Electronic Supplementary Material (ESI) for Catalysis Science & Technology.
This journal is © The Royal Society of Chemistry 2021

Catalysis Science & Technology

Supplementary information

Interfacial biocatalysis in bacteria-stabilized Pickering emulsions for microbial transformation of hydrophobic chemicals

Haisheng Xie[a], Wenyu Zhao[a], Daniel Chikere Ali[a], Xuehong Zhang[b], Zhilong Wang[a,*]

[a] State Key Laboratory of Microbial Metabolism, and Engineering Research Center of Cell & Therapeutic Antibody, Ministry of Education, School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan, Shanghai 200240, China

[b] State Key Laboratory of Microbial Metabolism, School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan, Shanghai 200240, China

*Corresponding author.
E-mail address: zlwang@sjtu.edu.cn (Z. Wang)

Calculation of contact angles using a drop-contour analysis software

Contact angles were precisely analyzed using the Drop Analysis-LB-ADSA plugin for the Image J software (NIH). The plugin was freely available (http://bigwww.epfl.ch/demo/dropanalysis/#soft). The procedure was presented briefly as follows. Photographic images of axisymmetric sessile drops were captured. The experimental drop profile and both side contact points should be clear. The theoretical profile should be best fitted to the experimental drop profile ending at the three-phase contact points, which was obtained by manually adjusting parameters including the apex position, the height and the curvature radius of the drop (Fig. S1a). The LB-ADSA plugin then calculated the theoretical profile based on the Young-Laplace equation and output the contact angles.

In this study, the contact angle of the cell lawn was obviously affected by the drying time (Fig. S1b). The contact angles reached the so-called "plateau state" between 2 and 4 h. Meanwhile, excess dehydration of cells (drying time >5 h) caused the contact angle to increase continuously. Thus, all the contact angles in the "plateau state" were measured during the drying time of 2–4 h.

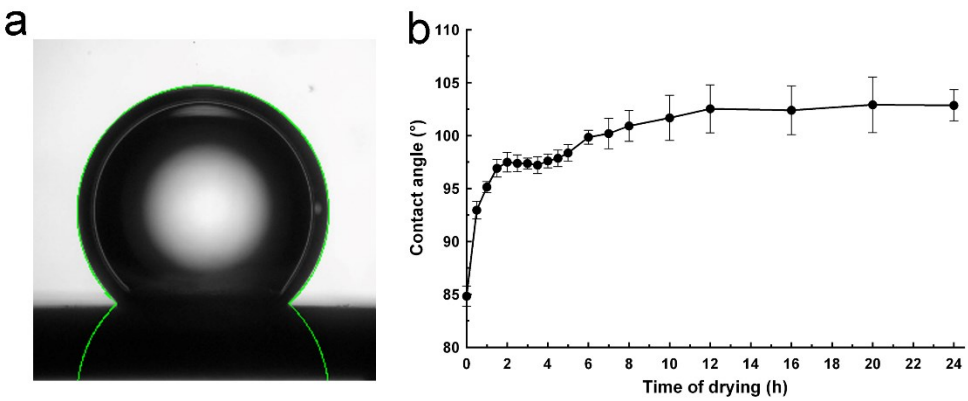


Fig. S1 (a) A theoretical profile fitting the experimental contour of a sessile drop. (b) Relationship between the three-phase contact angles of cell lawns in air and the time of drying. The cell lawn was dried in a chamber (30 ^{o}C, 50 %–60 % humidity).

