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# Enzyme-Loaded Mesoporous Silica Particles with Tuning Wettability as a Pickering Catalyst for Enhancing Biocatalysis

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**Abstract:** Pickering emulsion systems have created new opportunities for two-phase biocatalysis, however their catalytic performance is often hindered by biphasic mass transfer process relying on the interfacial area. In this study, lipase-immobilized mesoporous silica particles (LMSPs) are employed as both Pickering stabilizers and biocatalysts. A series of alkyl silanes with the different carbon length are used to modify LMSPs to obtain suitable wettability and enlarge the interfacial area of Pickering emulsion. The results show the water/paraffin oil Pickering emulsions stabilized by 8 carbon atoms silane grafted LMSPs (LMSPs_C8) with a three-phase contact angles of 95° get the relatively large interfacial area. Moreover, the conversion of enzymatic reaction catalyzed by LMSPs_C8 Pickering emulsion system is 3.4 times higher than that unmodified LMSPs with the reaction time of 10 min. Additionally, the effective recycling of LMSPs is achieved by simple low-speed centrifugation. As evidenced by a 6-cycles reaction of remaining 75% of relative enzymatic activity, the protection of 350–450 nm mesoporous silica particles can alleviate the inactivation of enzyme from the shear stress and make a benefit to form stabile Pickering emulsion. Therefore, the biphasic reactions in the Pickering emulsion system can be effectively enhanced through changing interfacial area only by the means of adjusting the wettability of biocatalysts.

**Keywords:** biocatalysis; immobilized lipase; Pickering emulsions; wettability; mesoporous silica particles

## 1. Introduction

Enzymes are biocatalysts with high efficiency for a wide variety of chemical reactions, providing high chemo-, stereo-, and regioselectivity in a mild and sustainable manner [1,2]. However, enzymes commonly exhibit activity in the aqueous phase, whilst a majority of substrates can only be soluble in the organic phase. Hence enzymatic reactions tend to happen at the interface of the organic-aqueous biphasic system [3]. Unfortunately, the enzymatic reaction of the biphasic systems is often limited by mass transfer process relying on interfacial area [4].

In order to address this limitation, colloidal particle stabilized Pickering emulsion as interfacial catalysis system has been developed [5]. Comparing with the traditional surfactant-based emulsion, Pickering emulsion has several advantages, such as environment-friendly, avoiding enzymes inactivation and simplifying separation procedure [6,7]. Moreover, the colloidal particle usually possesses a tunable wettability, it provides a wide variety of choices to stabilize the desired emulsion for biphasic reactions [8–10]. Meantime, the size of Pickering emulsion droplets can also be tuned with the change of colloidal particle concentration [11]. Up to now, there are two kinds of strategies

for biphasic enzymatic catalysis in Pickering emulsions. One includes free enzymes located in the inner aqueous phase [12–17]. The other includes enzyme-immobilized particles anchored around droplet interfaces [18–26], by contrast, it enables enzymes to be recycled, maximizes the contact area between enzymes and substrates, reduces diffusion distance of substrate molecules and improves the stability of enzymes. In the above two kinds of Pickering catalysis system, the changing of the organic-aqueous phases ratio or increasing particulate emulsifiers concentration is applied to improve interfacial area to enhance the enzymatic reaction. However, this will lead to the excessive waste of the particulate emulsifiers and not able to meet the demand of the system which emulsifier concentration and organic-aqueous phases ratio cannot be changed.

The mesoporous silica particles (MSPs) are widely used as enzyme carriers, due to outstanding biocompatibility, high specific area and controllable morphology [27,28]. Recently, Yang's group reports lipase-immobilized mesoporous silica particles (LMSPs) are utilized as Pickering interfacial biocatalysts [18]. However, in the process of wettability modification of LMSPs, the inactivation of the enzyme is inevitable due to the exposure of the biocatalyst to an organic solvent. Hence, the development of an effective strategy for tailoring LMSPs' wettability without damaging enzyme activity remains a challenge. According to the classical Pickering emulsion's theory, the biphasic interfacial area can be regulated by colloidal particle's wettability [11,29–31]. Nevertheless, as far as we know, LMSPs with tuning wettability as a Pickering catalyst for enhancing biocatalysis have not been reported to date.

Herein, LMSPs with tuning wettability are developed to act as a Pickering catalyst for the intensification of biphasic catalysis (Figure 1). By changing the chain length of silane grafted from C0 to C12, a set of LMSPs with finely tuned surface wettability from three-phase contact angle (TCA) of 25° to 145° can be obtained to form stable water/paraffin oil Pickering emulsions with the different droplet size (Figure 1a). Lipase PS from *Pseudomonas cepacia* (PCL), a versatile enzyme widely used even on the industrial scale, is chosen as a model enzyme to assess the catalytic performance, where tributyrin (Reactant) in oil phase is transformed to butyric acid (Product) in water under the catalysis of LMSPs at Pickering emulsion interface (Figure 1b). With the change of LMSPs' wettability, the conversion of reaction can also be improved by the increase of interfacial area.

