Pressure-Driven Enzyme Entrapment in Siliceous Mesocellular Foam

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Received September 14, 2005. Revised Manuscript Received November 29, 2005

A pressure-driven method was employed to immobilize enzymes on hydrophobic mesoporous silica supports. This approach gave rise to higher enzyme loading and reduced enzyme leaching, compared to conventional methods. *Candida antarctica* lipase B (CALB) was successfully entrapped in the cagelike pores of siliceous mesocellular foam using this method. The resulting supported enzyme catalyst demonstrated reusability and thermal stability superior to those of the commercial immobilized CALB catalyst, Novozyme 435.

Introduction

Enzyme-catalyzed asymmetric reactions have emerged as one of the most important fields in organic and pharmaceutical syntheses.[1−10] For example, the lipase-catalyzed kinetic resolution of racemic mixtures is a common way to achieve enantiomerically pure compounds.[1,2] Immobilization of enzymes onto solid supports may render these catalysts more mechanically robust and thermally stable and allow for ease of separation from the reaction media.[3−8] Through immobilization, the catalytic activity of these systems may also be enhanced as a result of reduced enzyme aggregation, especially in nonpolar organic media.[9,10]

Previous studies have revealed that the activity of the immobilized enzyme would be affected by many factors, such as the internal solvent environment, the interaction of the enzyme with the host materials, and the enzyme accessibility.[4] The sol−gel process is generally used to immobilize enzymes in an inorganic matrix.[9] However, the sol−gel derived solids do not usually possess well-defined pore sizes, which are important in ensuring enzyme accessibility to achieve high catalytic activity.[11] In contrast, mesoporous silica materials are excellent candidates as supports for enzymes as a result of their large surface areas, high pore volumes, and well-defined pore sizes (2−50 nm).[12−16] The pore diameters of mesoporous silica can be tailored to host specific enzymes according to their dimen-

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10.1021/cm0520618 CCC: $33.50
Published on Web 01/07/2006

sions. Different functional groups can also be grafted on the surface of mesoporous silica by reacting with the silanols. These surface functional groups can also impact the enzyme activity. For example, the introduction of hydrophobic functionalities on sol−gel derived silica has improved the esterification activity of the immobilized enzymes in organic media by 5 orders of magnitude, compared to free enzymes.[9] The hydrophobicity of the support promoted the access of the substrates to the enzymes, and the weak hydrophobic interaction between the enzymes and the support allowed the former to maintain their active conformation.[17] Because most enzyme-catalyzed reactions are carried out in organic media,[9,10] it would be important to develop an efficient method for immobilizing enzymes onto hydrophobic mesoporous silica. Conventionally, enzyme molecules have been entrapped by stirring the porous silica support particles in the enzyme stock solution.[7] Relatively low enzyme loading has been attained as a result of the low affinity of the aqueous enzyme solution with the hydrophobic silica support. Significant enzyme leaching from the support has also been observed, diminishing the reusability of the conventionally immobilized enzyme catalysts.

Besides the immobilization method, the choice of mesoporous silica structures has been rather limited in terms of pore size and pore connectivity. Entry of enzymes into the pores may be restricted by small pore dimensions, while enzyme leaching may be severe when the pores are too large relative to the enzyme molecule. Three-dimensional pore connectivity[12,13] is preferred over one-dimensional channel-like pores[14] because it would provide for greater substrate accessibility to the immobilized enzymes during catalytic reactions.

Herein we describe a multifaceted design of immobilized enzyme catalysts, which involved the selection of siliceous mesocellular foam (MCF) as the porous support, the morphological control of the support particles, the modification of the support surface, and the use of a pressure-driven method for entrapping enzymes. Instead of stirring the MCF particles in the enzyme solution, a high pressure (3000−5000 psi) was used to achieve high enzyme loading within the support (up to 275 mg/g of silica). The resulting catalyst demonstrated high activity, little enzyme leaching, and enhanced thermal stability. It was used to set up an efficient packed bed reactor for the kinetic resolution of 1-phenylethanol acylated with isopropenyl acetate.

Experimental Section

Materials. Spherical MCF particles[16] were synthesized by modifying the literature procedure,[12b] which would otherwise give rise to irregular particle morphology. Typically, 4 g of triblock copolymer P123 (BASF) was dissolved in an acidic solution (10 mL of HCl and 65 mL of H_2O). A total of 3.4 mL of 1,3,5-trimethylbenzene (Aldrich) was then added, and the resulting solution was heated to 37−40 °C with vigorous stirring. After 2 h of stirring, 9.2 mL of tetraethoxysilane (Aldrich) was added and stirred for 5 min. The solution was next aged at 40 °C for 20 h under a static condition. NH_4F (46 mg, Aldrich) in water (5 mL) was added to the solution, which was then transferred to an autoclave for aging at 100 °C for 24 h. The resulting precipitate was filtered, washed with water and ethanol, and dried. The white powder obtained was calcined at 550 °C in air for 6 h. For comparison, another mesoporous silica, FDU-12, was prepared according to the literature[13] (see details in Supporting Information). The free enzyme, *Candida antarctica* lipase B (CALB), was purchased from Roche. Novozyme 435, a commercial CALB catalyst immobilized on a polyacrylamide support, was provided by Novo Nordisk. Triethylamine, chlorodimethyloctylsilane, and chlorodimethyloctadecylsilane were purchased from Aldrich. 1-Phenylethanol and isopropenyl acetate were purchased from Sigma.

