to perform the microenvironmental modification (Scheme 1).

Scheme 1. Illustration of the Synthesis of Pho200-MS and Phi207-MS Materials Respectively Functionalized with Pho200 and Phi207 Polymer in the Mesochannels



Specifically, the SURFONAMINE-B200-modified organosilane (denoted as Pho200-organosilane) and SURFONAMINE-L207-modified organosilane (denoted as Phi207-organosilane) were synthesized (Figure S1), followed by the co-condensation of the organosilane with sodium silicate and tetraethoxysilane (TEOS) using P123 as the structure directing agent in HAc−NaAc buffer solution. The resulting materials were denoted as Pho200-MS-*x*% and Phi207-MS-*x*%, where *x* (*x* = 5 or 10) refers to the molar ratio of the organosilane in the initial mixture of silane precursors. According to this synthetic approach, the SURFONAMINE-B200 and SURFONAMINE-L207 polymers were respectively cross-linked into the mesoporous silica via covalent bonding rather than physical adsorption, which confirms the polymers stably anchored in the nanopores and do not leach out from the prepared materials.

Figure S2 displays the FT-IR spectra of Pho200-MS and Phi207-MS with different polymer content. All Pho200-MS and Phi207-MS samples present a broad band at ∼2977−2881 cm^{-1}, arising from the stretching vibration of C−H. Three characteristic peaks at 1453, 1380, and 1348 cm^{-1} due to the bending vibration of C−H were also clearly observed. In addition, it should be noted that the intensity of these peaks is all positively correlated with the amount of organosilane in the initial mixture. These results suggest that co-condensation under a mild acidic condition is a feasible approach to introduce long-chain polymers (M_w = 2000 g mol^{-1}) into the mesoporous silicas.

The mesostructure of Pho200-MS and Phi207-MS samples was characterized by XRD technique (Figure S3). There are three peaks in the low angle range which could be indexed as (100), (110), and (200) reflections of a two-dimensional hexagonal *P6mm* symmetry. Albeit the intensity of (110) and (200) peaks became attenuated with increasing the feeding amount of the organosilane, the sharp peak of (100) diffraction still remained very conspicuous, indicating that all these samples preserve an ordered mesoporous structure. As displayed in Figure 1, the TEM images of Pho200-MS-10% and Phi207-MS-10% clearly show highly ordered parallel cylindrical channels taken along the (110) direction. The results of XRD and TEM demonstrate that all the samples with

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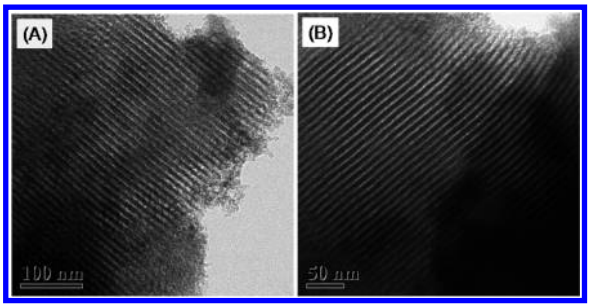

Figure 1. TEM images of (A) Pho200-MS-10% and (B) Phi207-MS-10%.

polymer modification possess highly ordered mesoporous structure.

Figure 2 presents the nitrogen adsorption−desorption isotherms of Pho200-MS and Phi207-MS samples. All curves

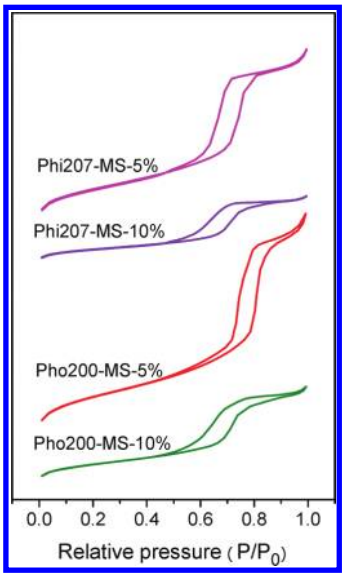

Figure 2. Nitrogen adsorption−desorption isotherms of Pho200-MS and Phi207-MS samples synthesized with different amount of organosilanes in the initial mixture.