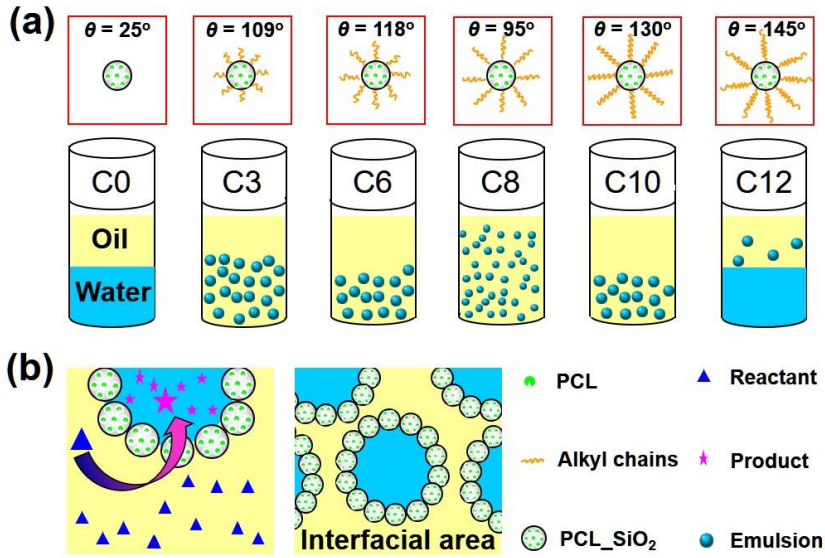


**Figure 1.** Schematic description of enhancement of biphasic reaction in Pickering emulsions via tuning biocatalysts wettability, (**a**) Pickering emulsions stabilized by lipase-immobilized mesoporous silica particles lipase-immobilized mesoporous silica particles (LMSPs) with the different carbon chain length of silane grafted (C0, C3, C6, C8, C10, C12), (**b**) Pickering interfacial catalysis with the controllable interfacial area.

## 2. Results and Discussion

### *2.1. Characterization of MSPs*

Prior to the enhancement of biphasic reaction in Pickering emulsions via tuning LMSPs wettability, the Characterization of MSPs, the encapsulation of PCL into MSPs, the effect of surface wettability of different silylating agents modified LMSPs on the Pickering emulsions were studied, and the optimum conditions were obtained and adopted as detailed in Sections 2.4–2.6.

Figure 2 shows TEM images of the prepared MSPs and exhibits the mesoporous morphology of particles in the magnified image. The MSPs with uniform particles sizes of 350–450 nm can be seen in Figure 2a. The radically arrayed pores throughout the materials (ca. 2–5 nm) are clearly observed (Figure 2b). From Figure 2, we can see the MSPs are very suitable as a carrier for enzyme immobilization.

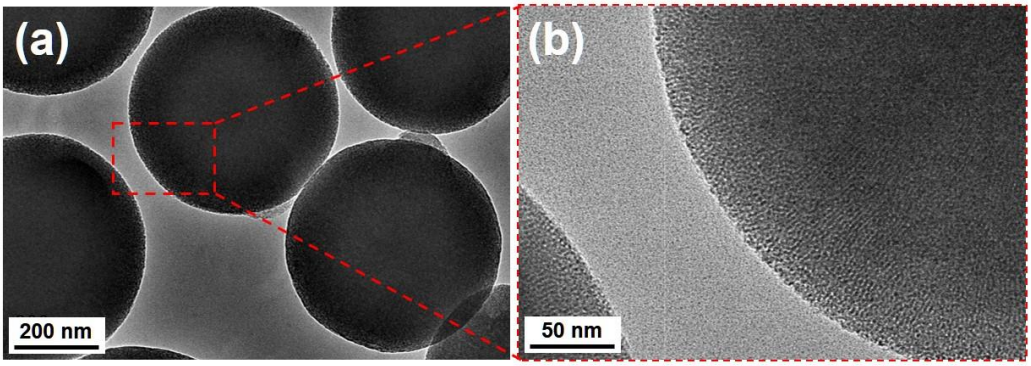


**Figure 2.** TEM images of the prepared MSPs (**a**), the mesoporous morphology in the magnified image (**b**).

### *2.2. Characterization of Encapsulation of PCL into MSPs*

The efficiency of the encapsulation of PCL into the MSPs was defined as the ratio between the amount of PCL in the MSPs and the amount of total PCL. The equation was shown as follow:

$$\text{Encapsulation efficiency (\%)} = 100(m - CV)/m, \quad (1)$$

m (mg) is the total amount of the PCL, C was the concentration of PCL in supernatant and washing solutions, V is the volume of the supernatant and washing solutions.