Characterization of Materials. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed on a JEOL JEM-3010 electron microscope (300 kV) and a JEOL JSM-7400F electron microscope (5 kV), respectively. Nitrogen sorption isotherms were obtained using a Micromeritics ASAP 2020M system; the samples were degassed at 100 °C for 40 h before the measurements. Photoacoustic Fourier transform infrared (PA-FTIR) spectra were recorded on a Digilab FTS 7000 FTIR spectrometer equipped with a MTEC-300 photoacoustic detector. The carbon, hydrogen, and nitrogen contents in the immobilized enzyme catalysts were examined with a CE440 CHN analyzer (Exeter Analytical).

Surface Modification of Mesoporous Silica. MCF and FDU-12 were rendered hydrophobic by surface treatment with long-chain alkyl groups. After degassing at 150 °C under vacuum overnight, MCF (3.0 g) was suspended in toluene (40 mL). Triethylamine (1.67 mL, 12.0 mmol) and chlorodimethyloctylsilane (1.42 mL, 6.0 mmol) were then added sequentially with stirring. The suspension was stirred at 60 °C for 24 h and filtered. The solid was washed with toluene, methanol, and acetone several times and dried under vacuum. The resulting modified MCF material was termed C_8-MCF. C_{18}-MCF was prepared by a similar procedure except that chlorodimethyloctadecylsilane (2.08 g, 6.0 mmol) was used instead of chlorodimethyloctylsilane. C_8-FDU-12 was prepared by the same procedure as C_8-MCF except that FDU-12 (3.0 g) was used instead of MCF.

Conventional Enzyme Immobilization. The modified mesoporous silica (0.6 g) was stirred rigorously in 50 mL of CALB stock solution (8 mg/mL) for 24 h. The suspension was filtered and washed with deionized water and hexane. After drying under vacuum, the sample was subjected to C, H, and N analyses for enzyme loading, based on the N content in the free enzyme. The result was confirmed by measuring the protein content in the supernatant post-immobilization via Bradford assay.

Enzyme Immobilization by the Pressure-Driven Method. The modified mesoporous silica (0.6 g) was dispersed in 2-propanol and packed into a high-performance liquid chromatography (HPLC) column (100 mm × 4.6 mm) using a slurry packer. After the 2-propanol in the column was removed thoroughly by washing with water, the enzyme stock solution (50 mL, 8 mg/mL) was cycled through the pre-packed silica column for 2 h under a high pressure of ∼4000 psi using a slurry packer. The enzyme-loaded mesoporous silica was then collected from the column, washed with deionized water, and dried under vacuum.

Catalytic Reaction. Kinetic resolution of 1-phenylethanol acylated with isopropenyl acetate was used to assess the catalytic activity. Through kinetic resolution, (*R*)-1-phenylethanol was selectively reacted, whereas (*S*)-1-phenylethanol remained unreacted because of differences in the transformation rates (Scheme 1). In

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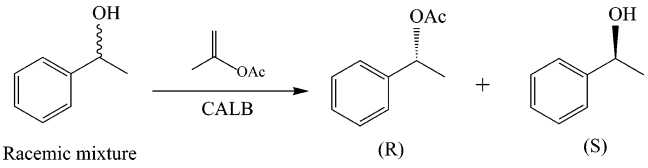
Scheme 1. Kinetic Resolution of 1-Phenylethanol with Isopropenyl Acetate by CALB



this reaction, an amount of catalyst containing a total CALB loading of 10 mg was dispersed in dry toluene (15 mL). 1-Phenylethanol (10.8 mmol) and isopropenyl acetate (17.4 mmol) were then added sequentially at room temperature. The reaction was monitored with gas chromatography (GC) until complete conversion was achieved. The enantiomeric excess (% ee) was then determined by HPLC. After 10 h of reaction, the catalyst was filtered, washed several times with toluene, and dried under vacuum. The dried catalyst was reweighed so as to determine the amount of substrates to be introduced to the recovered catalyst in the next reaction run.

Packed Bed Reactor. CALB was immobilized onto C_{18}-MCF by the pressure-driven method within a 250 mm × 4.6 mm HPLC column, which was then used directly as a packed bed reactor. A mixture of 1-phenylethanol (0.65 M) and isopropenyl acetate (0.97 M) solutions in toluene was continuously flowed through the packed bed reactor for 2 days. At the start of the reaction, the flow rate was adjusted to achieve full conversion of (*R*)-1-phenylethanol. The exit stream was continuously analyzed with GC to monitor the conversion.