can be identified as IV-type isotherms with a H1-type hysteresis loop at relative pressure around 0.55−0.85, validating the existence of open cylindrical mesopores. The BET surface area (S_{BET}), BJH pore diameter (D_p), and total pore volume (V_p) of these samples are summarized in Table S2. It could be noticed that all these parameters gradually decrease with increasing the amount of the organosilane in the initial mixture, probably attributed to the fact that the incorporation of polymer would occupy the space of the nanochannels to some extent. Besides that, all textural parameters of Pho200-MS samples appear larger than those of the corresponding Phi207-MS samples with identical amount of organosilane in the initial mixture. A likely scenario is that the long hydrophobic chains of Pho200-organosilane swelled the core of P123 micelles, therefore generating expanded unit cells for the resulting mesoporous silicas. This coincides with the pore expansion mechanisms proposed concerning TMB and other hydrophobic additives.[23,24]

The polymer content in Pho200-MS and Phi207-MS samples was qualitatively determined by thermal gravimetric (TG) analysis (Figure S4, Table S2). For both Pho200-MS and Phi207-MS samples, enhanced dosage of organosilane in the initial mixture resulted in increased weight loss in TG analysis, which is well consistent with the results of FT-IR as aforementioned, further evidencing a successful incorporation of long-chain polymers into the mesoporous silica.

Water vapor and benzene vapor adsorption experiments were carried out to determine the relative hydrophilicity/hydrophobicity of the Pho200-MS and Phi207-MS samples (Figure S5). Quantitative information with respect to each sample was acquired in terms of the ratio of the maximum adsorption amount of water to benzene, hereafter denoted as adsorbed water/benzene molar ratio (Table S2). It can be seen that water adsorption capacity gradually declined while benzene adsorption capacity concomitantly ascended in a sequence of MS, Phi207-MS-10%, Phi207-MS-5%, Pho200-MS-5%, Pho200-MS-10%, and Phenyl-MS, respectively, corresponding to an adsorbed water/benzene molar ratio of 3.97, 3.94, 2.27, 0.82, 0.41, and 0.22. This result convincingly confirms a considerable alteration from strong hydrophilicity (MS and Phi207-MS-10%), to moderate hydrophilicity (Phi207-MS-5%), moderate hydrophobicity (Pho200-MS-5% and Pho200-MS-10%), and finally strong hydrophobicity (Phenyl-MS). The highest hydrophilicity was given by MS while the highest hydrophobicity was afforded by Phenyl-MS. Phi207-MS and Pho200-MS are within the two boundaries and the hydrophilicity as well as hydrophobicity of the materials could be finely adjusted by controlling respectively the amount of hydrophilic Phi207-organosilane and hydrophobic Pho200-organosilane. That is to say, the hydrophilicity/hydrophobicity of the mesoporous silicas could be willingly and elaborately modulated via introducing polymers bearing specific balances of hydrophilicity/hydrophobicity as well as controlling the incorporation amount of these polymers.

3.2. Lysozyme (LYZ) Adsorption on Mesoporous Silicas with Different Surface Hydrophilicity/Hydrophobicity. The microenvironment (hydrophilicity/hydrophobicity) of Pho200-MS and Phi207-MS samples was investigated via the adsorption of hen egg white lysozyme (LYZ), which is a small globular protein (M_w = 14 400 Da) with a dimension of 3.0 × 3.0 × 4.5 nm^3.[4] In this work, all LYZ adsorption experiments were performed at the isoelectric point (pI = 11) of LYZ, thus rendering hydrophobic interaction the crucial driving force dominating the adsorption of LYZ onto the mesoporous silicas.[7,25−27] Paradoxical arguments have been documented concerning the function of hydrophobic interaction in relation to enzyme adsorption, even referring to simple enzymes such as LYZ.[27,28] To provide more clearly information for this issue, Pho200-MS and Phi207-MS samples with various hydrophobicity/hydrophobicity as well as MS and Phenyl-MS were employed to perform LYZ adsorption experiments. Primarily, LYZ adsorption isotherms were recorded. Thereafter, the adsorption data were analyzed according to the linear form of the Langmuir adsorption equation

$$\frac{C_e}{q_e} = \frac{1}{K_L} + \frac{\alpha_L}{K_L} C_e$$

where K_L (L g^{-1}) and α_L (L $mmol^{-1}$) are the Langmuir parameters, C_e (mM) is the LYZ concentration remaining in the solution at equilibrium, q_e (mmol g^{-1}) is the LYZ concentration in the solid adsorbent at equilibrium, and K_L/α_L gives the theoretical monolayer saturated adsorption amount