According to Equation (1), the efficiency of the encapsulation of PCL in the MSPs is calculated as 78%. Generally, the principal driving forces for enzyme adsorption inside pores are hydrophobic interaction, electrostatic attraction, hydrogen bonding, and van der Waals force [32–36]. As for PCL, due to its special interfacial activation effect, hydrophobic interaction is usually the dominant driving force playing a significant role in PCL uptake [32]. However, in our case the support MSPs is hydrophilic before silylating thus cannot offer hydrophobic affinity towards PCL. Due to the pI values of MSPs and PCL are both lower than the pH value of the buffer solution (pH = 8.0), little electrostatic attraction exists between the enzyme proteins and the pore-wall of MSPs. Therefore, the hydrogen bond formed between the large amount of Si-OH or Si-O-groups on the pore surface of MSPs and the amino or carboxylic groups on the external surface of PCL, as well as the van der Waals force, are probably the nature of interaction between the immobilized PCL and pore-wall of MSPs [33].

### *2.3. Characterization of Pickering Emulsions Stabilized by Different Silylating Agents Modified LMSPs*

Surface wettability of particles is thought to be crucial in stabilizing Pickering emulsions. In our previous studies, the most stable Pickering emulsion with the relatively large interfacial area is formed when the three-phase contact angle (TCA) of particles is close to 90° [37,38]. To get the optimum condition,

silylating agents with different carbon chain length (C0, C3, C6, C8, C10, C12) is used to modify the LMSPs. As shown in Figure 3a and Figure S3, in paraffin oil-water system, the TCA of C0, C3, C6, C8, C10, C12 are 25°, 109°, 118°, 95°, 130°, 145° respectively. Obviously, the TCA is increasing with the increase of the silane carbon chain length (from C0 to C6 and from C10 to C12). Notably, the TCA of 95° of C8 is very close to 90°. Interestingly, the result is similar to that in our previous studies, the C7 coating is having the more preferred TCA (105.5°) than C6 (108.5°) and C12 (140.5°), thus C7 is the optimum condition for modifying the $TiO_2$ particles to prepare stable Pickering emulsions [38]. We can deduce that, with the increase of the carbon chain length, there is always one Cn (n is the number of carbon element) coating having an optimum contact angle (close to 90°) which means the competition of hydrophilic/lipophilic interaction to the particles at the paraffin oil-water interface achieve a balance. However, because the paraffin oil is the mixture of alkane series with the different carbon chain length, the interaction between the paraffin oil and the silane grafted LMSPs is complicated. In the future, in our group, the balance relationship between hydrophilic and lipophilic interaction of LMSPs_C8 at the paraffin oil-water surface will be further studied via the computational chemistry. Thus, the LMSPs_C8 are chosen to stabilize the water/paraffin oil emulsions in the following experiments.

It is well known that TCA exhibited by particles at an oil−water interface is an important parameter governing the stabilization of a Pickering emulsion. To evaluate the ability of stabilizing the emulsions, water/paraffin oil Pickering emulsions stabilized by different silylating agents modified LMSPs are prepared. As shown in Figure 3b–d, the water and paraffin oil are in the separate phase state which means the pure PCL and LMSPs_C0 are not suitable for forming emulsions. Moreover, the Pickering emulsions appeared with the LMSPs_C3, LMSPs_C6, LMSPs_C8 and LMSPs_C10 are stable systems (Figure 3e–h). The thickness of the emulsions is C8 > C3 > C6 > C10 (Figure 3b), and the diameters of the emulsion droplet are C8 < C3 < C6 < C10 (Figure 3e–h). These results are attributes to the fact that known from Figure 3, the LMSPs_C8 with TCA of 95° exhibits powerful amphiphilicity, hence the Pickering emulsions are most stable. The TCA of LMSPs_C0 and C12 are 25° and 145° respectively that are much larger or less than 90° so that the emulsions cannot be formed (Figure 3d,i). In comparison, the TCA of LMSPs_C3 (109°), C6 (118°) and C10 (130°) are close to 90° (Figure 3b), thus Pickering emulsions are formed. However, these emulsions are less stable than LMSPs_C8 emulsions. To sum up, the TCA of a particulate stabilizer at the oil-water interface is a key parameter. When TCA approaches 90°, the emulsifiers tend to reside at the interface which is beneficial for the stability of oil-water Pickering emulsions with the relatively large interfacial area. Thus, LMSPs_C8 possesses most suitable wettability to form the stable Pickering emulsion.

