Electronic Supporting Information

Simultaneous enzymatic esterification and ester extraction in Pickering emulsions for the recovery of butanol from fermentation broth

Yaoyao Feng,[a] Pierre-Louis Carrette,[b] Christine Dalmazzone, [§a] Etienne Jourdier[§a]*

[a] IFP Energies nouvelles, 1 et 4 avenue de Bois-Préau, 92852 Rueil-Malmaison, France

[b] IFP Energies nouvelles, Rond-point de l'échangeur de Solaize, BP 3, 69360 Solaize, France

* Corresponding author: etienne.jourdier@ifpen.fr

[§] Co-last authors

FIGURE CAPTIONS

Figure S1. Optical micrographs of Pickering emulsions stabilized with R972 and R816 nanoparticles at variable concentration and the corresponding droplet size distribution, respectively. Emulsification conditions: 6 mL dodecane, 6 mL water, certain amounts of nanoparticles, 13500 rpm for 2 min.

Figure S2. Variation of transmission (ΔT) and backscattering (ΔBS) signals vs. sample height and time at 40 °C for water/dodecane emulsions stabilized by R972 nanoparticle (a, c and e) and R816 nanoparticle (b, d and f) from 0 h (purple curve) to 12 h (red curve). Emulsification conditions: 1.0 wt.% silica nanoparticles, 50/50 (v/v), 13500 rpm for 2 min, (a and b) using water as aqueous phase, (c and d) using 0.1 mol L^{-1} butanol and butyric acid (substrate) solution as aqueous phase, (e and f) using fermentation broth and the addition of extra butyric acid (butanol:butyric acid 1:3) as aqueous phase.

Figure S3. Optical micrographs of Pickering emulsions stabilized with R972 and R816 nanoparticles and the corresponding droplet size distribution, respectively. Emulsification conditions: 1.0 wt.% R972 or R816 nanoparticles, 50/50 (v/v), 13500 rpm for 2 min, (a and c) using 0.1 mol L^{-1} butanol and butyric acid solution as aqueous phase, (b and d) using fermentation broth and the addition of extra butyric acid (butanol:butyric acid 1:3) as aqueous phase.

Figure S4. Kinetic profile for enzymatic esterification of 0.23 mol L^{-1} artificial solution in W/O emulsion system and comparison with the kinetic profile obtained with the fermentation broth in W/O emulsion. Reaction conditions: 6 mL water (containing 0.23 mol L^{-1} butanol and 0.23 or 0.69 mol L^{-1} butyric acid), 6 mL dodecane, 1.0 wt.% silica nanoparticles, 0.3% v/v CaLB solution (relative to the volume of aqueous phase), the pH of the aqueous phase is fixed at 4.0, emulsification at 13500 rpm for 2 min, 40 °C for 6 h, 500 rpm.

Experimental Section

Methods

Characterization of Pickering emulsions stability by Turbiscan

The emulsion stability was characterized at 40 °C during 12 h by static multiple light scattering (SMLS) using a Turbiscan Lab (Formulaction, France) operating with a near-infrared light source (λ = 880 nm). The transmission detector captures the light that passes through the sample, whereas the backscatter detector collects the light scattered in the reverse direction by the sample. The light intensity of emulsion usually changes slightly, indicating a low change of droplet concentration and higher stability. More specifically, the delta backscattering (ΔBS) profiles present the emulsion coalescence, creaming or sedimentation process over time, while the delta transmission (ΔT) profiles can be used to analyze the formation of a clarified layer. Considering that ΔBS intensity is a function of the volume fraction of the emulsion phase and droplet size at a specific height in a glass vial, the ΔBS intensity decreases either with the droplet size or by decreasing the droplet concentration. The sharp decrease in ΔBS at the top layer of the emulsion suggests a decrease in the top concentration and clarification of the layer. The variation of ΔBS and ΔT over the time as a function of the height of the vial reflects the moving trend of droplets and predicates the stability of the Pickering emulsions.

Fermentation and broth characterization

The fermentation medium was obtained from a continuous cultivation of a genetically modified *Clostridium acetobutylicum* strain producing a mixture of isopropanol, butanol and ethanol (IBE fermentation).[S1] The seed liquid contains 2.5 g L^{-1} yeast extract, 2.9 g L^{-1} CH_3COONH_4, 1 g L^{-1} KH_2PO_4, 0.6 g L^{-1} K_2HPO_4, 1 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.007 g L^{-1} $FeSO_4{\cdot}7H_2O$, 0.05 g L^{-1} antifoam (Biospumex 36K, PMC Ouvrie), and 60 g L^{-1} glucose. The stirred tank reactor was equipped with temperature, pH and stirring speed control. The reactor was further performed for 1000 h at 34 °C with agitation.

