Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Data

Saponin-enhanced biomass accumulation and demulsification capability of the demulsifying bacteria *Alcaligenes* sp. S-XJ-1

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Surface free energy calculation:

Apolar Lifshitz-van der Waals (LW, γ_b^{LW}) component and polar electron-donor (γ_b^-) and electron-acceptor (γ_b^+) parameters of bacterial surface were estimated by putting the measured contact angles (θ_i) along with known total surface tension (γ_i^L) and parameters γ_i^{LW}, γ_i^-, and γ_i^+ of the three probe liquids (distilled water, formamide, and diiodomethane, Table S1) into the Young–Dupre equation (1).[1]

Table S1. Components and parameters of the surface free energy of probe liquids.

Probe liquids	Surface free energy (mJ/m^2)			
	γ^{LW}	γ^+	γ^-	γ^L
Water	21.8	25.5	25.5	72.8
Formamide	39	2.28	39.6	58
Diiodomethane	50.8	0	0	50.8

$$\sqrt{\gamma_i^{LW}} \times \sqrt{\gamma_b^{LW}} + \sqrt{\gamma_i^+} \times \sqrt{\gamma_b^-} + \sqrt{\gamma_i^-} \times \sqrt{\gamma_b^+} = (1 + \cos\theta_i) \times \frac{\gamma^L{}_i}{2} \quad (1)$$

The free energy of the interfacial interaction between two bacterial cells immersed in water (ΔG_{bwb}) for each sample was calculated by Eq. (2).[2]

$$\Delta G_{bwb} = -2 \times \left(\sqrt{\gamma_b^{LW}} - \sqrt{\gamma_w^{LW}} \right) - 4 \times \left(\sqrt{\gamma_b^+ \times \gamma_b^-} + \sqrt{\gamma_w^+ \times \gamma_w^-} - \sqrt{\gamma_b^+ \times \gamma_w^-} - \sqrt{\gamma_b^- \times \gamma_w^+} \right) \quad (2)$$

Literature cited

1. C. J. van Oss, *Colloids Surf., B*, 1995, **5**, 91–110.

2. K. M. Peng, J. Liu, L. J. Lu, W. Yin and X. F. Huang, *J. Adhes. Sci. Technol.*, 2016, **30**, 194–209.

Cell-surface substance analysis:

The translation of the elemental differences from XPS into the three major classes of proteins, lipids, and polysaccharides was presented as follows:

Chemical compositions on cell-surface were modeled in terms of three classes: proteins (Pr), polysaccharides (Ps) with a general formula $(C_6H_{10}O_5)_n$, and lipids (Lp) that refer to compounds containing mainly carbon and hydrogen. The compositions were calculated with the proportion of elements determined by XPS, according to the following set of equations (3–5):[3, 4]

$$[N/C] = 0.279(C_{Pr}/C) \quad (3)$$

$$[O/C] = 0.325(C_{Pr}/C) + 0.833(C_{Ps}/C) \quad (4)$$

$$[C/C] = (C_{Pr}/C) + (C_{Ps}/C) + (C_{Lp}/C) \quad (5)$$

Literature cited

3. J. Burgain, C. Gaiani, G. Francius, A. M. Revol-Junelles, C. Cailliez-Grimal, S. Lebeer, H. L. P. Tytgat, J. Vanderleyden and J. Scher, *Colloids Surf., B*, 2013, **104**, 153–162.

4. P. G. Rouxhet and M. J. Genet, *Surf. Interface Anal.*, 2011, **43**, 1453–1470.

Measurement of critical micelle concentration:

The surface tensions of saponin solutions were measured by a platinum plate method using an automatic surface tensiometer (DT-102; Huakun Instrument Co., Ltd., Shandong, China). The concentrations of solutions were between 0.006% and 0.5% (w/v). The CMC of saponin was the concentrations at which the surface tension first reached its minimum. This experiment was conducted in triplicate at a temperature of 27°C.

Adsorption and desorption tests:

Two kinds of biomass were used for the adsorption and desorption tests: one was the biomass cultivated with rape oil for 7 days (marked as "biomass-R"), and the other was the biomass cultivated with rape oil and saponin additives (0.1%, 0.5%, and 1%) for 7 days (marked as "biomass-RS"). In the adsorption tests, equal amounts of inactive biomass-R (as an "adsorbent") were mixed with saponin (0.01%, 0.03%, 0.05%, 0.1%, 0.5%, and 1%, w/v) in PBS (pH=7) for 24 h at 130 rpm before centrifugation. In the desorption tests, equal amounts of inactive biomass-RS were added to an equal volume of deionized water and mixed for 5 min at 1000 rpm before centrifugation. After centrifugation, the saponin concentration in the supernatant was determined by spectrophotometry,[5] and the harvested bacteria were used to estimate demulsification. The adsorbed or desorbed amount of saponin per gram biomass and the adsorption or desorption ratio were evaluated. The values presented are averages of triplicate samples.