Effect of formulation parameter on Pickering emulsion structure

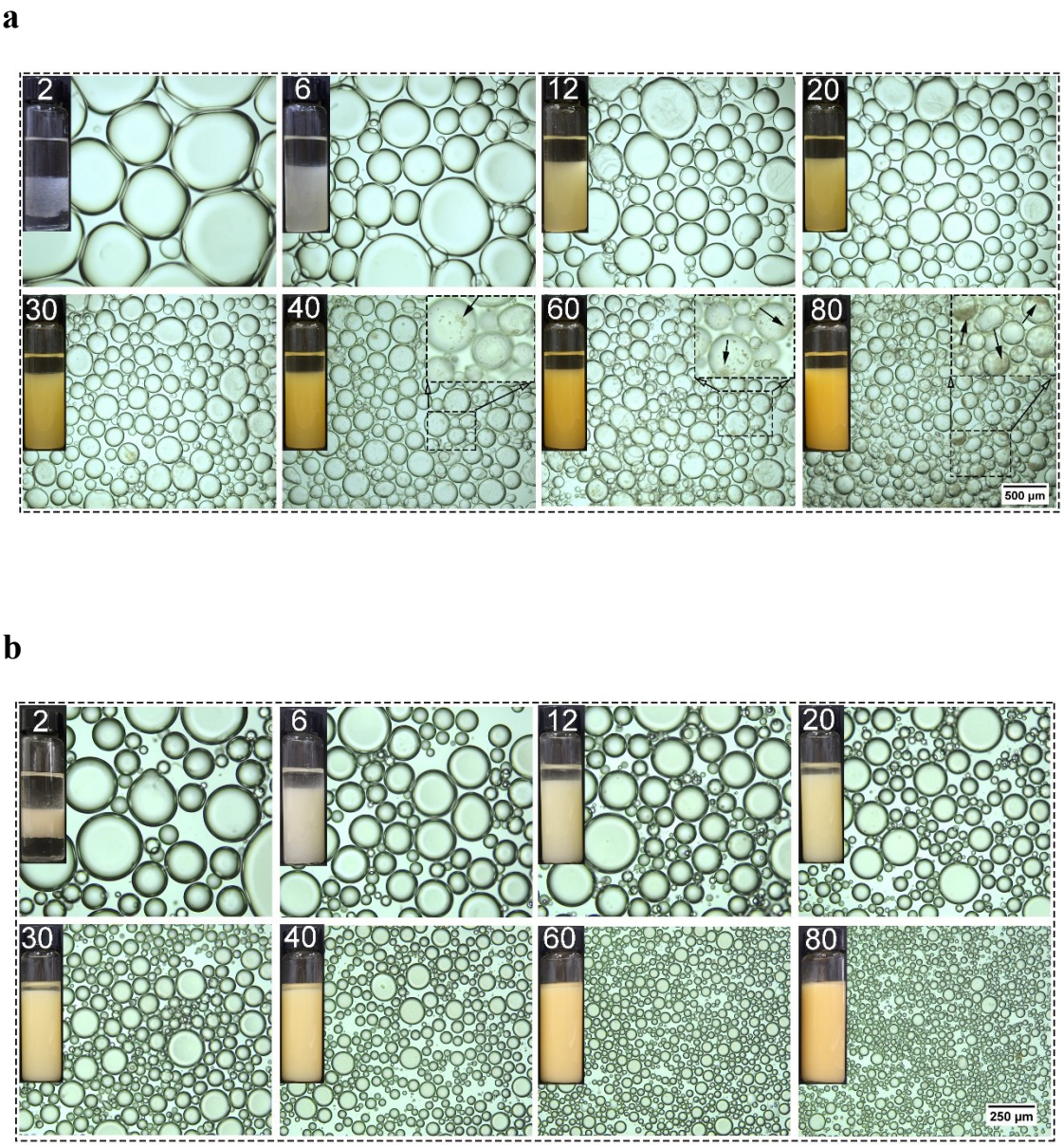
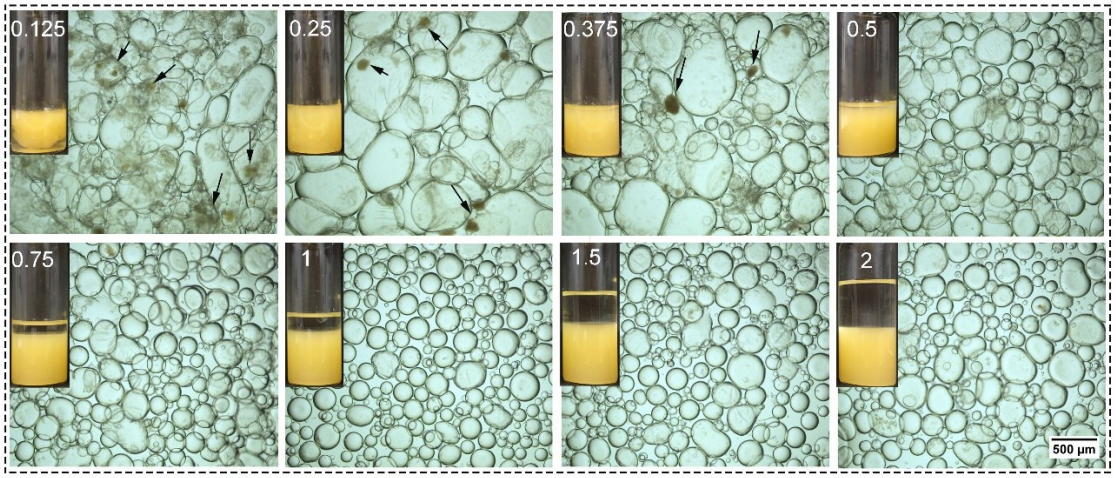