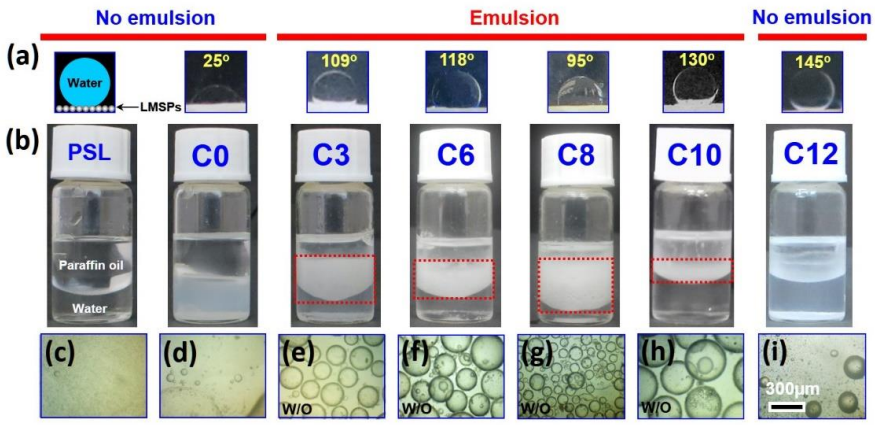


**Figure 3.** The three-phase contact angle (TCA) of C0, C3, C6, C8, C10 and C12 modified LMSPs (**a**), appearance (**b**) and corresponding optical microscopy images of Pickering emulsions prepared with pure PCL (c), LMSPs_C0 (**d**), LMSPs_C3 (**e**), LMSPs_C6 (**f**), LMSPs_C8 (**g**), LMSPs_C10 (**h**) and LMSPs_C12 (**i**), all at a particle concentration of 1% w/v, a volume ratio of paraffin oil (containing 3 mg·mL$^{-1}$ tributyrin) and water 1:1.

### *2.4. Conversion of Tributyrin Hydrolysis Reaction with the Catalysis of LMSPs_C8 in Pickering Emulsion or Biphasic System*

Tributyrin hydrolysis reaction is chosen to display the enhancement of enzyme reaction caused by LMSPs. Although the products, such as glycerol dibutyrateand and glycerol monobutyrate, are well-known emulsifiers, in this study, 6 mL paraffin oil containing 3 mg·mL$^{-1}$ tributyrin (substrate) as oil phase, 6 mL phosphate buffer solution as water phase, it can be deduced that the product concentration of glycerol dibutyrate ($\leq$ 5 μmol·mL$^{-1}$) and glycerol monobutyrate ($\leq$ 5 μmol·mL$^{-1}$) are very low. Thus, these products under this condition with very low concentration hardly impact on the stability of Pickering emulsions. To demonstrate the remarkable difference in the conversion of tributyrin hydrolysis reaction between the biphasic system and Pickering emulsion system, LMSPs_C8 is chosen for the catalysis of tributyrin hydrolysis reaction at room temperature with mild stirring (50 rpm) for 10 min. Meantime, we evaluate the conversion after 10 min with a relative low conversion of the biphasic system as control. As shown in Figure 4a and b, in the biphasic system, the LMSPs_C8 are located at the oil-water interface, whilst the stable Pickering emulsions are formed after homogeneous emulsification and the diameter of the emulsion droplet is about 100 μm. The aggregates of LMSPs_C8 are observed at the emulsion interface in Figure 4c. In order to explore the conversion of tributyrin hydrolysis reaction in the Pickering emulsions system and the biphasic system, NaOH solution was titrated manually to determine the concentration of the product. The conversion was determined as follows.

$$\text{Conversion (\%)} = V_{\text{NaOH}} \times C_{\text{NaOH}} \times 100\% \,/\, (C_S \times V_S) \quad (2)$$

$V_{\text{NaOH}}$ is the volume of the used NaOH, $C_{\text{NaOH}}$ is the concentration of the NaOH, $C_S$ is the molarity of tributyrin, $V_S$ is the volume of the oil phase.

As shown in Figure 4d, according to Equation (2), the conversion of tributyrin hydrolysis reaction in the Pickering emulsions system (26.9%) is much greater than that in the biphasic system (7.8%). This result is attributed to the fact that the total interface areas of Pickering emulsion system are much greater than that of the biphasic system. Thus, more products can be generated in the Pickering emulsion system.

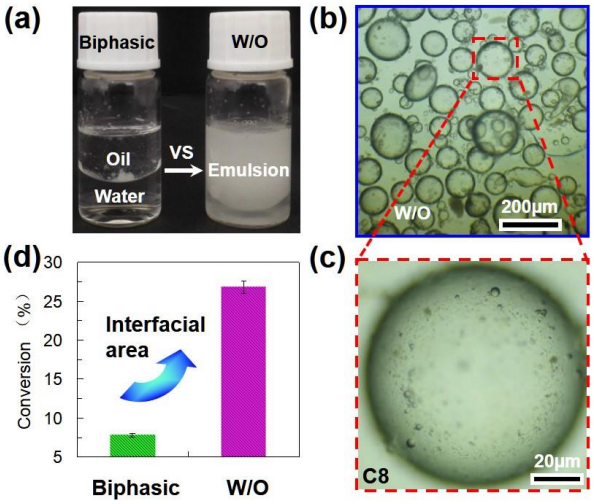


**Figure 4.** Conversion of tributyrin hydrolysis reaction with the catalysis of LMSPs_C8 in Pickering emulsion and biphasic system. Photographs of before and after emulsification (**a**), optical microscope image of Pickering emulsions prepared with LMSPs_C8 (**b**), the surface morphology in the magnified image (**c**), conversion of tributyrin hydrolysis reaction in the Pickering emulsions system and biphasic system (**d**). All at a particle concentration of 1% w/v, a volume ratio of paraffin oil (containing 3 mg·mL$^{-1}$ tributyrin) and water 1:1, the enzymatic reaction at room temperature with mild stirring (50 rpm) for 10 min.

