Supporting Information

Biphasic Biocatalysis using a CO$_2$-Switchable Pickering Emulsion

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EXPERIMENTAL

**Reagents and materials:** Silica nanoparticles (primary particle diameter 20 nm) with a purity > 99.8% were purchased from Wuxi Jinding Longhua Chemical Co., Ltd. (China). 2,2,4-trimethylpentane (HPLC, 99.8%) and N,N-Dimethylidodecylamine (DMA, > 97%) were obtained from Adamas Reagent Co., Ltd. Oleic acid (99%) and fluorescein isothiocyanate isomer I (FITC, 95%) were purchased from Beijing J&K Company (China). Lipase from *Candida rugose* (CRL) was purchased from Sigma Aldrich. Olive oil with a saponification value of 196 mg KOH/g and an acid value of 4.0 mg KOH/g was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Cupric acetate (anhydrous, 97%) was purchased from Energy Chemical. High purity N₂ and CO₂ were provided by Wuxi Xinnan Chemical Gas Co., Ltd. Ultrapure water (18.2 MΩ cm, pH = 6.1 at 25°C) used in all experiments was produced from a Simplicity Pure Water System (Merck Millipore, Shanghai).

**Conductivity measurement of DMA solution:** The conductivity of DMA dissolved in a water-acetone solution (1:1 by volume) was measured using a conductivity meter (FE30, Mettler Toledo) at room temperature. Due to poor solubility of DMA in water, acetone was added in a mixed solution. During bubbling of CO₂, the vessel containing DMA solution was put into a water/ice bath, whereas it was at room temperature when bubbling N₂. It can be seen that the conductivity of the solution gradually increased when bubbling CO₂ since the amine group of the surfactant becomes protonated. In contrast, deprotonation of DMA-CO₂ by bubbling N₂ through the solution led to a decrease of the conductivity.
Determination of adsorption isotherm of surfactant on silica: Surface tension was employed to determine the adsorption of DMA-CO$_2$ onto silica particles. The surface tension of DMA-CO$_2$ solutions in the absence and presence of silica particles (0.1 wt.%) was measured using the du Noüy ring method at 25 °C. The equilibrium concentration of surfactant in a series of silica dispersions (0.1 wt.%) after adsorption for 24 h was calculated based on the surface tension of the dispersions using the surface tension of DMA-CO$_2$ in the absence of silica particles as calibration.

Preparation of emulsions: Silica nanoparticles were dispersed via an ultrasonic processor (JYD-650, Shanghai) at 50 W for 15 s at a nominal concentration of 0.005 g silica/mL in pure water or surfactant solutions of different concentration. In preparing emulsions, equal volumes of aqueous phase containing either surfactant or silica or both and pure isooctane were put in a 20 mL glass vessel (2.5 cm in diameter, 6 cm in length). Following homogenization with an ultra-turrax homogenizer (IKA T18 basic, S18N-10G head) at 8000 rpm for 2 min, emulsions stabilized by either surfactant or silica or both were obtained.

Characterization of emulsions: The emulsion type was determined by the drop test. A drop of emulsion was added to water. Generally, water continuous emulsions would rapidly disperse in water. To further identify the emulsion type, emulsions containing a low amount of Nile red were observed using a fluorescence microscope (Nikon 80i, Japan). Microscopy images of the emulsion droplets were also recorded using a VHX-1000 microscope system (Keyence Co.).
**Contact angles at oil-water-solid interface:** A glass slide was cut into strips 1.5 cm wide and soaked in 30% aqueous sodium hydroxide for 24 h. They were then washed with deionized water and dried. Then, a clean glass strip was placed inside a glass cuvette of 35 mm (L) × 25 mm (D) × 20 mm (H), with its two ends being supported by standing legs. The cuvette was then filled with surfactant solution until the glass strip was immersed. After 12 h (for adsorption), an isoctane droplet (0.5 μL) was released from a U-shaped needle in solution below the glass strip, which was then captured to form an inverted sessile droplet. The contact angle through the oil droplet was measured using a Dropmeter A-100 contact angle instrument and converted to the contact angle through the aqueous phase. The temperature was maintained at 25 °C using an air bath during measurement.

**Labelling of CRL with FITC:** 250 μL of anhydrous dimethyl sulfoxide containing 10 mg FITC was slowly added into a glass vessel (25 mL) containing a solution of CRL prepared in phosphate buffer solution (pH = 7.4). After addition, the labeling reaction was performed in the dark for 24 h at 4 °C. To separate the unbound FITC from the conjugate, the solution was transferred to a semi-permeable membrane (molecular weight cut off 3500 Da, diameter 22 mm, MD-34, Shanghai Baoman Biotechnology Co., Ltd. China).