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of LYZ (Q_0). Figure S6 shows the plots of specific sorption (C_e/q_e) against C_e with regard to different mesoporous silicas. It can be seen that Langmuir isotherm can accurately describe LYZ adsorption behaviors on all these samples since their correlation coefficients (R^2) are all in excess of 0.99, which means that the hydrophilicity/hydrophobicity of the support has a negligible impact on LYZ adsorption type in the range involved in our research.

It has been widely recognized that the adsorption capacity of LYZ is sensitive to the surface hydrophilicity/hydrophobicity of the absorbent; however, confusing results have been proposed that whether LYZ favoring hydrophobic microenvironment or hydrophilic microenvironment, principally due to the criteria adopted in the evaluation.[27,28] As presented in Table 1, Q_w denoting the tentative amount of LYZ immobilized on per unit weight of support does not exhibit notable difference for Pho200-MS-10% and Phi207-MS-10%. Q_0 also defined with weight unit but referring to the theoretical monolayer adsorption capacity of LYZ (derived from the ratio of K_L/α_L as aforementioned) suggests that Pho200-MS-10% has a slightly higher loading capacity compared with Phi207-MS-10%. However, the question is that when employing the weight unit to normalize the adsorbed amount of LYZ, there would be a great influence from the specific surface area of the support. That is, a larger surface area profits a higher adsorption capacity in view of the monolayer adsorption pattern for LYZ molecules suggested by the Langmuir-type adsorption isotherms. Herein, we established our evaluation criterion in terms of the value of Q_s, which stands for the enzyme loading capacity at equilibrium per unit surface area of the support determined experimentally. As presented in Table 1, the Q_s of Phi207-MS-10% (0.17 μmol m^{-2}) is visibly higher than that of Pho200-MS-10% (0.11 μmol m^{-2}), clearly demonstrating an essential superiority of hydrophilic microenvironment over hydrophobic microenvironment in LYZ adsorption, quite opposite to the conclusion derived from the comparison of Q_w or Q_0 values. Meanwhile, it should be noted that the obviously higher value of α_L for Phi207-MS-10% also indicates a stronger interaction between LYZ and the hydrophilic support.[29]

To further elucidate the effect of hydrophilicity/hydrophobicity of the support on LYZ immobilization, LYZ adsorption experiment as a function of time was also carried out (Figure 3). Taking textural properties of these samples into consideration, the pore size, specific surface area, and pore volume of Pho200-MS-10% and Phenyl-MS are all evidently larger than that of MS, which should favor a much easier adsorption process and accordingly a more rapid adsorption rate for LYZ owing to the less diffusion limitations. However, the fact was that the kinetics curve of Phi207-MS-10% featured a sharp initial rise in adsorption amount and an adsorption plateau stage, inferring a high affinity between LYZ and the hydrophilic surface. As for Pho200-MS-10% and Phenyl-MS, the initial adsorption rate was much lower and the adsorption amount of LYZ continuously increased with time without the appearance of a saturation state, suggesting a weak surface–protein affinity.[25] Taken together, these results further manifest that LYZ molecule has a favoring proclivity to the hydrophilic support other than hydrophobic support, which is very reasonable in view of the hydrophilic nature of the external surface of LYZ.[26]

Table 1. Adsorption Capacity and Parameters of Langmuir Sorption Isotherms for LYZ Adsorption onto Pho200-MS-10% and Phi207-MS-10%

	Q_w[a] (μmol g^{-1})	Q_0[b] (μmol g^{-1})	Q_s[c] (μmol m^{-2})	α_L[d] (L $mmol^{-1}$)
Pho200-MS-10%	20.86	22.20	0.11	135.5
Phi207-MS-10%	20.71	19.03	0.17	352.6

[a]The amount of LYZ molecules immobilized on per unit weight of support in the adsorption experiment. [b]The theoretical adsorption capacity of LYZ per unit weight of support according to the value of K_L/α_L in Langmuir adsorption equation. [c]The tentative amount of LYZ adsorbed on per unit area of solid surface. [d]Parameter in Langmuir adsorption equation.