Before determining fermentation broth composition, the residual cells were separated from the aqueous phase by centrifuging at 5000 rpm for 20 min at 4 °C. After pretreatment to remove solid biomass, the fermentation broth turned into a clear and yellow solution. The concentrations of butanol, ethanol, isopropanol and acetone produced were determined by Scion 456-GC (Scion Instruments) equipped with a flame ionization detector and a PoraBOND-Q column (25 m length, 0.32 mm internal diameter, 0.5 µm film thickness) using propanol as an internal standard.

The concentration of the carboxylic acids was measured using a high performance liquid chromatograph (HPLC, LC-20AD Shimadzu) equipped with an autosampler (injection=10 µL), and a refractive index (RI) detector and UV/Vis detector set at 210 nm. The column was packed with HPX-87H (Bio-Rad) and the column temperature was maintained at 60 °C. The mobile phase was 0.01N H_2SO_4 with the flow rate of 0.6 mL min^{-1}. Broth samples were diluted in H_2O and filtered at 0.2 µm.

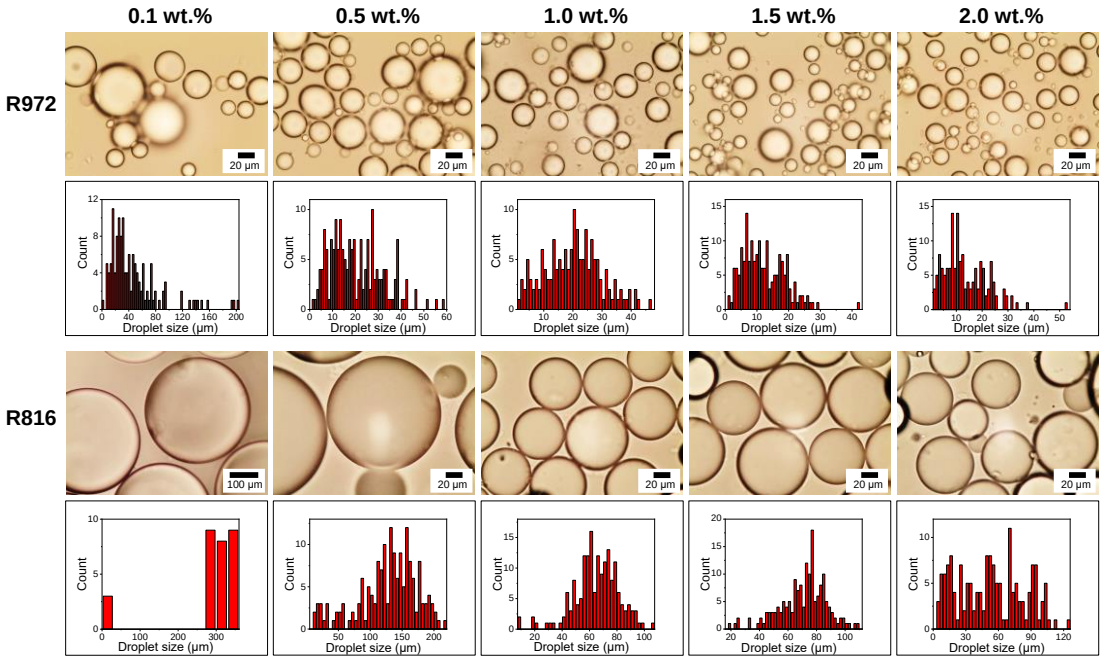

Figure S1. Optical micrographs of Pickering emulsions stabilized with R972 and R816 nanoparticles at variable concentration and the corresponding droplet size distribution, respectively. Emulsification conditions: 6 mL dodecane, 6 mL water, certain amounts of nanoparticles, 13500 rpm for 2 min.