Fig. S2 Effect of cell concentration on Pickering emulsion structure. Microscopic images of the Pickering emulsions consisting of 4 ml water phase, 4 ml oil phase of PDMS (a) or BEHP (b); photographs of the corresponding Pickering emulsions were inserted on the left; the cell concentration was varied (indicated in each vial, g/L_{water}). The emulsions stood for 24 h after preparation to take the photographs and microscopic images. In the water–PDMS emulsions at relatively high cell concentrations, the inserted picture in the upper-right corner was the magnification micrographs of the region in a small box, where the black arrows indicated large cell aggregates.

a



b

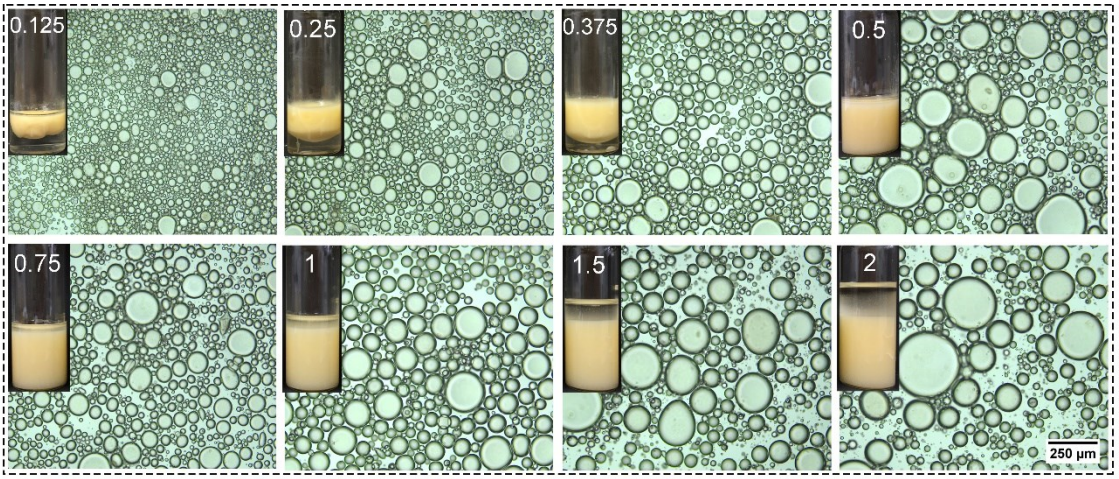


Fig. S3 Effect of volume ratio of oil-to-water on Pickering emulsion structure. Microscopic images of the Pickering emulsions with the oil phase PDMS (a) or BEHP (b); photographs of the corresponding Pickering emulsions were inserted on the left; the water phase was fixed to 4 ml while the volume ratio of oil to water ($R_{O/W}$) was varied from 0.125 to 2 (indicated on each vial), the cell concentration was constant (30 g/L_{water}). The emulsion stood for 24 h after preparation to take the photographs and microscopic images. In the water–PDMS emulsions at relatively low $R_{O/W}$, large cell aggregates were indicated with black arrows.

Effect of pretreating cells with Triton X-100 on biocatalytic activity in the aqueous system.