Literature cited

5. J. Yan, Z. L. Wu, Y. L. Zhao and C. S. Jiang, *Sep. Purif. Technol.*, 2011, **80**, 300–305.

Figure S1. The surface tensions and CMC of saponin solutions.

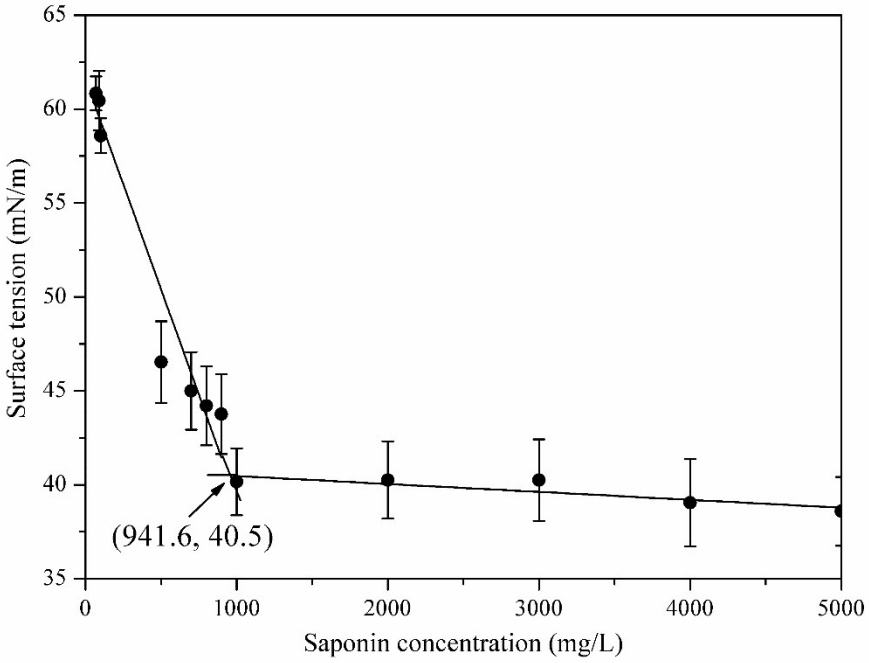


Figure S2. The relationship between contact angle and demulsification ratio.