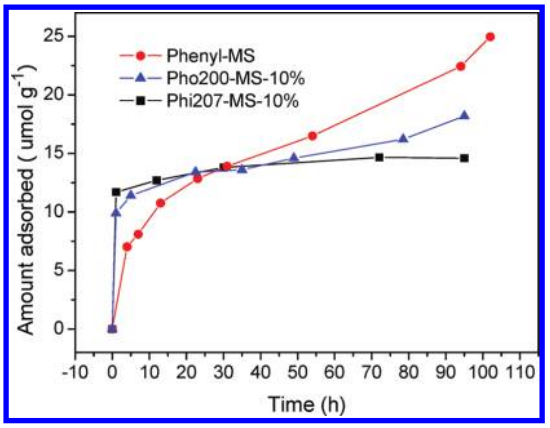

Figure 3. LYZ adsorption onto Phi207-MS-10%, Pho200-MS-10%, and Phenyl-MS as a function of time.

3.3. Lipase Immobilized on Mesoporous Silicas with Different Surface Hydrophilicity/Hydrophobicity. The great potentials of lipases in chemical and pharmaceutical industries have been recognized in recent years because of their excellent performance in both hydrolytic and synthetic reactions.[30] In our research, lipase from *Pseudomonas cepacia* (PCL) was chosen to explore the impact of the support hydrophilicity/hydrophobicity on the catalytic performance of the immobilized enzyme. In this section, four typical mesoporous silicas—MS, Phi207-MS-10%, Pho200-MS-10%, and Phenyl-MS—with diverse hydrophobicity were employed.

It has been documented that the adsorption isotherms of lipase onto various functionalized mesoporous silicas all displayed characteristic Langmuir shape,[17,31] which implies that the adsorbed amount of PCL could also be normalized in term of the specific surface area of the support to acquire the intrinsic affinity between lipase and the support. For this reason, the PCL adsorption amount per surface area was calculated for each sample and illustrated in Figure 4A. It can be seen that Pho200-MS-10% with adsorbed water/benzene molar ratio of 0.41 afforded the highest adsorption amount; neither the more hydrophobic Phenyl-MS nor the hydrophilic MS and Phi207-MS could achieve a higher adsorption capacity. Based on the postulation that other than electrostatic interaction or hydrogen bonding the hydrophobic interaction is the driving force for the adsorption of lipase onto mesoporous silicas,[17] it is rational to assume that the difference in PCL adsorption amount is directly associated with the difference in support hydrophilicity/hydrophobicity. The maximum PCL loading capacity observed for Pho200-MS-10% is considered to profit

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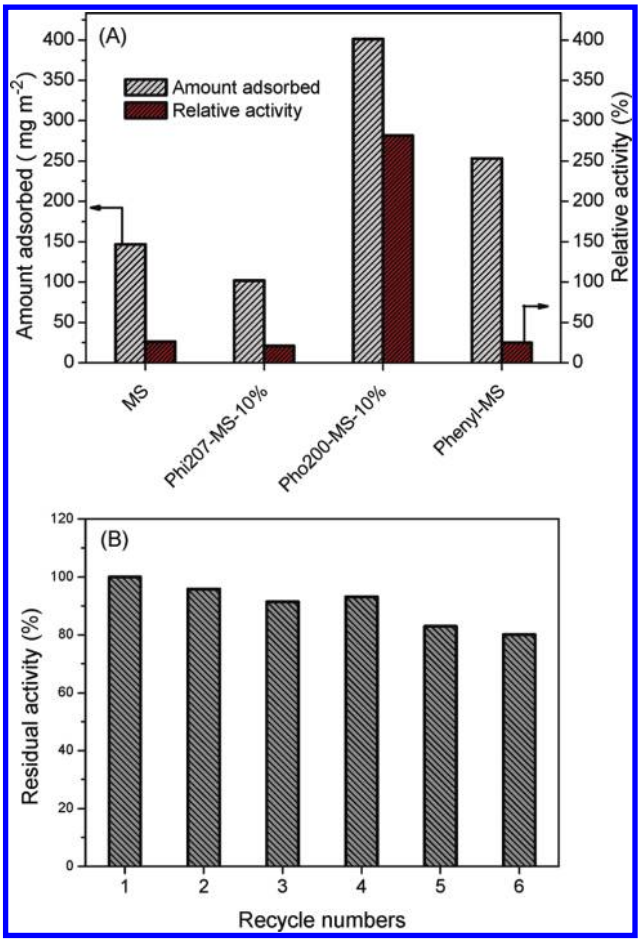