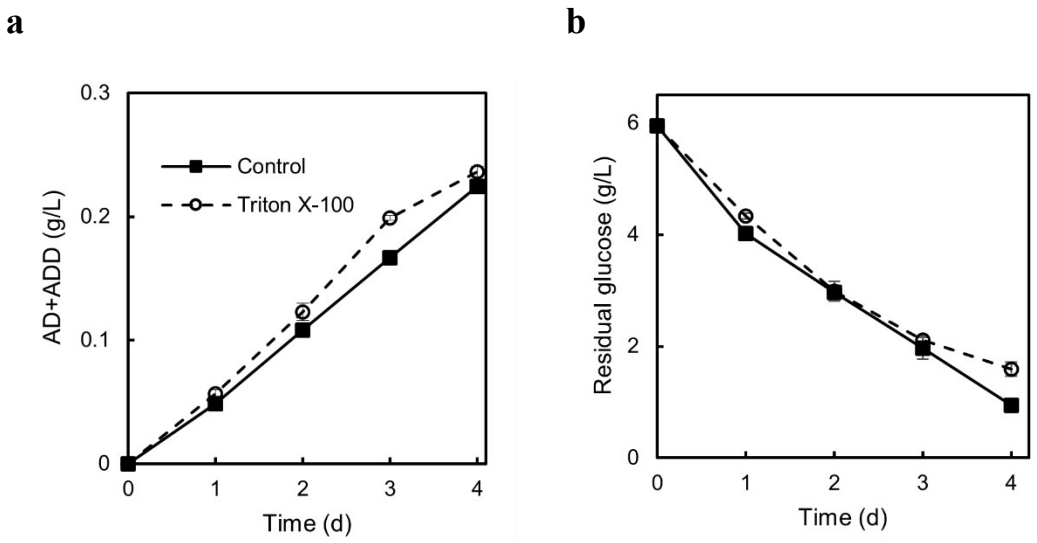


Fig. S4 Biocatalytic activity of the Triton X-100 pretreated cells in the aqueous system. (a) Microbial transformation of cholesterol to AD(D); (b) glucose degradation. The wet paste (6 g/L_{water}) was incubated in PBS (50 mM, pH 7; control) or Triton X-100 aqueous solution (0.2 g/L Triton X-100 in 50 mM, pH 7 PBS; pretreated cells) for 24 h, and then the cells were collected and washed with PBS for 5 times to remove the surfactant. The biocatalysis were carried out in PBS (50 mM, pH 7) with 10 g/L cholesterol or 6 g/L glucose, 6 g/L cells, and at 200 rpm, 30 ^{o}C for 3 days.