### *2.5. Conversion of Tributyrin Hydrolysis Reaction in Different Silylating Agents Modified LMSPs Stabilized Pickering Emulsion*

To explore the optimum conditions for the tributyrin hydrolysis reaction in the Pickering emulsions, the effects of substrate concentration ($C_S$) on the conversion of the reaction catalyzed by the free PCL were studied. We can calculate the conversion according to Equation (2). As shown in Figure S1, with the increase of the tributyrin concentration (from 3 to 6 mg $mL^{-1}$), the conversion values increased from 6.71 to 9.23%, also indicating that the $C_S$ is not excessive. As a result, 3 mg·$mL^{-1}$ tributyrin is chosen in our study.

In order to ensure there is only a single variable in the experiments, the effect of silylating on the conversion is studied. As shown in Figure S2, the conversions of tributyrin hydrolysis reaction catalyzed by the LMSPs_C0, C3, C6, C8, C10, C12 in the paraffin oil-water biphasic system are closed to each other which means the process of silylating has no obvious effects on the conversion.

Subsequently, the effect of total interfacial areas of the Pickering emulsion on the conversion is studied. As shown in Figure 5, the conversions of LMSPs_C3, C6, C8, C10 are all greater than those of C0 and C12. It is because the total area of oil-water interface in the Pickering emulsion state is much greater than that in the biphasic state which lead to the shortening of mass transfer distance. As a result, the tributyrin hydrolysis reaction is enhanced that is reflected in the increase of the conversion. In addition, in the Pickering emulsions, the conversions show C8 > C3 > C6 > C10, the result is attributed to that the extent of TCA away from 90°. Notably, the conversion of LMSPs_C8 stabilized Pickering emulsion is 3.4 times higher than that in the biphasic system with the reaction time of 10 min, because the Pickering emulsions with LMSPs_C8 reach the relatively large interfacial area.

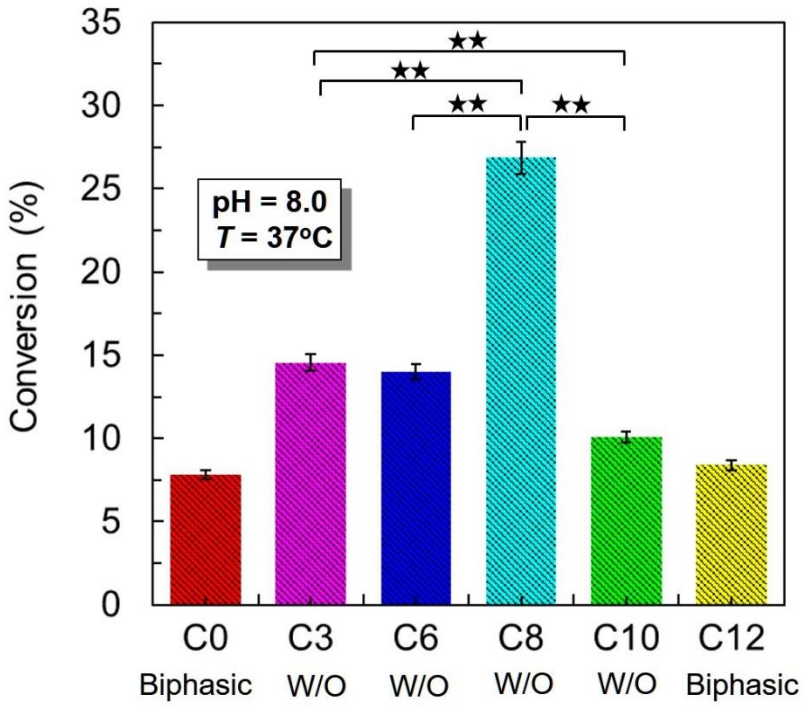


**Figure 5.** Conversion of tributyrin hydrolysis reaction in different silylating agents modified LMSPs stabilized Pickering emulsion, all at a particle concentration of 1% w/v, a volume ratio of paraffin oil and water 1:1. One-way ANOVA and t-tests were used for statistical analysis. Asterisks indicate significant differences. * $P < 0.05$, ** $P < 0.01$.

### *2.6. Effect of the Cycles of Reaction on relative activity*

The surface of the MSPs spreads over ordering pore structure. Thus, the specific surface area is very large which made MSPs as an effective and reusable carrier for enzyme immobilization. To

demonstrate the effect of LMSPs recycle times on the relative enzymatic activity, LMSPs_C8 is chosen as a model. The reaction is repeated for six times and the relative enzymatic activity is measured after each batch. As shown in Figure 6, the emulsion droplets at run 1 are homogeneous and the average diameter is about 100 μm (Insert (a)). After six cycles, the average diameter of emulsion droplets at run 6 is close to that at run 1 (Insert (b)). In addition, with the increase of the recycle times, although the relative enzyme activity is decreasing, the degree of reduction is small and after three cycles the relative enzyme activity becomes stable. Moreover, at the run 6, only 25% of relative enzyme activity lost, which is superior than the reported results after 5 cycles 65% of relative enzyme activity lost [19]. These results are attributes to that compared with the 20 nm mesoporous Silica used in the reported paper, the average diameter of the LMSPs is about 450 nm. The big size of mesoporous silica particles provides a relative mild environment for enzyme encapsulation, protect the enzyme from inactivation by shear force during the process of emulsification and centrifugation, make a benefit to form stabile Pickering emulsion.