Figure 4. (A) Adsorbed amount and relative activities of PCL immobilized on mesoporous silicas with different microenvironment. (B) Reusability of PCL immobilized on Pho200-MS-10% in the hydrolysis of triacetin.

from the moderate hydrophobic microenvironment (MHM) of the support.

Activity assay of PCL was performed by hydrolysis of triacetin in an aqueous solution at 30 °C.[22] Figure 4A exhibits the relative activities of PCL immobilized on the four representative mesoporous silicas with the activity of free PCL taken as 100%. Relative activities of 26.2%, 20.7%, 281%, and 24.8% for PCL entrapped in MS (MS-PCL), Phi207-MS-10% (Phi207-MS-10%-PCL), Pho200-MS-10% (Pho200-MS-10%-PCL), and Phenyl-MS (Phenyl-MS-PCL) were obtained, respectively. The high activity of Pho200-MS-10%-PCL is supposed to be correlated with the "interfacial activation" of PCL, which has been evidenced by X-ray crystallography and computer simulation.[9−11,32] That is, when PCL or many other lipases are adsorbed on a hydrophobic interface, an amphiphilic α-helical loop, or "lid", covering the active site of the lipase in the native state (close state), will roll back and bring about a full access to the catalytic triad (open state), therefore resulting in a markedly increase in lipase activity. Meanwhile, the conformational change of the lid is accompanied by a substantial augment in the nonpolar area surrounding the active site. Specifically, in the natural state, the hydrophilic side of the lid is the external surface exposed to the aqueous solution while the hydrophobic side is the internal surface toward the active groove. However, the interfacial activation results in a complete exposure of the hydrophobic side and a partly burial of the hydrophilic side, which greatly expands the nonpolar surface around the catalytic triad by ~750 A^2.[10] Therefore, there would be three patterns depending on the microenvironment surrounding the immobilized PCL: (1) If PCL molecules are entrapped in a hydrophilic microenvironment (MS and Phi207-MS-10%), the lid would shield the active site from the substrates as in the native state, thus producing a depressed activity. (2) When PCL molecules are adsorbed on a support with MHM (Pho200-MS-10%), the movement of the lid would uncover the catalytic triad and induce a remarkable increment in PCL activity. (3) However, as for the support with too strong hydrophobicity (Phenyl-MS), the intense hydrophobic interaction between PCL and the support would not only open the lid but also interact with and disturb the catalytic triad environed by a vast hydrophobic surroundings in the "open state" as discussed above, therefore leading to a conformation destruction of the active site and consequently a pronounced decrease in PCL activity.[31] In a word, the activity of immobilized PCL is heavily determined by the degree of support hydrophobicity. The key point to obtain a significant enhancement in lipase activity is the solely conformational change of the lid without involving the active center in the interfacial activation. That is to say, the degree of support hydrophobicity should be well controlled to a certain range; otherwise, a straight interaction and destruction relating to the active center would take place. In this work, taking advantage of water and benzene adsorption experiments, we quantitatively determined the degree of support hydrophobicity in terms of the adsorbed water/benzene molar ratio as aforementioned. Therefore, a tentative conclusion could be drawn that a moderate hydrophobicity with an adsorbed water/benzene molar ratio of 0.41 could promote a conspicuous increase in PCL activity, while a higher degree of hydrophobicity with an adsorbed water/benzene molar ratio of 0.22 will lower the PCL activity, validating that there is a transition point concerning the degrees of support hydrophobicity which lies in the range of 0.22−0.41 defined via the water/benzene adsorption ratio.