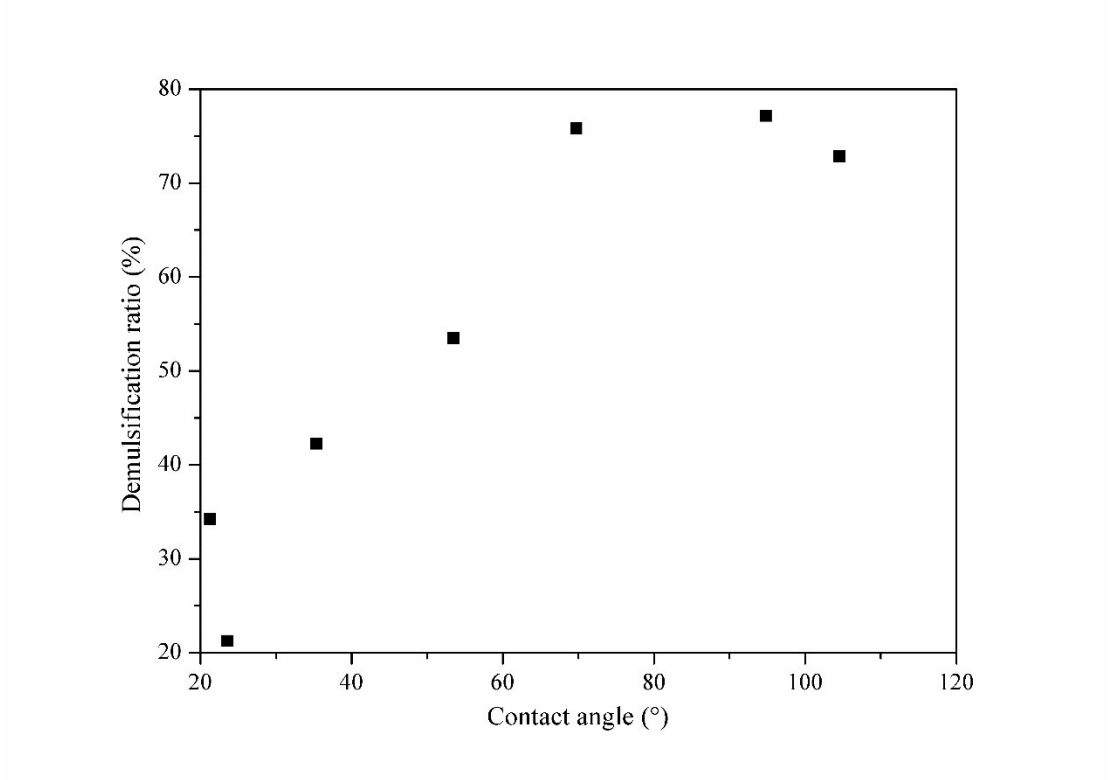
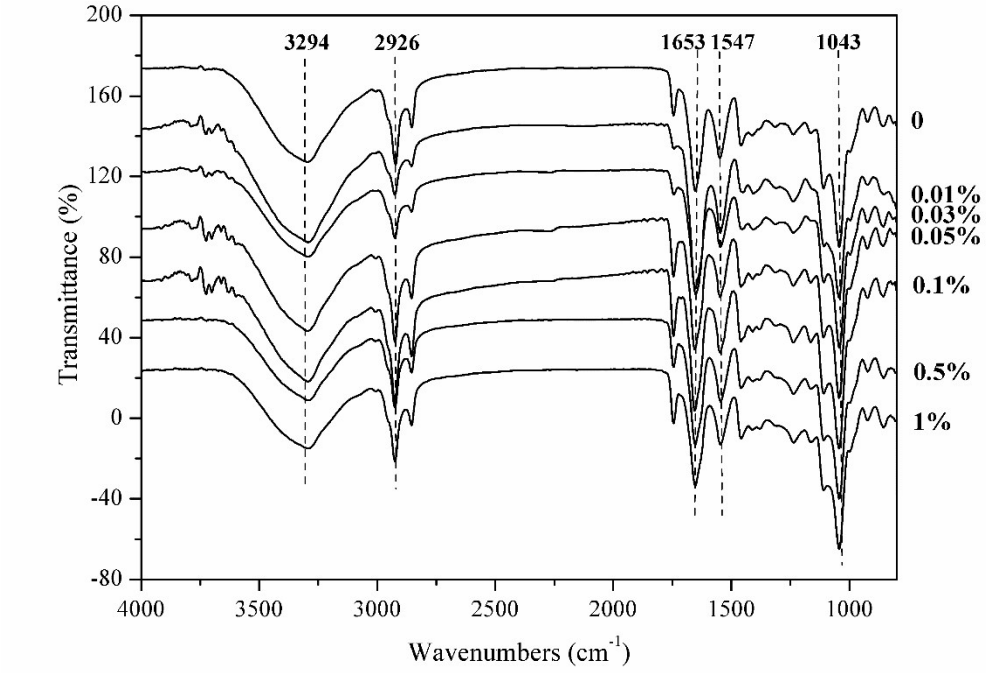


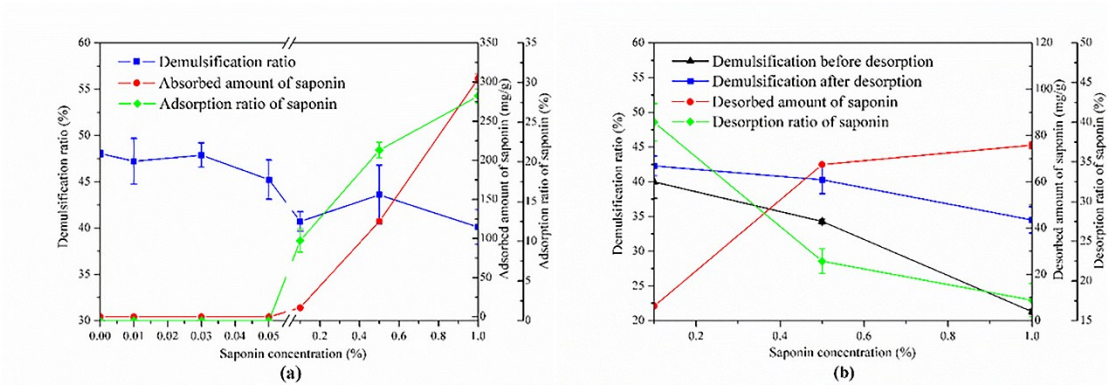
Figure S3. The effects of saponin at different concentrations on cell-surface functional groups.