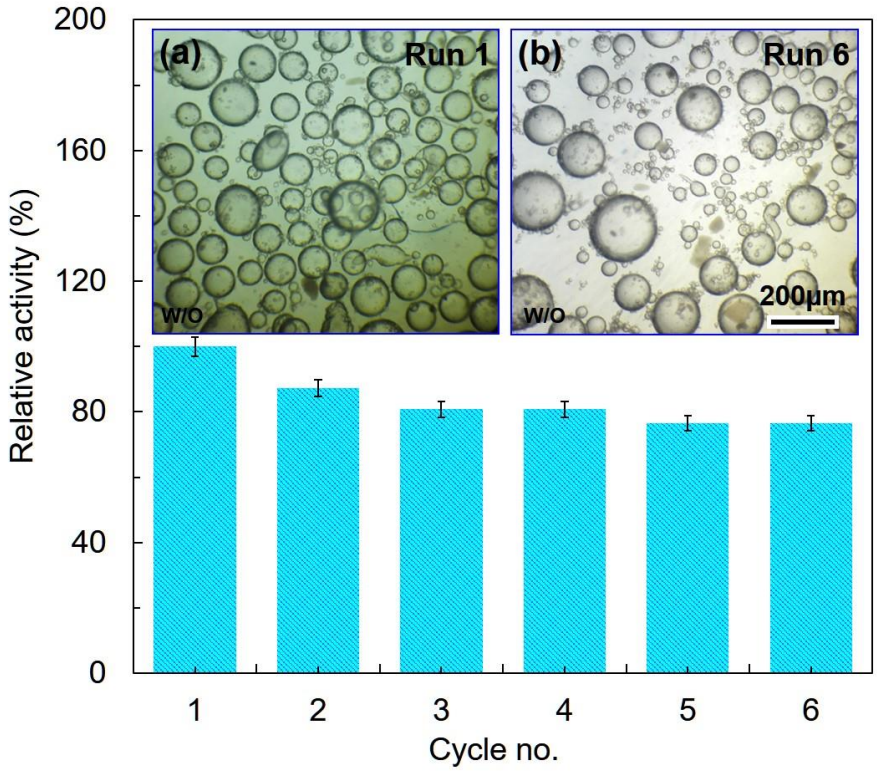


**Figure 6.** Relative activity of LMSPs_C8 at the interface of W/O Pickering emulsions over six cycles. Insect figure (**a**) and (**b**) is the micrograph of Pickering emulsion in first and sixth cycle. All at a particle concentration of 1% w/v, a volume ratio of paraffin oil and water 1:1.

## 3. Materials and Methods

### *3.1. Chemicals and Reagents*

Lipase PS from *Pseudomonas cepacia* (PCL) and tributyrin were purchased from Hangzhou Chuangke Biotechnology Co., Ltd. (Hangzhou, China) and Aladdin reagent Co., Ltd. (Shanghai, China) respectively. Paraffin oil and hexane were acquired from Chengdu Shiyang chemical reagent company (Chengdu, China) and Guangdong Guanghua technology Co., Ltd. (Guangdong, China) respectively. Trimethylchlorosilane (C3) was obtained from Sinopharm chemical reagent Co., Ltd. (Beijing, China). Hexyltrichlorosilane (C6), octyl trichlorosilane (C8), decyltrichlorosilane (C10) and dodecyltrichlorosilane (C12) were commercially available from Tokyo Kasei Industry Co., Ltd. (Tokyo, Japan). Cetyltrimethyl ammonium bromide (CTAB) was purchased from Chengdu Beisite reagent Co.,

Ltd. (Chengdu, China). Tetraethoxysilane (TEOS) was purchased from Chengdu Kelong Chemical Reagents Co., Ltd. (Chengdu, China). Deionized water (18.2 MΩ, Milli-Q, Millipore) was used throughout the study.

### 3.2. Preparation of MSPs

The MSPs were prepared according to the following steps. The CTAB solution was prepared by dissolving 2.5 g of CTAB powder into 50 mL of deionized water with magnetic stirring (500 rpm) at room temperature until the solution became clear. Then, 75 mL of the anhydrous ethanol was added into the solution and the suspension was magnetically stirred (75 rpm) for 10 min at room temperature. After that, 13 mL ammonium hydroxide was added slowly with magnetic stirring (250 rpm) at room temperature for 15 min to get the uniform dispersed solution. 5 mL TEOS was added into the dispersed solution with magnetic stirring (550 rpm) at room temperature for 2 h to get the suspension with white sediments. After the suspension was naturally precipitated for 2 h, the supernatant was discarded. Followed by centrifuging at 7500 rpm and washing three times with deionized water, the as-prepared product was obtained. Then, the as-prepared product was centrifuged at 15,000 rpm and washed three times with anhydrous ethanol. The products were taken from the bottom of the centrifuge tube and dried at 90 °C for 12 h in air. Finally, the dried products were calcined inside a Muffle furnace at 650 °C for 5 h to obtain uniform MSPs.