Results and Discussion

Characterization of Mesoporous Silica Supports. MCF and FDU-12 were chosen as the support materials for this enzyme immobilization study for several reasons. First, they have large and tunable pore sizes (20−50 nm and 10−15 nm, respectively), which can accommodate enzymes of different dimensions. Thus, they are different from most of the mesoporous silica materials, which are <10 nm in pore sizes and limited to the hosting of only small enzymes within their pores.[18] Second, MCF and FDU-12 both possess a three-dimensional, interconnected pore structure, which would facilitate mass transfer and molecular diffusion. Third, their pores are cagelike and interconnected by windows smaller than the diameter of the cage itself.[12,13] This would allow for the entrapment of enzymes with sizes close to (or slightly larger than) that of the windows (Scheme 2), while minimizing enzyme leaching from the pores due to the restriction of the small windows.

Considering the dimensions of CALB (6.9 nm × 5.0 nm × 8.7 nm), MCF and FDU-12 with window sizes of ∼10 nm were prepared in this study by controlling the synthesis conditions (see details in Experimental Section and Supporting Information). Conventional MCF has an irregular particle morphology with a very broad particle size distribution.[12] By modifying the synthesis conditions,[16] we have derived spherical MCF particles with a fairly uniform particle size of ∼5 μm (Figure 1a). Figure 1b illustrates the high porosity and uniform pore size of the spherical MCF particle. The MCF sample was ∼25 nm in pore diameter (Figure 1c), which was consistent with the average pore size (26.7 nm) obtained from

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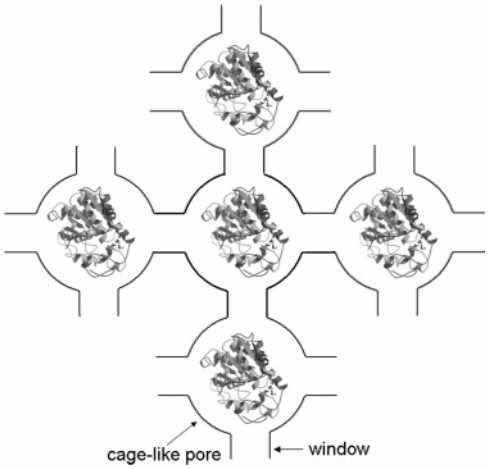
Scheme 2. Enzymes Entrapped in the Cagelike Mesopores of MCF and FDU-12[a]



[a] This scheme gives an ideal representation of the entrapment of enzymes in the cagelike pores. The hydrophobic interaction between the enzymes and the long-chain alkyl groups (C_8 or C_{18}) on the silica surface has been omitted.

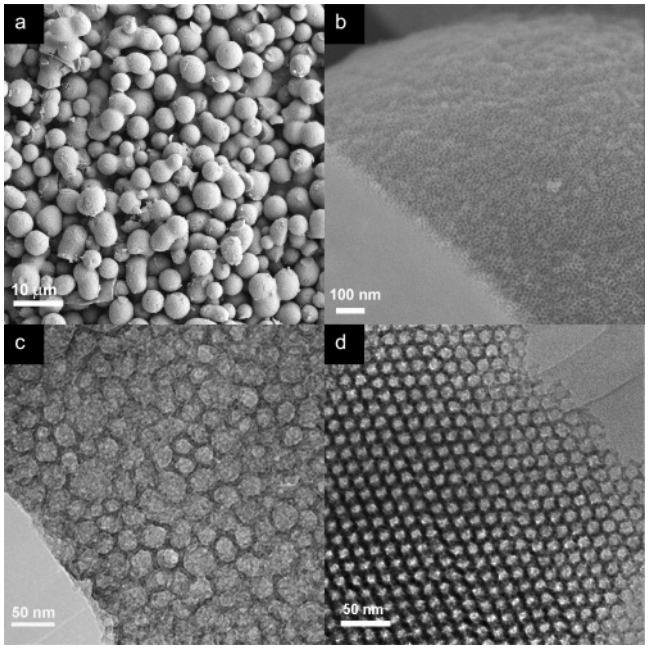


Figure 1. SEM micrographs of (a) spherical MCF particles and (b) a single spherical MCF particle at high magnification. TEM micrographs of (c) MCF and (d) FDU-12 taken at [110] incidence.

the N_2 adsorption isotherm (Figure 2). The window size was determined from the N_2 desorption isotherm to be 14.5 nm by the simplified Broekhoff-de Boer (BdB-FHH) method.[15] The MCF particles possessed a pore volume of 2.16 cm^3/g and a BET surface area of 535 m^2/g (see Table 1).

Calcined MCF showed a PA-FTIR spectrum typical of silica (Figure 3a). After modification with C_{18}, new PA-FTIR peaks associated with C−H vibration were observed at ∼2900 and 1450 cm^{-1}, while the silanol (SiO−H) peak at 3750 cm^{-1} was significantly reduced (Figure 3b). The modification of MCF with C_8 and C_{18} groups decreased both the pore diameter and the window diameter by ∼2 nm and ∼4 nm, respectively (see Table 1). Although C_8-MCF and C_{18}-MCF showed lower surface area and pore volume than the unmodified MCF (see Table 1), they were still highly