Figure 4B presents the reusability of PCL immobilized on Pho200-MS-10% in hydrolysis of triacetin. More than 80% of the initial activity remained for Pho200-MS-10%-PCL after six consecutive runs, demonstrating a great advantage of the immobilized PCL over the free PCL solution in recycling without obviously compromising its activity.

The thermal stability of the free PCL and the immobilized PCL was assayed by measuring their residual activities at 30 °C after the incubation at an elevated temperature (in a temperature range of 40−70 °C). As presented in Figure 5, the activity of free PCL declined distinctly with incubation temperature and merely 20% of its initial activity was preserved after incubation at 70 °C. However, Pho200-MS-10%-PCL retained more than 90% of its initial activity even after the thermal treatment at 60 °C and not until 70 °C did a visible loss in activity was observed (still remained 58% of the initial activity). The residual activities of Phenyl-MS-PCL after suffering the thermal incubation turned out evidently lower than that of the free PCL. With regard to the two hydrophilic supports, MS-PCL presented a parallel thermal stability to the free PCL while PCL immobilized on Phi207-MS-10% which had a comparable hydrophilicity to MS yielded a much depressed resistance to temperature increment. According to the lower adsorption capacity observed for Phi207-MS-10% than for MS (Figure 4A), a rational interpretation could be postulated that in the case of Phi207-MS-10% PCL molecules

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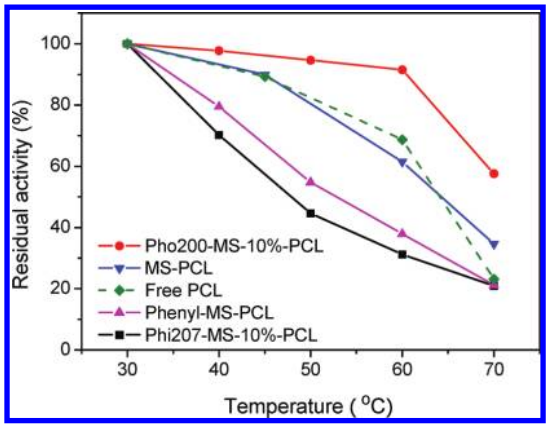


Figure 5. Residual activity of the free PCL (dashed) and the PCL immobilized on mesoporous silicas with different microenvironment (solid) after the incubation at different temperatures for 2 h.

were generally adsorbed on the external surface of the support or crowded at the entrance of the mesopores other than migrating into the inner mesochannels, ascribed to the small pore diameter (6.5 nm) and the existence of the long polymer chains in the mesopores. Therefore, PCL immobilized on Phi207-MS-10% gained little protection from silica frameworks and accordingly more vulnerable to deleterious circumstance than MS-PCL did. The above results indicate that Pho200-MS-10%-PCL was thermally more stable than the free PCL and the PCL immobilized on other supports. The improved resistance to thermal inactivation of Pho200-MS-10%-PCL is reconciled with previous reports that the hydrophobic support shows better stability against thermal inactivation.[15,31] This is easy to comprehend considering the extensive hydrophobic surroundings of the active center in the "open state", which reduced the pliability of the enzyme molecules due to the decreased water content and allowed a multipoint hydrophobic attachment of enzyme to the support, therefore promoting a rigidification of the enzyme structure.[15,33−35] As for Phenyl-MS-PCL, we conjectured it was the strong hydrophobicity of the support that responsible for the low thermal stability. The "open state" conformation would render the active site with a deformed structure more susceptible to the external environment, attributed to the loss of the shield provided by the lid in the "close state".[9] In brief, the above discussion clarifies a correlation between the support hydrophobicity and the thermal stability of the immobilized PCL. The support with MHM is preferable for an improvement in PCL thermal stability.