### 3.3. Encapsulation of PCL into the MSPs

The PCL solution was prepared by dissolving 0.02 g PCL powder into 10 mL of phosphate buffer solution (PBS, pH = 8.0, 50 mM). 0.1 g of MSPs was added into the prepared PCL solution and the suspension was magnetic stirred (250 rpm) for 3 h at 4 °C. The mixture was then centrifuged at 8000 rpm for 5 min to remove the supernatant. The precipitates were collected and washed with deionized water three times, and then dried at drying oven (37 °C). According to the standard curve (Figure S4), the amount of PCL in supernatant and washing solutions could be directly determined by UV spectrophotometer at a wavelength of 205 nm. The encapsulation efficiency of PCL can be calculated according to Equation (1).

### 3.4. Evaluation of Different Silylating Agents Modified LMSPs Surface Wettability

The prepared LMSPs were first modified by different silylating agents. LMSPs_C3 were prepared by adding 0.1 g LMSPs into 20 mL trimethylchlorosilane solution (0.2 mol·$L^{-1}$ trimethylchlorosilane in hexane). Then the solution was stirred for 4 h at room temperature, followed by centrifuging at 5000 rpm to remove the supernatant, and dried in a drying oven at 37 °C. Similarly, the modified LMSPs (C6, C8, C10, C12) were obtained as the above steps but replacing C3 as C6, C8, C10, C12 respectively. Moreover, as the control group, LMSPs_C0 were prepared as the same processes with the others but without using any silylating agents.

The surface wettability characteristics of the prepared LMSPs were investigated by recording the TCA. The TCA of LMSPs at the oil−water interface was measured by using the compressed disk method [39]. An approximately 2 mm thickness disc-like LMSPs aggregation made by pressing with tablet press was laid in a crystal vessel. Then, 10 mL paraffin oil contained 3 mg·$mL^{-1}$ tributyrin was introduced into the vessel. After that, a 3 μL aliquot of water droplet was dropped onto the disc like LMSPs aggregation. The snapshot was photographed when the droplet was stationary. An average value from more than five parallel measurements on different sites of the same conditions was obtained, and the experiments were performed under ambient conditions.

### 3.5. Preparation of Pickering Emulsion Stabilized by Different Silylating Agents Modified LMSPs

Phosphate buffer solution (PBS, pH = 8.0, 50 mM) was used as the dispersed phase. Paraffin oil (6 mL) containing 3 mg·$mL^{-1}$ tributyrin and 0.06 g the modified LMSPs_C0, C3, C6, C8, C10, C12 with ultrasonic treatment to obtain a suspension with good dispersion were used as the continuous

phase in the different experiment groups respectively. The paraffin oil phase was added to the aqueous phase (1:1, volume ratio), followed by homogeneous emulsification at 18,000 rpm for approximately 1 min using homogenizer (S10, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) to obtain W/O emulsions (emulsion volume in total is about 12 mL).

### *3.6. The Effect of the Substrate Concentration on the Conversion with Biphasic Condition*

Two groups of enzyme and substrate solutions were added into two beakers respectively. One is 6 mL paraffin oil containing 3 mg·mL$^{-1}$ tributyrin and 6 mL phosphate buffer solution (PBS, pH = 8.0, 50 mM) containing 2.0 mg·mL$^{-1}$ PCL. The other is 6 mL paraffin oil containing 6 mg·mL$^{-1}$ tributyrin and 6 mL phosphate buffer solution (PBS, pH = 8.0, 50 mM) containing 2.0 mg·mL$^{-1}$ PCL. After reacted at room temperature with mild stirring (50 rpm) for 10 min, enzyme inactivator that was prepared by mixing anhydrous ethanol, acetone and deionized water (1:1:1, volume ratio) together and 3 drops of phenolphthalein indicator (1 wt. % phenolphthalein in ethanol solution) were added. At last, 0.01 mol·L$^{-1}$ NaOH was titrated manually to determine the concentration of the product. The conversion can be calculated according to Equation (2).

### *3.7. The effect of Wettability of LMSPs on the Conversion with Biphasic Condition*

Added 6 mL paraffin oil containing 3 mg·mL$^{-1}$ tributyrin and 0.06 g the modified LMSPs (C0, C3, C6, C8, C10, C12) with ultrasonic treatment to obtain a suspension with good dispersion into six beakers respectively. Then, 6 mL phosphate buffer solution (PBS, pH = 8.0, 50 mM) were added into each beaker respectively. After reacted at room temperature with mild stirring (50 rpm) for 10 min, the enzyme inactivator and 3 drops of phenolphthalein indicator were added. Finally, 0.01 mol·L$^{-1}$ NaOH was titrated manually to determine the concentration of the product. The conversion can be calculated according to Equation (2).