Effect of cell surface wettability on Pickering emulsion structure

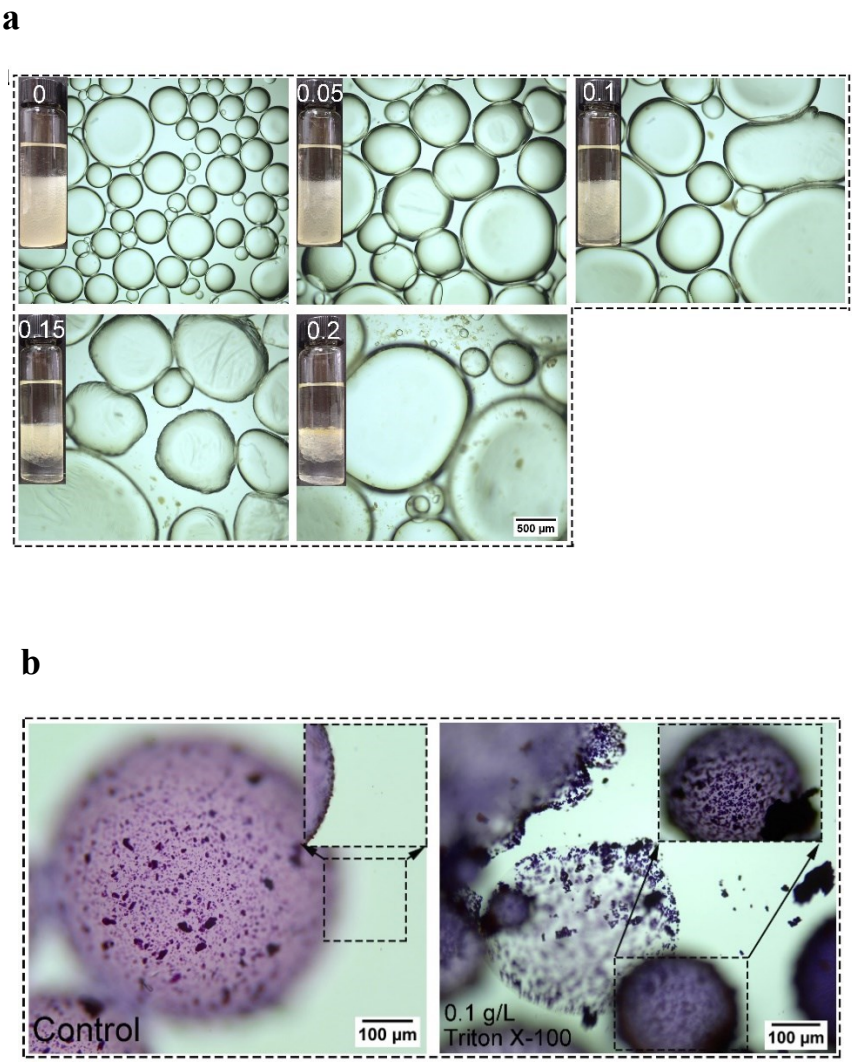


Fig. S5 Pretreated cell stabilized Pickering emulsions with oil phase PDMS. (a) Microscopic images of the pretreated cell stabilized Pickering emulsions (4 ml water, 4 ml PDMS and 6 g/L_{water} cells). Photographs of the corresponding Pickering emulsions were inserted on the left (Triton X-100 concentrations (g/L) for pretreatment of cell paste were indicated on each vial). (b) Microscopic images of the cells (stained with fuchsin) adhering to the interface of the Pickering emulsions. The inserted pictures were the micrographs of the region in the small box, but at different focus depth. In the control (cells pretreated in PBS without Triton X-100), there was nearly no cells in the continuous oil phase and the cell aggregates at the interface were small. In contrast, the Pickering emulsion stabilized by the pretreated cell (0.1 g/L Triton X-100) were relatively unstable, with larger cell aggregates in the continuous oil phase and at the interface.