3.4. Kinetic Resolution of (*R*,*S*)-1-Phenylethanol Catalyzed by Lipase Immobilized on Mesoporous Silicas with Different Surface Hydrophilicity/Hydrophobicity.

In addition to the outstanding performance in the hydrolysis reaction, lipase is also an excellent chiral biocatalyst for highly enantioselective resolution of racemic substrates in nonpolar media. To figure out the relationship between support hydrophobicity and the catalytic activity of the immobilized PCL, the kinetic resolution of racemic 1-phenylethanol with vinyl acetate as the acyl donor and hexane as the reaction medium was performed at 30 °C over the four typical immobilized PCL. (Since the existence of water is known to retard the esterification activity of lipase, the catalytic performance of the free PCL solution was not concerned.) As shown in Figure 6A and Table 2, it is not surprising to

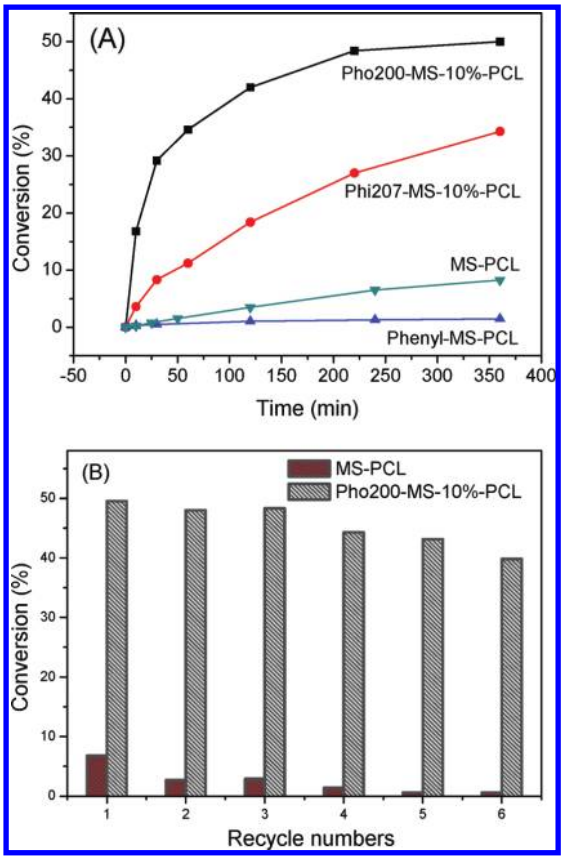
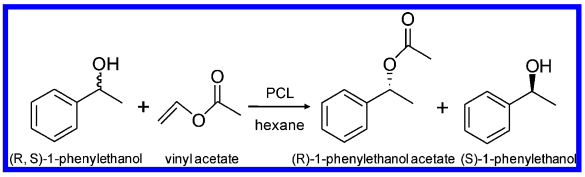


Figure 6. (A) Conversions as a function of time for the immobilized PCL and (B) the reusability of PCL immobilized on MS and Pho200-MS-10% in the kinetic resolution of (*R*,*S*)-1-phenylethanol.

Table 2. Kinetic Resolution of (*R*,*S*)-1-Phenylethanol Catalyzed by the Immobilized PCL[a]



catalyst	initial rate (mmol/(min $mg_{protein}$))	conv[b] (%)	ee[c] (%)
Pho200-MS-10%-PCL	0.225	50.0	99
Phi207-MS-10%-PCL	0.055	34.3	99
MS-PCL	0.005	8.2	99
Phenyl-MS-PCL	0.002	1.5	99

[a]Typical reaction conditions: (*R*,*S*)-1-phenylethanol (0.5 mmol) and vinyl acetate (2.0 mmol) were stirred in 5 mL of dry hexane at 30 °C. [b]Conversion after 6 h. [c]Determined in terms of the ester product.

notice that Pho200-MS-10%-PCL displayed the highest initial rate (0.225 mmol/(min $mg_{protein}$)) and conversion (49.5%) in comparison with other immobilized PCL. The lowest catalytic activity was given by Phenyl-MS-PCL, which is probably attributed to its destructive catalytic triad owing to the strong hydrophobicity of the support as mentioned above. Phi207-MS-10%-PCL exhibits enhanced initial rate (0.055 mmol/(min $mg_{protein}$)) and conversion (34.3%) compared to MS-PCL. This is probably due to the distribution of the enzyme molecules (mainly on the outside surface of Phi207-MS-10% as aforementioned), which afforded a free access of the reactants