### *3.8. Assessments of the Catalytic Performance and Reusability of the Pickering Emulsion Stabilized by Modified LMSPs*

Added 6 mL paraffin oil containing 3 mg·mL$^{-1}$ tributyrin and 0.06 g the modified LMSPs_C8 with ultrasonic treatment to obtain a suspension with good dispersion. Then, 6 mL phosphate buffer solution (PBS, pH = 8.0, 50 mM) was added into the above suspension, followed by homogeneous emulsification at 18,000 rpm for approximately 1 min using homogenizer (S10, NingBo Scientz Biotechnology Co., Ltd., Ningbo, China) to obtain W/O emulsions (emulsion volume in total is about 12 mL). After reacted at room temperature with mild stirring (50 rpm) for 10 min, the enzyme inactivator and 3 drops of phenolphthalein indicator were added. Finally, 0.01 mol·L$^{-1}$ NaOH was titrated manually to determine the concentration of the product. When performing the recyclable catalytic reaction in Pickering emulsion, the C8 reaction mixture was separated by centrifugation (5000 rpm, 10 min) before the enzyme inactivator was added, the supernatant and precipitate were respectively collected for the assessments enzymatic reaction activity and recycle LMSPs_C8. One activity unit (1U) is defined as the enzyme amount need for producing 1μmol butyric acid per minute. The relative activity (%) represents the ratio of residual enzymatic activity to the initial enzymatic activity. In the meantime, the remaining solid sample was respectively washed with hexane and deionized water for three times and then dried at room temperature prior to the next cycle.

### *3.9. Characterization Techniques*

The particle diameters of the mesoporous silica particles were determined using a Malvern Zetasizer Nano-ZS90 instrument (Worcestershire, United Kingdom). The morphologies of MSPs were investigated using a field emission transmission electron microscope (FE-TEM; JEM-2100F, JEOL, Tokyo, Japan). The samples were prepared by dispersing in anhydrous ethanol and coating on a carbon-coated copper grid. The grid was then allowed to dry before being imaged. All of the emulsion droplets were viewed using an XSP-24 (Phoenix Co., Ltd., Shangrao, China) research microscope

equipped with a Moticam 2000 camera. The microscopic pictures were captured using Motic Images Plus 2.0 software (Version 2.0, Motic Instruments Inc., Richmond, Canada) and then processed and analyzed using Image Pro Plus software. SPSS 12.0 (SPSS Inc., SPSS Inc., Chicago, IL, USA) was used to perform statistical data analysis. The results, including the error bars in the graphs, were given as the mean ± standard deviation.

## 4. Conclusions

In summary, we describe the construction of enzyme-loaded mesoporous silica particles with tuning wettability as a Pickering catalyst for the intensification of biocatalysis. By changing the chain length of silane grafted, the wettability of LMSPs can be fine-tuned to optimize the interfacial area of Pickering emulsions. The C8 silane chain grafted LMSPs with three-phase contact angle of 95° hold the relative great conversion of enzymatic reaction, due to the relatively large interfacial area of Pickering emulsion. Moreover, the LMSPs catalysts can be simply recycled with high relative enzyme activity which was much better than that reported in previously published papers. We believe that the versatile approach can provide a green platform for the enhancement of a wide variety of biphasic biocatalytic reactions.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4344/9/1/78/s1, Figure S1. Effect of substrate concentration on the conversion with the free PCL. Figure S2. Effect of the chain length of silane grafted LMSPs on the conversion of enzymatic reaction catalyzed by the LMSPs_C0, LMSPs_C3, LMSPs_C6, LMSPs_C8, LMSPs_C10, LMSPs_C12 in the paraffin oil-water biphasic system. Figure S3. TCA of C0, C3, C6, C8, C10 and C12 modified LMSPs. One-way ANOVA and t-tests were used for statistical analysis. Asterisks indicate significant differences. * $P < 0.05$, ** $P < 0.01$. Figure S4. Standard curve of the absorbance of PCL at 205 nm.

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## Abbreviations

| | |
|---|---|
| MSPs | The mesoporous silica particles |
| LMSPs | Lipase-immobilized mesoporous silica particles |
| LMSPs_Cn | n carbon atoms silane grafted LMSPs |
| TCA | The three-phase contact angle |
| PCL | Lipase PS from *Pseudomonas cepacia* |
| W/O | Water-in-oil |
| $V$ | The volume of the supernatant and washing solutions |
| $C_S$ | The molarity of tributyrin |
| C3 | Trimethylchlorosilane |
| C6 | Hexyltrichlorosilane |
| C8 | Octyltrichlorosilane |
| C10 | Decyltrichlorosilane |
| C12 | Dodecyltrichlorosilane |

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