Supplementary information

Petroleum hydrocarbon degrading bacteria associated with chitosan as effective particle-stabilizers for oil emulsification

Haiyue Gong, Yiming Li,* Mutai Bao, Dong Lv and Zhining Wang

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, 266100, Shandong Province, China

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, 266100, Shandong Province, P.R. China. E-mail address: liym@ouc.edu.cn; Tel:+86 -532-66782509; Tel: +86 -532-66782509

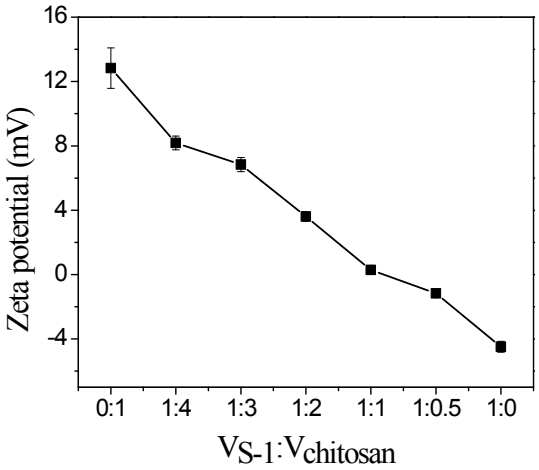

Fig. S1. Zeta potential of *Bacillus cereus* S-1 suspensions at different bacteria and chitosan volume ratios ($C_{S\text{-}1} = 5.06\times10^8$ cell mL^{-1}, $C_{chitosan} = 0.015\%$ w/v).

Contact angle measurements

Contact angle was measured for *Bacillus cereus* S-1 cells treated with and without chitosan. Washed cell suspension (40 mL) with and without chitosan was filtered through a 0.22 μm pore size nylon membrane filters leaving a smooth bacterial lawn deposited on the filter paper. A smooth bacterial lawn was deposited on the filter paper and was dried on an LB agar plate at room temperature for 1 h. For chitosan case, before drying the bacterial lawn was rinsed in pure water to remove the free chitosan. The filter was then immersed in a n-tetradecane bath. Immediately after placing a 2 μL droplet of distilled water, the three-phase contact angle between the aqueous drop, bacterial lawn and n-tetradecane was measured using a OCA15 Pro (Dataphysics, Germany). The measurements were carried out on at least three separate locations for each sample.

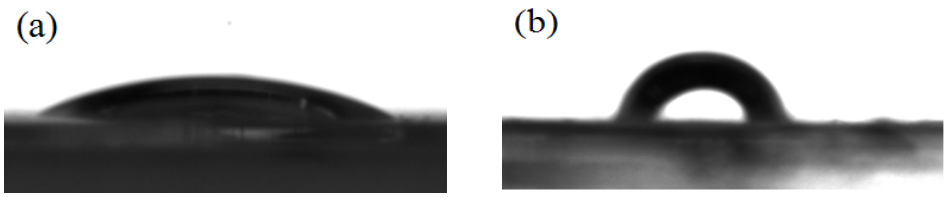

Fig. S2. Three phase contact angle of *Bacillus cereus* S-1 cell. (a) Untreated (control) cell lawn, θ = 25.1º; (b) cells pre-treated with = 0.015% w/v chitosan solution (θ = 81.2º).

Fig. S3. Photographs of n-tetradecane-in-water emulsion stabilized by *Bacillus cereus* S-1 (bottle a) and 0.01% w/v chitosan (bottle b) alone in 0.85% NaCl solution after 5min (V_{oil}:V_{water} = 1:2).

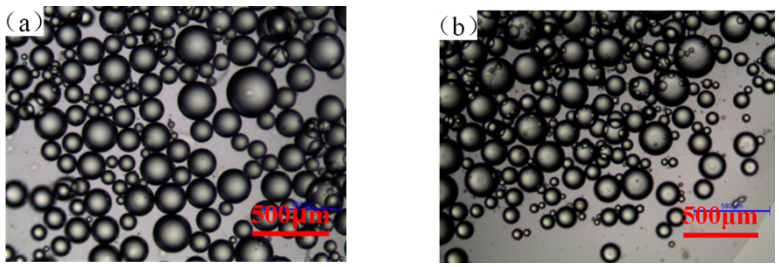

Fig. S4. The optical microscopic images of emulsions stabilized by *Bacillus cereus* S-1 and chitosan complex in the ASW after 7d (a) and 31d (b).