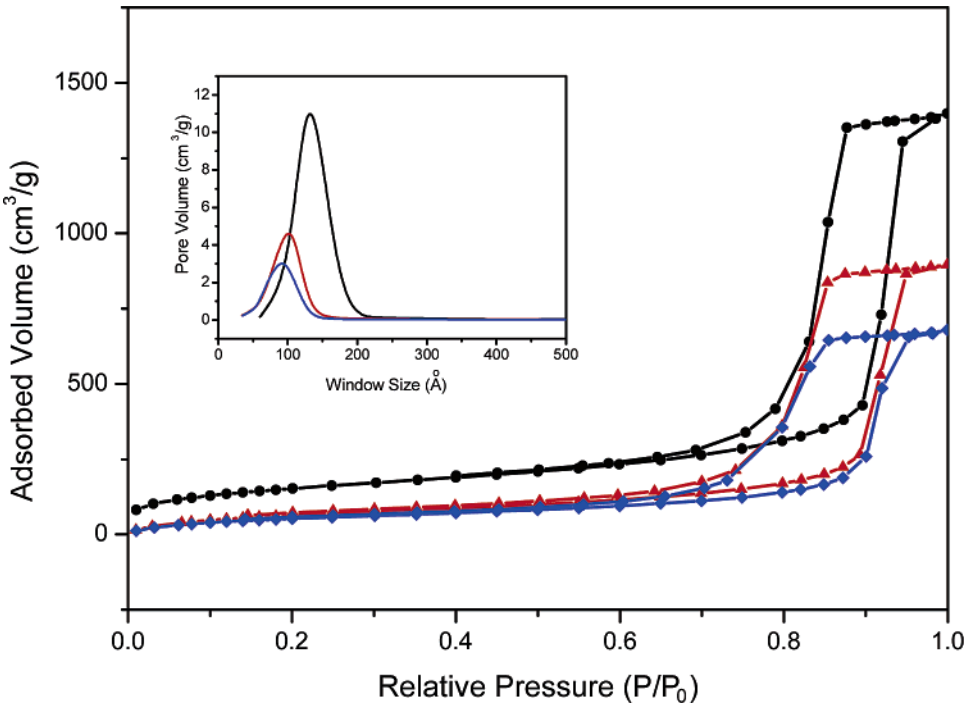


Figure 2. Nitrogen adsorption−desorption isotherms and (inset) window size distributions of (●) MCF, (▲) C_{18}-MCF, and (♦) CALB/C_{18}-MCF prepared by the pressure-driven method. The window sizes were calculated from the desorption branches of the N_2 sorption isotherms based on the BdB sphere model.[15]

Table 1. Characteristics of the Mesoporous Silica Materials before and after Modification and Loading with CALB by the Pressure-Driven Method[a]

	pore size (nm)	window size (nm)	surface area (m^2/g)	pore volume (cm^3/g)
MCF	26.7	14.5	535	2.16
C_8-MCF	24.3	12.5	332	1.60
C_{18}-MCF	22.5	10.6	252	1.27
CALB/C_{18}-MCF	20.0	9.3	208	1.05
FDU-12	14.0	9.0	509	1.25
C_8-FDU-12	12.0	7.5	319	0.81
CALB/C_8-FDU-12	12.0	6.0	194	0.50

[a] Determined from N_2 sorption isotherms. The pore sizes were calculated from the adsorption branches based on the BdB sphere model. The window sizes were calculated from the desorption branches based on the BdB sphere model.

porous with narrow pore size distributions (see Figure 2).

FDU-12 has a pore size and a window size of 14 and 9.0 nm, respectively (Figure 1d, Table 1). Its pore volume and BET surface area were 1.25 cm^3/g and 509 m^2/g, respectively. FDU-12 was modified only by C_8, which decreased the window size to 7.5 nm. The window size would be too small to entrap CALB if FDU-12 were to be modified by C_{18}.

Enzyme Loading. Compared to the conventional approach, the pressure-driven method resulted in significantly higher enzyme loading on C_8-MCF, C_{18}-MCF, and C_8-FDU-12 within a shorter period of time (Figure 4). For example, 24 h of stirring led to an enzyme loading of 92 mg/g onto C_8-MCF, whereas an enzyme loading of 275 mg/g onto C_8-MCF was achieved in 2 h with the pressure-driven method. C_8-FDU-12, which has a smaller pore size and pore volume than C_8-MCF, only provided about half the enzyme loading as C_8-MCF. However, C_8-FDU-12 also demonstrated much higher enzyme loading by the pressure-driven method

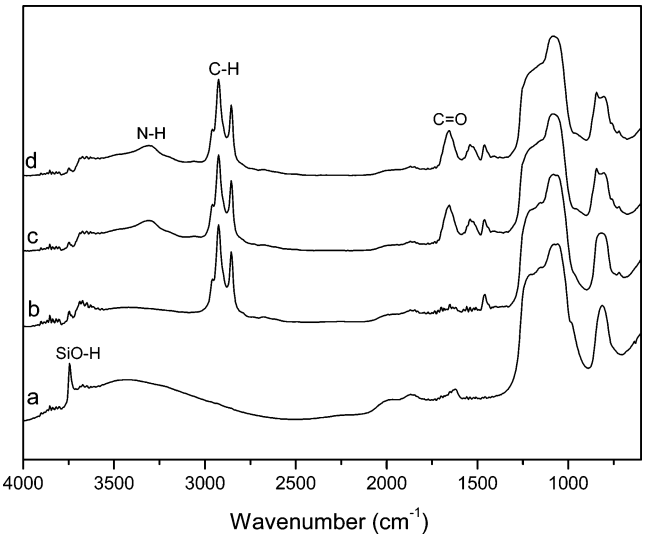


Figure 3. PA-FTIR spectra of (a) calcined MCF, (b) C_{18}-MCF, and CALB/C_{18}-MCF prepared by the pressure-driven method (c) before and (d) after thorough washing with 1000 mL of deionized water.