**Assessment of the catalytic performance of CRL in Pickering emulsion:** Typically, the isoctane-in-water Pickering emulsions ($V_{oil} = V_{water} = 4$ mL) used as an enzymatic reaction medium were stabilized by a mixture of silica (0.5 wt.%), DMA-CO$_2$ (0.6 mM) and CRL (1 mg/mL) unless specified otherwise. In general, isoctane (4 mL)
containing olive oil (5 vol.%) was added to the aqueous phase containing a mixture of silica (0.5 wt.%), DMA-CO$_2$ (0.6 mM) and CRL (1 mg/mL). Following homogenization, the reaction was carried out in a water bath without stirring (DC-0506, Shanghai Hengping Instrument and Meter Factory) at 30°C.

At the end of the reaction, the Pickering emulsion was bubbled with N$_2$ at a rate of 150 mL min$^{-1}$ at 25 °C. A certain volume of the upper oil phase was diluted in isooctane (5 mL) and then vigorously shaken for 2 min after the addition of 1 mL of Cu(Ac)$_2$-pyridine solution (5 wt.%). In addition, the six reaction systems for benchmarking were treated the same to halt the reaction in order to analyze the yield of the product. The produced fatty acid was determined by UV-vis spectrophotometry using the calibration curve of oleic acid in isooctane (absorbance at $\lambda = 705$ nm). For the hydrolysis of olive oil, the conversion was calculated using:

$$H = \frac{n_{\text{acid}} \times 56.1 - m_{\text{oil}} \times 4}{m_{\text{oil}} \times (196 - 4)} \times 100\%$$

where $H$ is conversion, $n_{\text{acid}}$ (mmol) is the amount of fatty acid determined from the calibration curve for oleic acid, $m_{\text{oil}}$ (g) is the mass of olive oil, 196 (mg KOH/g) is the saponification value of olive oil and 4.0 (mg KOH/g) is the acid value of olive oil (Figure S15).

As for the esterification of octanol with oleic acid, the reaction conditions were the same as those of the hydrolysis except for the concentration of octanol (100 mM) and oleic acid (50 mM). The yield was determined in the same way described above.

**Recycled catalytic reactions in Pickering emulsions:** For olive oil hydrolysis
reaction: The catalytic reaction was performed following the same procedure as described above. The Pickering emulsion was broken by bubbling \( \text{N}_2 \) (150 mL min\(^{-1}\) at 25 °C) at the end of each catalytic cycle. Isooctane was collected by evaporation the oil phase. The trace of in-active “surfactant” DMA was separated and collected by rinsing the crude product (such as fatty acid) with carbonated water for three times. The product was purified by recrystallization. The aqueous phase containing enzyme and silica was recycled for the next cycle. Olive oil and DMA were then added into a certain volume of isooctane. A new cycle of the reaction was started after the Pickering emulsion was reformed by bubbling \( \text{CO}_2 \) through the oil–water mixture at 25 °C for 10 min followed by re-homogenization for 2 min. The recycling process of esterification was similar as the hydrolysis reaction.

**Hydrolysis of olive oil catalyzed by CRL in other systems.**

(a) *Biphasic reaction with nothing else under stirring* (I, Figure 2). CRL was first added into water with stirring. An equal volume of olive oil in isooctane (5 vol.%) was then added into the aqueous solution, and the reaction system was stirred at 600 rpm for a period time.

(b) *Biphasic reaction with DMA-CO\(_2\) (0.6 mM) under stirring* (II, Figure 2). DMA (0.6 mM) was added into water with stirring. After bubbling \( \text{CO}_2 \), the original opaque aqueous solution was transformed to a transparent DMA-CO\(_2\) solution. An equal volume of olive oil in isooctane (5 vol.%) and CRL were then added into the aqueous solution, and the reaction system was stirred at 600 rpm for a period time.
(c) *Biphasic reaction with silica nanoparticles (0.5 wt.%) under stirring* (III, Figure 2). Silica nanoparticles (0.5 wt.%) were first dispersed in water *via* an ultrasonic processor. Then an equal volume of olive oil in isoctane (5 vol.%) and CRL were added, and the reaction system was stirred at 600 rpm for a period time.

(d) *Biphasic reaction with DMA-CO$_2$ (0.6 mM) and silica (0.5 wt%) under stirring* (IV, Figure 2). DMA (0.6 mM) was added into water with stirring. After bubbling CO$_2$, the original opaque aqueous solution was transformed to a transparent DMA-CO$_2$ solution. Silica nanoparticles (0.5 wt.%) were then dispersed in the DMA-CO$_2$ aqueous solution *via* an ultrasonic processor. An equal volume of olive oil in isoctane solution (5 vol.%) and CRL were then added into the dispersion, and the reaction system was stirred at 600 rpm for a period time.
**Figure S1.** Inter-conversion between inactive form (left) and active form (right) of $N,N$-dimethyldodecylamine (DMA) at room temperature.