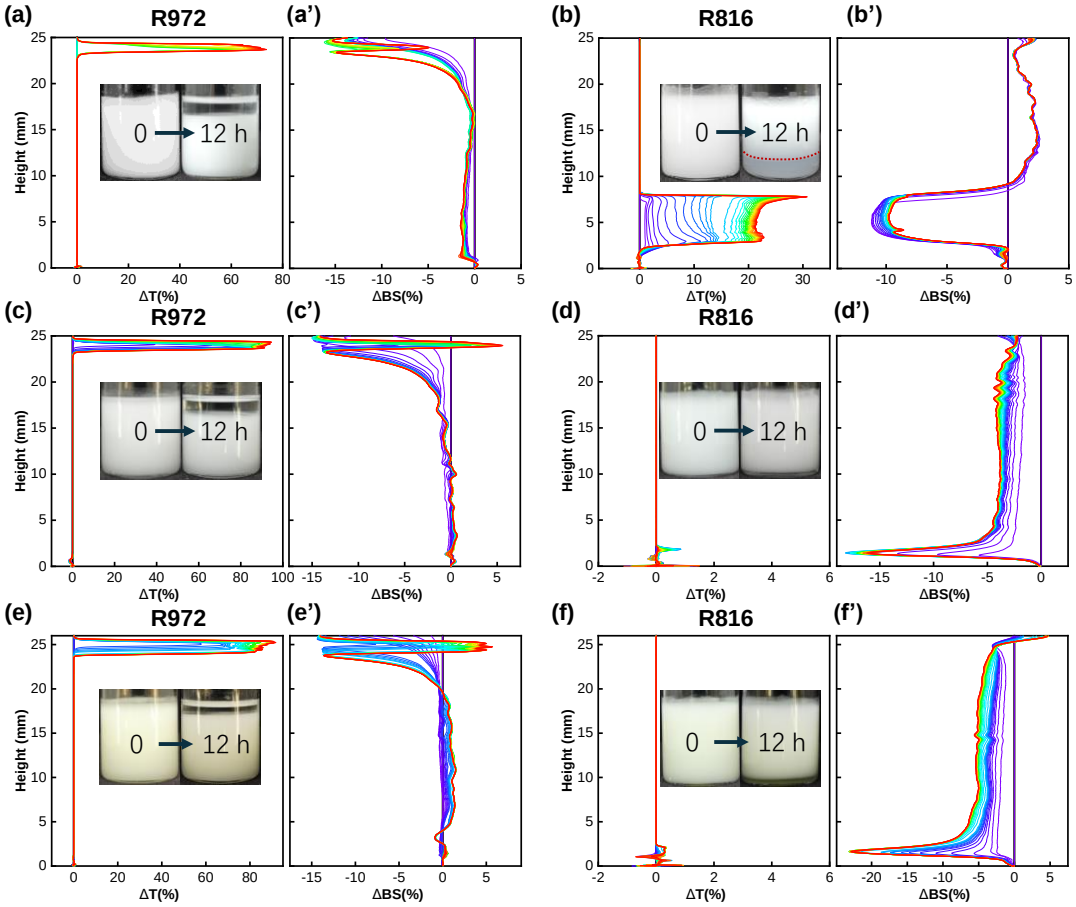

Figure S2. Variation of transmission (ΔT) and backscattering (ΔBS) signals vs. sample height and time at 40 °C for water/dodecane emulsions stabilized by R972 nanoparticle (a, c and e) and R816 nanoparticle (b, d and f) from 0 h (purple curve) to 12 h (red curve). Emulsification conditions: 1.0 wt.% silica nanoparticles, 50/50 (v/v), 13500 rpm for 2 min, (a and b) using water as aqueous phase, (c and d) using 0.1 mol L^{-1} butanol and butyric acid (substrate) solution as aqueous phase, (e and f) using fermentation broth and the addition of extra butyric acid (butanol:butyric acid 1:3) as aqueous phase.