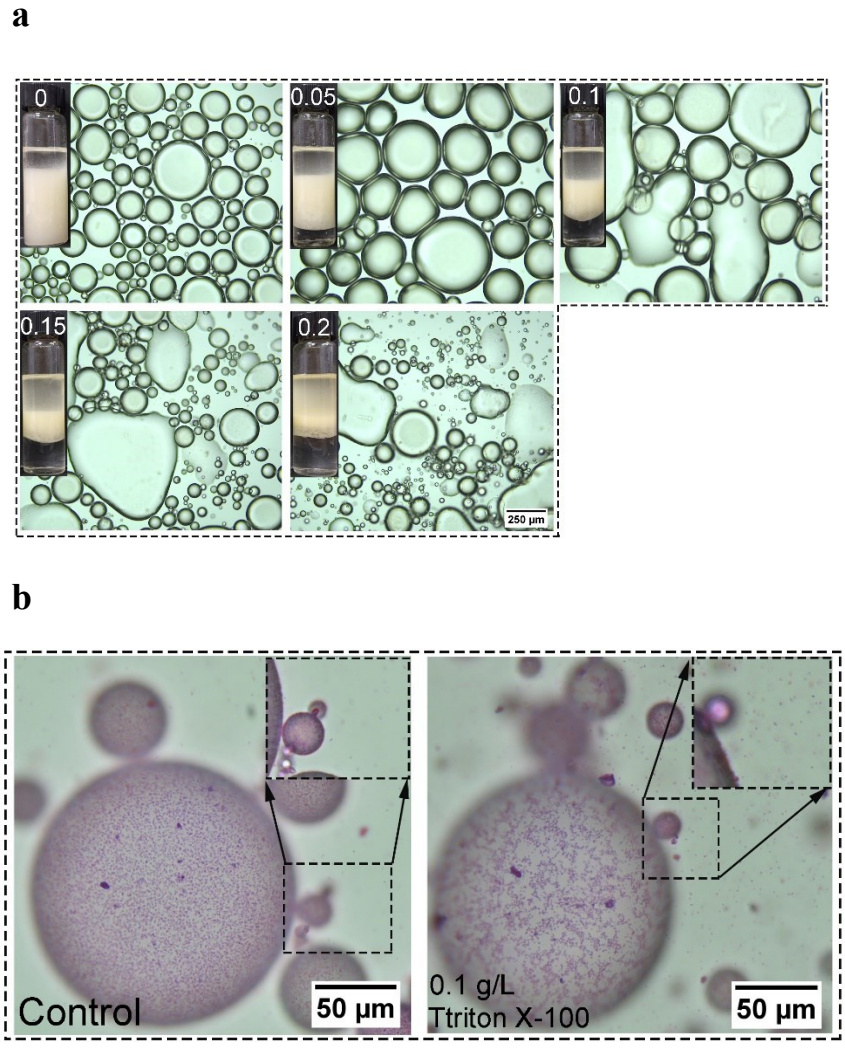


Fig. S6 Pretreated cell stabilized Pickering emulsions with oil phase BEHP. (a) Microscopic images of the pretreated cell stabilized Pickering emulsions (4 ml water, 4 ml BEHP, 6 g/L_{water} cells). Photographs of the corresponding Pickering emulsions were inserted on the left (Triton X-100 concentrations (g/L) for pretreatment of cell paste were indicated on each vial). (b) Microscopic images of the cells (stained with fuchsin) adhering to the interface of the Pickering emulsions. The inserted pictures were the micrographs of the region in the small box, but at different focus depth. In the control (cells pretreated in PBS without Triton X-100), there were only a few cells in the continuous oil phase while most cells adhered to the interface to form a dense cell monolayer. In the Pickering emulsions stabilized by the pretreated cells (0.1 g/L Triton X-100), there were a lot of cells in the continuous oil phase while only a few cells adhered unevenly to the interface with larger void not filled with cells.

Pickering emulsions formed at the end of microbial fermentation of cholesterol

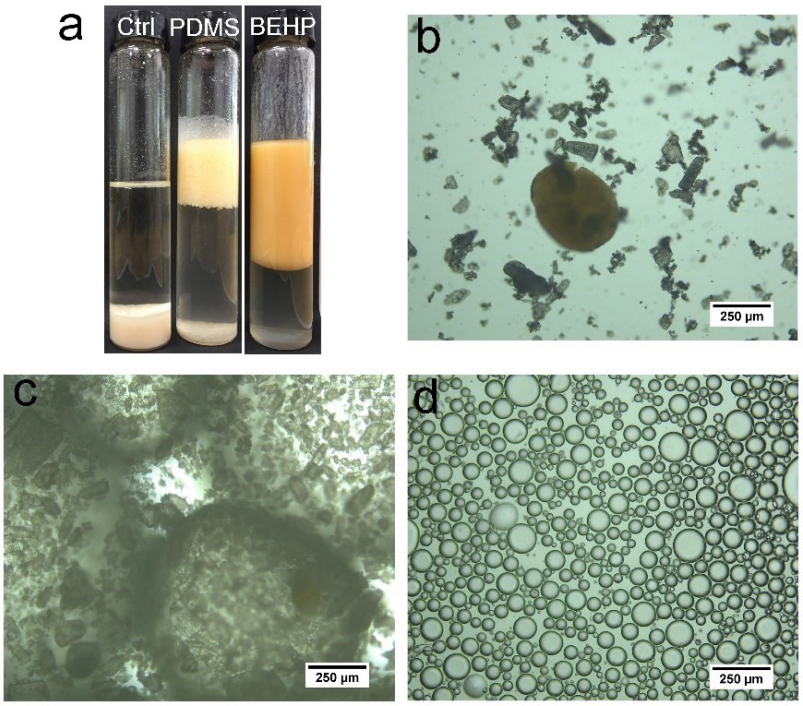


Fig. S7 Bacteria-stabilized Pickering emulsions at the end of microbial fermentation of cholesterol. 40 ml fermentation aqueous medium was used as the control; 10 ml PDMS or BEHP was added into 40 ml fermentation aqueous medium to construct the oil–water biphasic systems. With the progress of microbial fermentation, the growing cells emulsified the oil–water biphasic systems to form the Pickering emulsions. The observation was carried out on the ninth day for the control and the water–PDMS emulsion, and on the sixth day for the water–BEHP emulsion. (a) Photographs of the Pickering emulsions after standing for 24 h. Microscopic images of the control (b), the water–PDMS emulsion (c) and the water–BEHP emulsion (d); in the control, there were a large amount of solid cholesterol and a few large cell aggregates; in the water–PDMS emulsion, the water droplets were very big, and a lot of solid cholesterol and some cell aggregates adhered to the surfaces of the water droplets; in the water–BEHP emulsion, there were numerous small water droplets in the continuous oil phase and the cholesterol was completely exhausted.