**Figure S2.** Conductivity variation of water-acetone solution (1:1 by vol.) containing 8 mM DMA upon alternately bubbling CO$_2$ (30 mL min$^{-1}$) and N$_2$ (100 mL min$^{-1}$) for three cycles at 20 °C.

**Figure S3.** $^1$H NMR (Bruker Advance III 400 MHz, D$_2$O : MeOD = 1:1, 25 °C, TMS) spectra of DMA before and after bubbling CO$_2$. The protons in DMA are assigned by the letters a-f. The chemical shifts of several specific protons located at different positions in DMA are shown in Table S1.
Table S1. Chemical shifts of the protons in DMA before and after bubbling CO$_2$.

<table>
<thead>
<tr>
<th>Position</th>
<th>DMA</th>
<th>DMA-CO$_2$</th>
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<tbody>
<tr>
<td>a</td>
<td>2.238</td>
<td>2.848</td>
</tr>
<tr>
<td>b</td>
<td>2.329</td>
<td>3.070</td>
</tr>
<tr>
<td>c</td>
<td>1.489</td>
<td>1.711</td>
</tr>
<tr>
<td>e</td>
<td>1.303</td>
<td>1.359</td>
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Figure S4. Variation of zeta potential of aqueous silica dispersions (0.1 wt.%) as a function of DMA concentration with and without CO$_2$. 
**Figure S5.** (a), (b) Appearance of isooctane-water emulsions (1:1 by vol.) stabilized by DMA-CO$_2$ alone taken (a) immediately and (b) 24 h after preparation. (c), (d) Appearance of isooctane-water emulsions (1:1 by vol.) stabilized by a mixture of silica nanoparticles (0.5 wt.%) and DMA-CO$_2$ taken (c) immediately and (d) 24 h after preparation. Concentration of DMA-CO$_2$ in water from left to right is 0, 0.2, 0.4, 0.8, 1, 3 and 5 mM, respectively.

**Figure S6.** Fluorescence microscopy image of isooctane-in-water Pickering emulsion stabilized by a mixture of silica (0.5 wt.%) and DMA-CO$_2$ (0.6 mM) with the oil phase stained with Nile red.
Figure S7. (a) Surface tension of DMA-CO$_2$ solutions with and without silica (0.1 wt.%) as a function of initial surfactant concentration at 25 °C. (b) Adsorption isotherm of DMA-CO$_2$ on silica particles derived from data in (a).

Figure S8. Inverted photos of isoctane drops captured by a glass strip immersed in aqueous solutions of DMA-CO$_2$. Concentration of DMA-CO$_2$ from left to right is 0.01, 0.03, 0.06, 0.1, 0.3 and 0.6 mM. Contact angles quoted are those through the aqueous phase.
Figure S9. Optical micrographs of isooctane-in-water emulsions (1:1 by vol.) after 1 week stabilized by a mixture of silica nanoparticles (0.5 wt.%) and DMA-CO$_2$ at different concentrations given.

Figure S10. Digital photographs of isooctane-in-water Pickering emulsions ($V_{\text{oil}} = V_{\text{water}} = 4$ mL) stabilized by silica nanoparticles (0.5 wt.%) in combination with DMA-CO$_2$ and CRL (1 mg/mL) (a) immediately after preparation, (b) 24 h after preparation and (c) after addition of olive oil (5 vol.%) in isooctane taken 24 h after preparation. [DMA-CO$_2$]/mM in water from left to right: 0.2, 0.4, 0.8, 1, 3 and 5.
Figure S11. Micrographs of Pickering emulsions stabilized by silica nanoparticles (0.5 wt.%) in combination with DMA-CO$_2$ (at different concentrations given) and CRL (1 mg/mL) taken 24 h after preparation: (a) without olive oil, (b) with olive oil (5 vol.%) in isooctane.

Figure S12. Fluorescence microscopy image of the Pickering emulsion stabilized by a mixture of silica (0.5 wt.%), DMA-CO$_2$ (0.6 mM), olive oil (5 vol.%) and CRL (1 mg/mL) labelled with FITC.
**Figure S13.** Optical micrographs of isooctane-in-water Pickering emulsions utilized as olive oil hydrolysis reaction medium at different ratios of oil:water with silica (0.5 wt%), DMA-CO$_2$ (0.6 mM) and CRL (1 mg/mL).

**Figure S14.** Optical micrographs of Pickering emulsions in which the hydrolysis of olive oil was carried out. (a) After the reaction was performed for 30 min in cycle 1, (b) after bubbling N$_2$ through the emulsion at room temperature for 40 min, (c) after the reaction was performed for 30 min in cycle 2.

**Figure S15.** (a) Absorbance spectra of oleic acid at different concentrations given in isooctane. (b) Absorbance at 705 nm as a function of oleic acid concentration.
Figure S16. Hydrolysis conversion of olive oil in a Pickering emulsion as a function of time for different cycles numbered.