than by the conventional method (Figure 4). When the concentration of CALB stock solution was reduced from 8 to 4 mg/mL, the resulting enzyme loading was slightly lowered in all cases. Nevertheless, the pressure-driven method still loaded significantly more enzymes onto the three different hydrophobic mesoporous supports than the conventional stirring approach. Thus, while the enzyme loading was affected by the concentration of enzyme stock solution, it was most significantly impacted by the method of enzyme loading. When the pressure-driven method was used, substantially more enzymes could be loaded onto the mesoporous silica supports, especially those that have ultralarge pore sizes and high pore volumes. The aqueous enzyme stock solution possibly did not have a high affinity with the

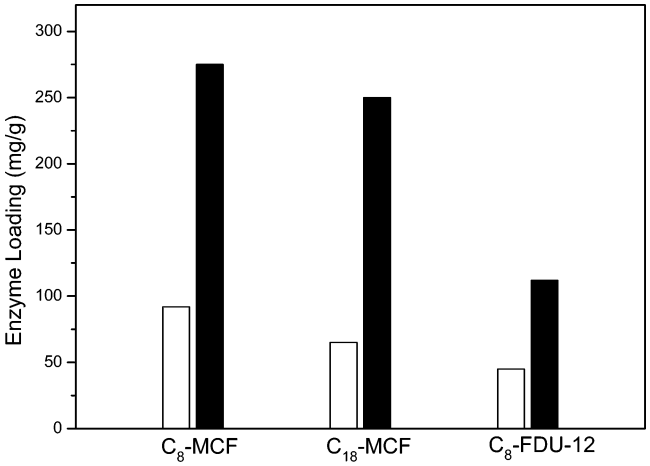


Figure 4. CALB loading on the modified mesoporous silica supports using (□) the conventional stirring method and (■) the pressure-driven method.

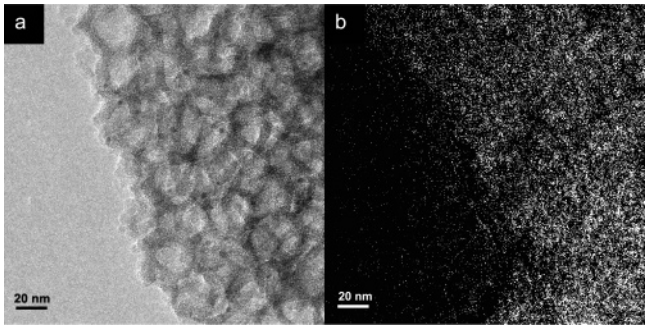


Figure 5. (a) TEM micrograph and (b) the corresponding nitrogen mapping (by electron energy loss spectroscopy) of CALB/C_{18}-MCF prepared by the pressure-driven method.

hydrophobic supports; thus, it was more effective to force the enzymes to enter the mesopores by high pressures.

CALB/C_{18}-MCF prepared by the pressure-driven method still exhibited a high BET surface area (208 m^2/g) and pore volume (1.05 cm^3/g) (Figure 2 and Table 1). The pore diameter of C_{18}-MCF was only reduced from 22.5 to 20.0 nm after the enzyme loading, possibly because the tertiary structure of CALB was unfolded under the vacuum treatment associated with the N_2 adsorption−desorption experiment.[7c] Figure 5 illustrates the uniform nitrogen mapping over the CALB/C_{18}-MCF sample, indicating the homogeneous distribution of the nitrogen-containing enzymes within the mesoporous silica matrix. CALB/C_{18}-MCF also showed PA-FTIR peaks at 1650 cm^{-1} and 3300 cm^{-1} (Figure 3c), which were associated with the amide groups of the enzymes, confirming the enzyme incorporation.

The surface area and pore volume of C_8-FDU-12 were decreased significantly by ~40% after CALB immobilization (Table 1), suggesting that some of the pores in this mesoporous support were blocked by the enzymes as a result of the small window size of C_8-FDU-12.