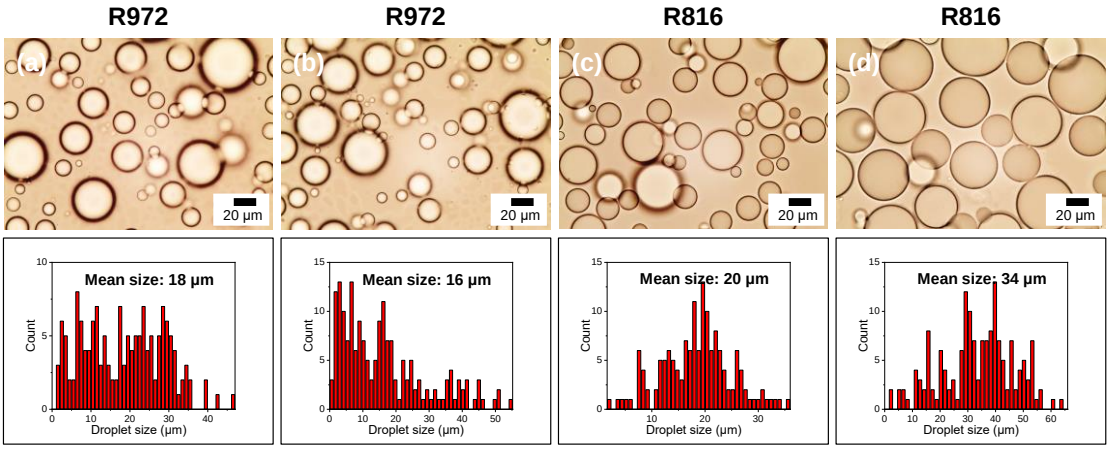

Figure S3. Optical micrographs of Pickering emulsions stabilized with R972 and R816 nanoparticles and the corresponding droplet size distribution, respectively. Emulsification conditions: 1.0 wt.% R972 or R816 nanoparticles, 50/50 (v/v), 13500 rpm for 2 min, (a and c) using 0.1 mol L^{-1} butanol and butyric acid solution as aqueous phase, (b and d) using fermentation broth and the addition of extra butyric acid (butanol:butyric acid 1:3) as aqueous phase.

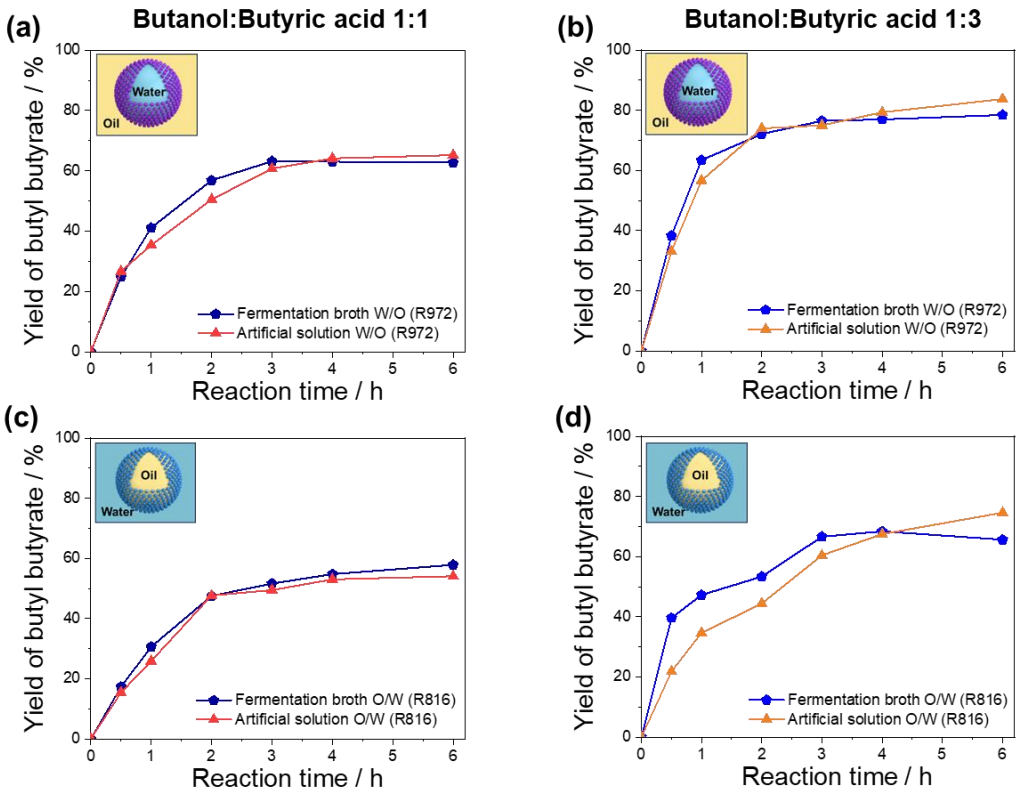

Figure S4. Kinetic profile for enzymatic esterification of 0.23 mol L^{-1} butanol artificial solution with alcohol:acid ratio 1:1 (a and c) or 1:3 (b and d) in W/O emulsion system (a and b) or O/W emulsion system (c and d) and comparison with the kinetic profile obtained with the fermentation broth in the same conditions. Reaction conditions: 6 mL water (containing 0.23 mol L^{-1} butanol and 0.23 or 0.69 mol L^{-1} butyric acid), 6 mL dodecane, 1.0 wt.% R972 or R816 silica nanoparticles, 0.3% v/v CaLB solution (relative to the volume of aqueous phase), the pH of the aqueous phase is fixed at 4.0, emulsification at 13500 rpm for 2 min, 40 °C for 6 h, 500 rpm.

References

S1. F. Collas, W. Kuit, B. Clément, R. Marchal, A. M. López-Contreras and F. Monot, Amb Express, 2012, 2, 1–10.