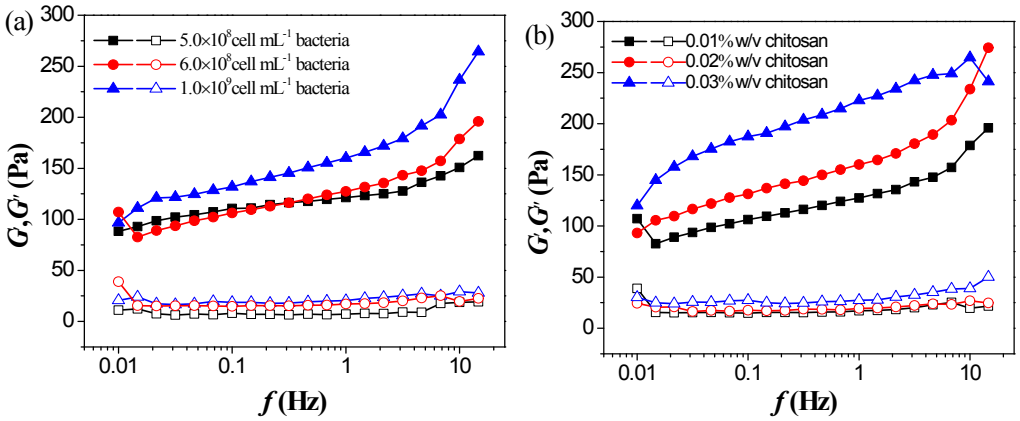

Fig. S5. Frequency dependence of the storage moduli G′ (filled symbols) and loss moduli G″ (open symbols) of oil-in-NaCl solution emulsions (V_{oil}:V_{water} = 1:2).

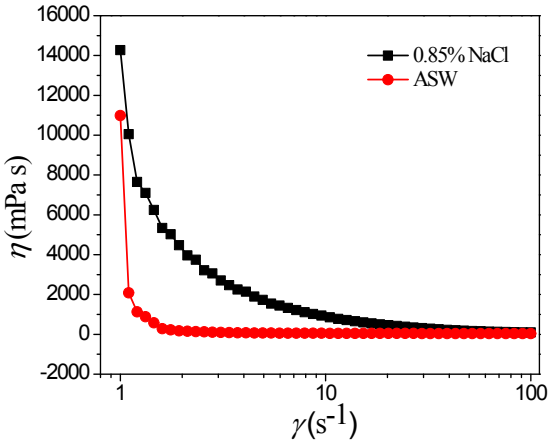

Fig. S6. Viscosity dependence of oil-in-water emulsions on shear rate ($C_{S\text{-}1}$ = 5.04×10^8 cells mL^{-1}, $C_{chitosan}$ = 0.01% w/v, V_{oil}:V_{water} = 1:2).

Table S1 Morphological, physiological and biochemical properties of *Bacillus cereus* S-1 isolated from petroleum-polluted seafloor sediment.

Item	*Bacillus cereus* S-1
Colony color	Milky-white
Colony surface	Wet smooth
Shape of cells	Long rod
Transparency	Non-transparent
Gram staining	+
Colonial morphology	Irregular
Spore staining	+
Catalase test	+
Glucose oxidation	Oxidized
MR test	-
Gelatin liquefaction	+
V-P test	+
Hydrolyzed Starch	-
Hydrated cellulose	-

+, Positive; -, negative.

Table S2 Bacteria growth of *Bacillus cereus* S-1 in different conditions after 7 days[a].

$C_{\text{n-tetradecane}}$ (g L^{-1})	C_{chitosan} (% w/v)	Bacterial count ($\times 10^8$ cells mL^{-1})
0	0	0.66
1	0	1.17
1	0.013	1.01

[a] Initial bacterial count was 1.92×10^8 cells mL^{-1}.