Activity and Selectivity in the Kinetic Resolution of 1-Phenylethanol. For the acylation of 1-phenylethanol by isopropenyl acetate (Scheme 1), a fixed amount of CALB was introduced as free or supported catalysts. CALB/C_8-MCF and CALB/C_{18}-MCF prepared by the pressure-driven method showed similar catalytic activity (Figure 6). In both cases, complete conversion of (*R*)-1-phenylethanol to (*R*)-1-phenylethyl acetate (i.e., 50% conversion of the

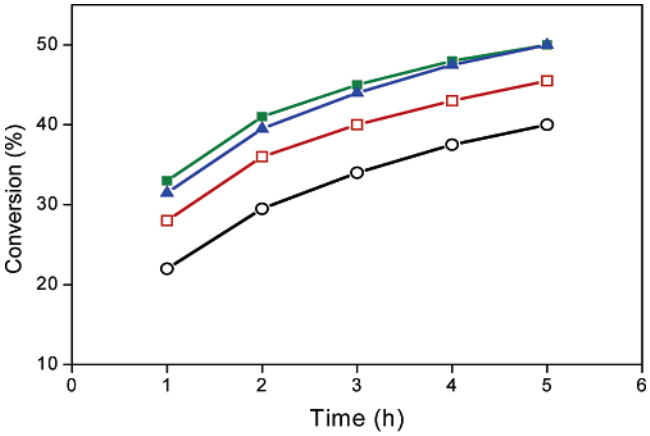


Figure 6. Catalytic conversions over (□) free CALB, and (▲) CALB/C_8-MCF, (■) CALB/C_{18}-MCF, and (○) CALB/C_8-FDU-12 prepared by the pressure-driven method.

(*R*)-1-phenylethanol and (*S*)-1-phenylethanol racemic mixture) was achieved after 5 h. In contrast, free CALB showed a slower reaction rate, giving 45.5% conversion in 5 h. This is possibly because the free hydrophilic CALB formed aggregates in toluene, leading to a lower active surface area. By loading CALB uniformly within the pores of MCF, aggregation was avoided or reduced. The weak hydrophobic interaction between the immobilized CALB and the pore surface of MCF allowed CALB to maintain its active conformation, and, therefore, high activity. CALB/C_8-FDU-12 gave an even lower reaction rate (40% conversion in 5 h) than free CALB (Figure 6). This could be due to the relatively low porosity and the blocked porous network of C_8-FDU-12 after CALB immobilization (Table 1), limiting the mass transfer in the catalytic reaction. This study indicated that MCF was more suitable than FDU-12 for CALB immobilization given its high porosity and ultralarge pore size. Thus, subsequent studies were focused on MCF-supported catalysts.

CALB/C_8-MCF, CALB/C_{18}-MCF, and CALB/C_8-FDU-12 prepared by the pressure-driven method all showed similarly high enantioselectivities (ee_s > 99.7% and ee_p > 99.7%) as the free CALB (see Figure S1 in Supporting Information). This confirmed that the pressure-driven method for enzyme loading did not alter the selectivity of CALB.

Enzyme Leaching over Multiple Reaction Runs. CALB/C_{18}-MCF prepared by the conventional stirring method showed a substantial decrease in 6-h conversions (from 50% to 37%) over five runs (Figure 7a). Elemental analysis revealed that over 65% of the CALB loaded in C_{18}-MCF was lost after five runs, suggesting that the decrease in activity was mainly due to enzyme leaching. In contrast, CALB/C_{18}-MCF prepared by the pressure-driven method only showed a minor decrease in conversion (from 50% to 46.5%) after eight runs or 80 h of reaction (Figure 7b). Elemental analysis showed that only 10% of the CALB loaded by pressure has leached out of the C_{18}-MCF support after 8 runs. Thus, the pressure-driven method not only significantly improved the enzyme loading, but also greatly reduced the enzyme leaching.

The enzyme molecules were driven by pressure to settle deep within the porous framework of MCF. The cage-like

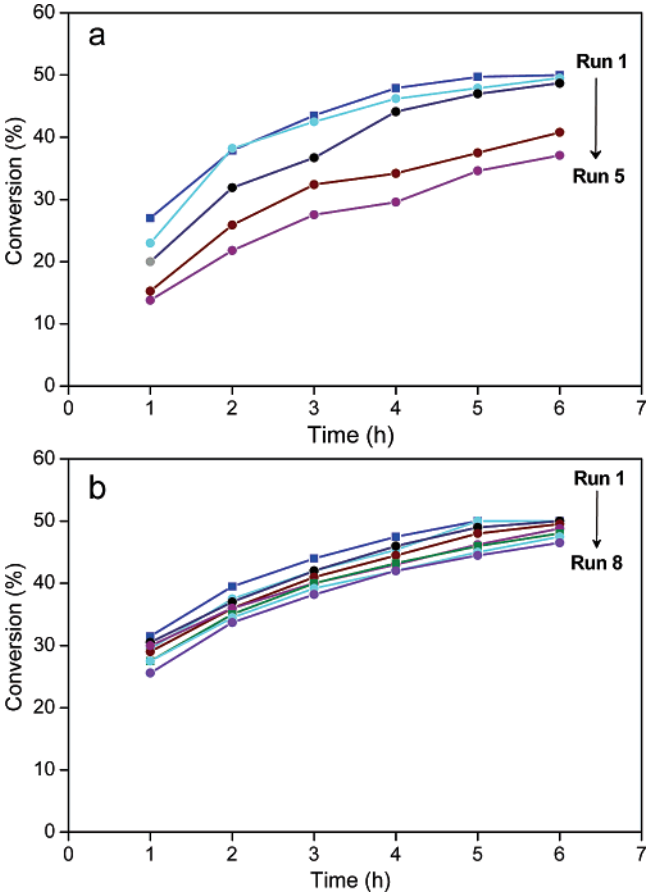


Figure 7. Conversion vs time in sequential runs over CALB/C_{18}-MCF prepared by (a) the conventional immobilization method and (b) the pressure-driven method.

pores of MCF physically inhibited the entrapped CALB from leaching. In contrast, a substantial amount of the CALB molecules was probably weakly adsorbed on the outer surface of the mesoporous particles when the conventional stirring method was used. These enzymes would be leached from the support relatively easily during the reaction runs.