Results:

Obvious peaks were observed at 3294, 2926, 1653, 1547, and 1043 cm^{-1} for all samples, and the corresponding functional groups were –OH, C-H, amide I (amide bond in the C=O stretching vibration), amide II bond (amide bond of the NH bending vibration), and O−C, respectively.

Figure S4. Adsorption (a) and desorption (b) of saponin and corresponding changes in demulsification.



Results:

Demulsification was affected by the adsorption of saponin at high concentrations (0.1–1%) on *Alcaligenes* sp. S-XJ-1 cells, while the cells exhibited almost no adsorption for saponin at low concentrations (0.01–0.05%) (Fig. S4a). The desorption of saponin from *Alcaligenes* sp. S-XJ-1 improved demulsification (Fig. S4b), which also indirectly proved that the adsorption of saponin on bacteria did inhibit demulsification.

Figure S5. The relationship between protein content and the contact angle of the cell-surface.

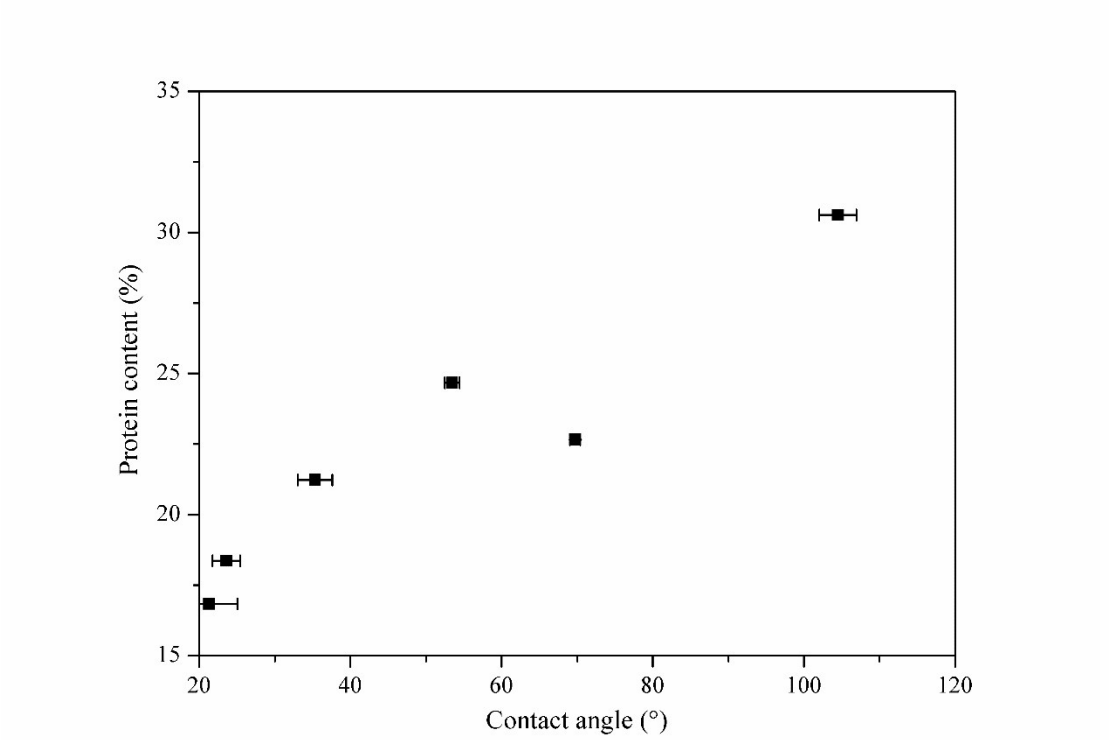


Table S2. Chemical compositions of the demulsifying strain S-XJ-1 cultivated with saponins at various concentrations.

Saponin concentration	Total carbon	Total nitrogen	Total oxygen	Molar ratio vs total carbon	
(%)	(%)	(%)	(%)	N/C	O/C
0	75.10	4.20	18.61	0.0559	0.2478
0.01	74.10	6.18	18.88	0.0834	0.2547
0.03	74.79	5.27	19.01	0.0705	0.2542
0.05	76.03	3.89	18.97	0.0512	0.2495
0.1	77.22	3.64	18.01	0.0472	0.2332
0.5	75.59	2.91	20.92	0.0386	0.2768
1	76.50	3.16	19.24	0.0413	0.2515