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to the enzyme molecules. All of the four immobilized PCL acquired more than 99% enantiomeric excess (ee) to yield the (*R*)-1-phenylethanol acetate irrespective of the hydrophilic/hydrophobic properties of their supports (Table 2). Meanwhile, it should be mentioned that to clearly distinguish the discrepancy among divergent immobilized PCL, the reaction bulk in our experiment was far from saturated by biocatalyst. Increasing the addition amount of the immobilized PCL, Pho200-MS-10%-PCL for example, the reaction time could be dramatically reduced to less than 1.5 h (data not shown) other than 6 h to reach the complete conversion.

The influence of the support hydrophilicity/hydrophobicity on the reusability of the immobilized PCL in kinetic resolution of (*R*,*S*)-1-phenylethanol was also investigated represented by Pho200-MS-10%-PCL and MS-PCL (Figure 6B). In both cases, no visible loss in enantioselectivity was observed (data not shown). However, the conversion of MS-PCL presented an apparent decrease from 6.8% to 0.5% after six successive recycles, whereas Pho200-MS-10%-PCL still remained a conversion of 39.8%, clearly demonstrating that Pho200-MS-10% with MHM shows a higher recycling stability relative to MS with hydrophilic mesochannels. There are generally two reasons for the observed decrease in conversion: the deformation of protein structure and enzyme leaching. The FT-IR spectra of the Pho200-MS-10%-PCL and MS-PCL after six recycles (Pho200-MS-10%-PCL-recycle and MS-PCL-recycle, respectively) and the fresh PCL solution (free PCL) are presented in Figure S7. The amide I band (1650 cm^{-1}) and amide II band (1540 cm^{-1}) observed for the free PCL were related to the secondary structure of the lipase. It can be seen that there was no shift of the two characteristic bands with regard to the Pho200- MS-10%-PCL-recycle, indicating the protein conformation of PCL accommodated in Pho200-MS-10% was maintained even after six successive recycles. Therefore, the lipase leaching is probably accounting for the observed decrease in conversion of Pho200-MS-10%-PCL after six recycles. As for the MS-PCL-recycle, a shift of the amine I band (from 1650 to 1638 cm^{-1}) could be clearly discerned in the FT-IR spectrum, demonstrating the deformation of the enzyme structure was also an important factor leading to the decrease in conversion observed for MS-PCL.

4. CONCLUSIONS

In summary, the mesoporous silicas functionalized with hydrophilic or hydrophobic long-chain polymer were synthesized by co-condensation method in a mild acidic medium. The results of the water and benzene adsorption experiments validated that the modification with polymer bearing specific balance of hydrophilicity/hydrophobicity provides a facile approach to endow the microenvironment of the mesoporous silicas with desired hydrophilicity/hydrophobicity. A series of LYZ adsorption experiments indicated that the hydrophilic microenvironment has a stronger affinity toward LYZ, therefore preferring a more rapid adsorption rate and a higher adsorption capacity. More importantly, the typical mesoporous silicas possessing intense hydrophilicity, intermediate hydrophobicity, and intense hydrophobicity were chosen to explore the influence of the support hydrophilicity/hydrophobicity on the catalytic performance of the immobilized PCL. The results convincingly demonstrated that PCL immobilized on the support with MHM afforded the best performance, not only in hydrolytic activity but also in adsorption capacity, thermal stability, reusability, and even the catalytic activity in nonpolar solvent. Our research suggests that the highly stable, active, and recyclable heterogeneous biocatalysts could be achieved by accommodating enzyme molecules into the solid supports with finely turned microenvironment via polymer modification.

■ ASSOCIATED CONTENT

Supporting Information

FT-IR spectra of synthesized organosilanes, TG analysis, water vapor and benzene vapor adsorption isotherms, and linear-fitted LYZ adsorption isotherms of the mesoporous silicas with various hydrophobicity/hydrophilicity. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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