In a separate experiment, we immobilized CALB onto C_{18}-MCF using the pressure-driven method in a HPLC column and then flushed the as-prepared column with 1000 mL of deionized water under a moderate pressure of ~1000 psi. Elemental analysis indicated that such a thorough washing hardly decreased the CALB loading in C_{18}-MCF; the PA-FTIR C═O stretching peak was also not affected in intensity (Figure 3d). This finding confirmed that the enzymes were firmly entrapped within the modified MCF support by the pressure-driven method and were robust against leaching.

The pressure-driven method also resulted in CALB/C_{18}-MCF with a higher catalytic activity than the stirring method. For example, in the first run, the catalysts prepared by the pressure-driven method and stirring method showed 1-h conversions of 31.5% and 27%, respectively, although the same amount of CALB was introduced to both reaction systems (see Figure 7). This could be because a more homogeneous dispersion of CALB was achieved throughout the C_{18}-MCF particles by the pressure-driven method, whereas some agglomeration or multilayer adsorption of CALB might be present at the outer shell of the support particles with the stirring method.

Reusability of Catalysts. The reusability of CALB/C_8-MCF and CALB/C_{18}-MCF prepared by the pressure-driven method was compared to that of Novozyme 435. Because the actual enzyme loading in Novozyme 435 was unknown, we adjusted the amounts of catalysts introduced to the reaction system so that all three catalysts gave 33% conversion after 1 h in the first run. Compared to Novozyme 435, CALB/C_8-MCF and CALB/C_{18}-MCF showed superior 1-h conversions over subsequent runs (see Figure S2 in Supporting Information), illustrating that CALB immobilized onto mesoporous silica by the pressure-driven method has better long-term stability and reusability than the commercial CALB/polymer catalyst.

Thermal Stability. The catalytic applications of enzymes are often hampered by their poor thermal stability, which may be improved through enzyme immobilization. To examine their thermal stability, free and supported CALB catalysts were tested for catalytic activity before and after 15 h of heat treatment at 80 °C. CALB/C_{18}-MCF prepared by the pressure-driven method showed negligible loss in activity from the heat treatment (Figure 8a). In contrast, CALB/MCF-C_{18} prepared by the conventional stirring method and Novozyme 435 showed a substantial reduction in activity after the thermal treatment (Figure 8b,c). Free CALB was severely deactivated upon heat treatment and lost most of its activity (Figure 8d). The deactivation of CALB at high temperature was attributed to the change of its conformation (enzyme distortion).[7c] Because the pore size of the MCF support was similar to the dimensions of CALB, the entrapped CALB was confined within the mesopores, which might suppress the change in enzyme conformation at high temperature. When immobilized by the conventional stirring method, some of the CALB would be located on the outer surface of the mesoporous silica particles; these enzymes would not be properly stabilized against heat treatment. This study demonstrated the effectiveness of the pressure-driven method in enzyme immobilization to achieve enhanced thermal stability for enzyme catalysts.

Packed Bed Reactor. A standard HPLC column (250 mm × 4.6 mm) was employed in the preparation of CALB/C_{18}-MCF by the pressure-driven method. The catalyst-packed column was then tested for continuous reaction. Full conversion of (*R*)-1-phenylethanol to (*R*)-1-phenylethyl acetate was achieved even at a fairly high flow rate of 1.5 mL/min, giving a high initial reaction rate of 3.2 mM 1-phenylethanol/(min·g enzyme). The activity and enantioselectivity of the reaction remained constant during the first 10 h of reaction. After 48 h of reaction at a constant flow rate of 1.5 mL/min, the reaction rate decreased by 9%.

It should be noted that our spherical MCF particles were particularly suitable for use in a packed bed reactor because their uniform size and morphology allowed for homogeneous packing with a moderate backpressure. For example, at a flow rate of 1.5 mL/min, the pressure drop over a 25-cm-long column was 1050 psi. In contrast, conventional MCF with irregular particle morphology and broad particle size distribution would have given rise to a significantly higher backpressure in a packed bed reactor. Commercial Novozyme 435 was also not suitable for the packed bed reactor because

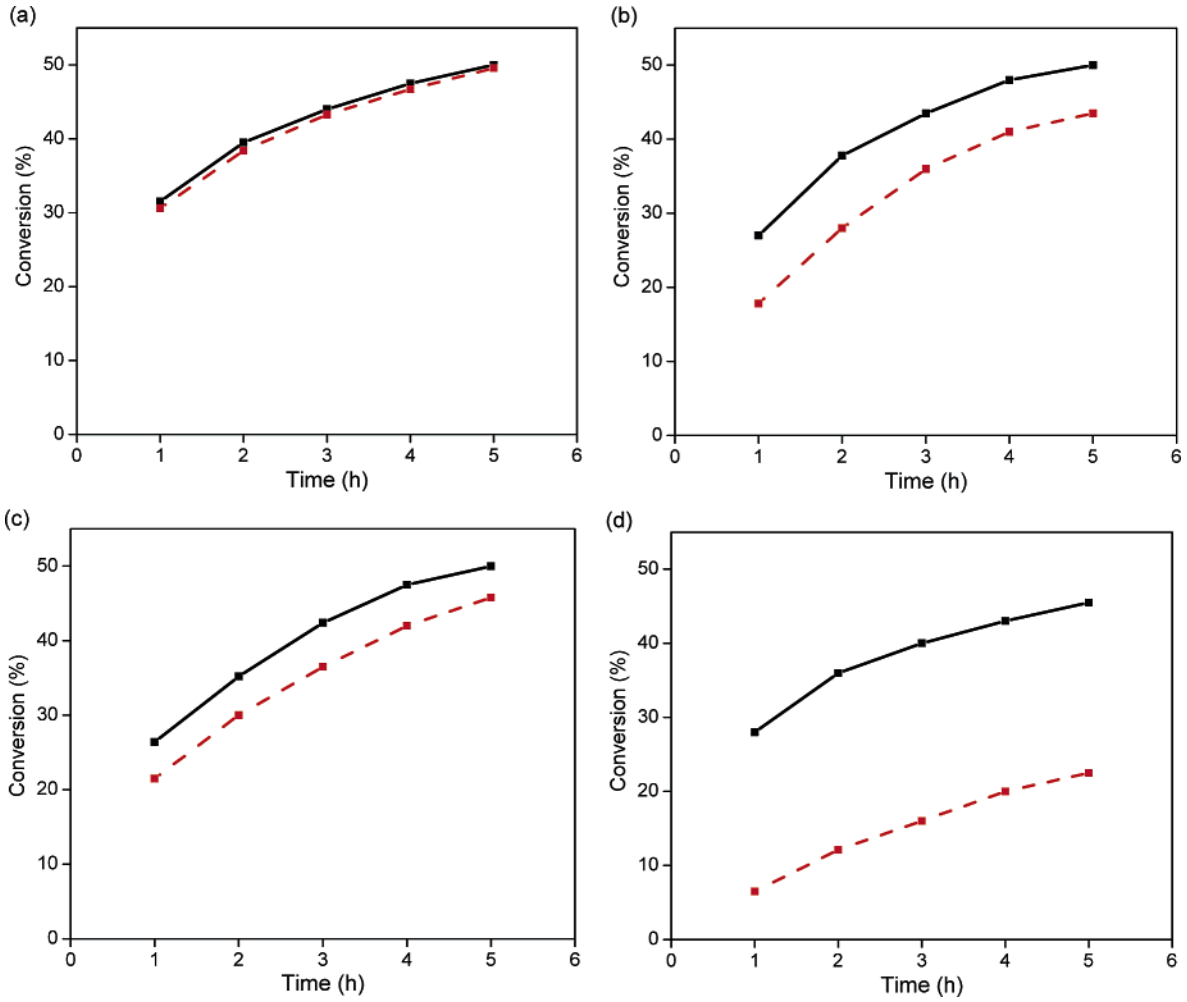


Figure 8. Conversion vs time over CALB/C_{18}-MCF prepared by (a) the pressure-driven method and (b) the conventional method, (c) Novozym 435, and (d) free CALB. Catalysts were tested (solid lines) before and (dashed lines) after heat treatment at 80 °C.

its polymer support would swell in the organic solvents, resulting in an unstable pressure in the reactor.

Some proteins might be denatured by high pressures; this was not the case for lipase. The pressure-driven method has led to improved performance for lipase immobilization. Further studies would be conducted to examine its general applicability to other enzymes.

Conclusions

This study illustrated that spherical MCF particles served as an excellent support for enzyme immobilization. The ultralarge cagelike mesopores of this silica support were ideal for entrapping enzymes. A pressure-driven method was developed to effectively immobilize enzymes uniformly and robustly within the hydrophobic MCF particles. Compared to the conventional stirring method, the novel pressure-driven method not only greatly increased the enzyme loading, but also significantly reduced the enzyme leaching over multiple reaction cycles. Lipase CALB immobilized on hydrophobic MCF particles by the pressure-driven method showed excellent activity and selectivity in the kinetic resolution of 1-phenylethanol. It also demonstrated superior reusability and thermal stability compared to commercial Novozyme 435 catalyst. CALB/C_{18}-MCF prepared by the pressure-driven method was also successfully applied in a packed bed reactor for continuous reaction with high activity and selectivity.

Acknowledgment. This work was supported by the Institute of Bioengineering and Nanotechnology (Agency for Science, Technology and Research, Singapore).

Supporting Information Available: Synthesis of FDU-12; HPLC chromatograms; 1-h conversion achieved in sequential runs by Novozym 435, and CALB/C_8-MCF and CALB/C_{18}-MCF prepared by the pressure-driven method (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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