Supporting Information

Biomineralization of Varied Calcium Carbonate Crystal by Synergistic Effect of Silk Fibroin/Magnesium ions in a Microbial System

Tao Chen[a], Peiheng Shi[a], Yi Li[a], Tao Duan[b], Yu Yang[c], Xianyan Li[b], Wenkun Zhu[a,b*]

[a] *Nuclear Waste and Environmental Safety Key Laboratory of Defense, Southwest University of Science and Technology, Sichuan Mianyang 621010, P.R. China.*

[b] *State Key Laboratory of Environmentally Friendly Energy Materials, Southwest University of Science and Technology, Mianyang 621010, P.R.China.*

[c] *Mianyang People's Hospital, Mianyang 621010, P.R.China.*

**corresponding author: zhuwenkun@swust.edu.cn.*

1. Experiments

1.1 Mineralization experiments in the absence of additives

1 L of liquid medium was prepared using deionized water, glucose 20 g, peptone 10 g, NaCl 5 g. The above liquid medium was placed in a sterilizer and sterilized at 120 ° C for 20 minutes, then taken out and placed in a sterile operation table and cooled naturally. Then 0.05 mol/L of $Ca(NO_3)_2$/Urea was added to the above liquid medium by the microporous membrane filter sterilization method. Finally, 5 ml of the bacterial liquid was inoculated into the above liquid medium. The liquid medium was incubated at 30 °C for 6, 12, and 18 h, respectively. The mineralized sample was obtained by static and suction filtration, and the mineralized sample was washed three times with distilled water and absolute ethanol, and then dried at 35 °C for 12 h.

1.2 Mineralization experiments in the presence of magnesium ions

1 L of liquid medium was prepared using deionized water, glucose 20 g, peptone 10 g, NaCl 5 g. The above liquid medium was placed in a sterilizer and sterilized at 120 ° C for 20 minutes, then taken out and placed in a sterile operation table and cooled naturally. Then 0.05 mol/L of $Ca(NO_3)_2$/Urea and 2g/L of $MgCl_2$ was added to the above liquid medium by the microporous membrane filter sterilization method. The remaining experimental steps consistent with before.

1.3 Mineralization experiments in the presence of SF

1 L of liquid medium was prepared using deionized water, glucose 20 g, peptone 10 g, NaCl 5 g. The above liquid medium was placed in a sterilizer and sterilized at 120 ° C for 20 minutes, then taken out and placed in a sterile operation table and cooled naturally. Then 0.05 mol/L of $Ca(NO_3)_2$/Urea was added to the above liquid medium by the microporous membrane filter sterilization method. SF solution (4 mL) was injected into the above liquid medium. The remaining experimental steps consistent with before.

1.4 Mineralization experiments by chemical methods

1.41 Preparation of calcium carbonate by chemical method in the absence of additives

In a typical procedure, the solutions of calcium nitrate (0.05 mol/L, 30 mL) and an anhydrous sodium carbonate solution (0.05 mol/L, 30 mL) were mixed with mechanic stirring for 5 min to obtain a mixed solution. The mineralized sample was obtained by static and suction filtration, and the mineralized sample was washed three times with distilled water and absolute ethanol, and then dried at 35 °C for 12 h.

1.42 Preparation of calcium carbonate by chemical method in the presence of magnesium ions

In a typical procedure, the solutions of calcium nitrate (0.05 mol/L, 30 mL) and magnesium chloride hexahydrate (2g/L, 2ml) were mixed with mechanic stirring for 5 min to obtain a mixed solution. Then, an anhydrous sodium carbonate solution (0.05 mol/L, 30 mL) was added rapidly to the mixed solution with stirring under ambient condition. The mineralized sample was obtained by static and suction filtration, and the mineralized sample was washed three times with distilled water and absolute

ethanol, and then dried at 35 °C for 12 h.

1.43 Preparation of calcium carbonate by chemical method in the presence of SF

In a typical procedure, the solutions of calcium nitrate (0.05 mol/L, 30 mL) and SF solution (4 mL) were mixed with mechanic stirring for 5 min to obtain a mixed solution. Then, an anhydrous sodium carbonate solution (0.05 mol/L, 30 mL) was added rapidly to the mixed solution with stirring under ambient condition. The mineralized sample was obtained by static and suction filtration, and the mineralized sample was washed three times with distilled water and absolute ethanol, and then dried at 35 °C for 12 h.

1.44 Preparation of calcium carbonate by chemical method in the Presence of SF/Magnesium ions

In a typical procedure, the solutions of calcium nitrate (0.05 mol/L, 30 mL), SF solution (4 mL) and magnesium chloride hexahydrate (2g/L, 2ml) were mixed with mechanic stirring for 5 min to obtain a mixed solution. Then, an anhydrous sodium carbonate solution (0.05 mol/L, 30 mL) was added rapidly to the mixed solution with stirring under ambient condition. The mineralized sample was obtained by static and suction filtration, and the mineralized sample was washed three times with distilled water and absolute ethanol, and then dried at 35 °C for 12 h.

2. $CaCO_3$ Crystals Growing in the absence of additives

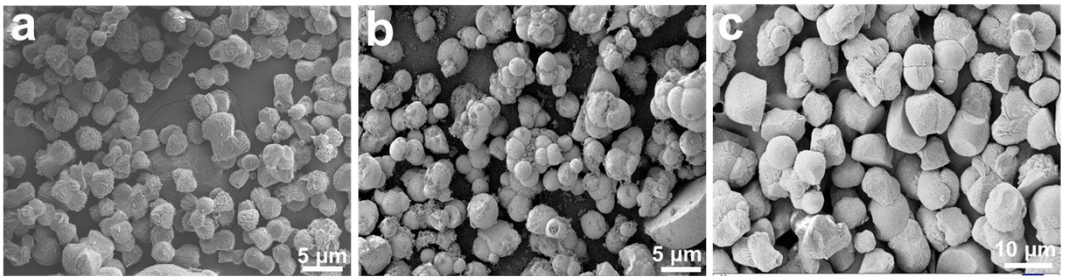
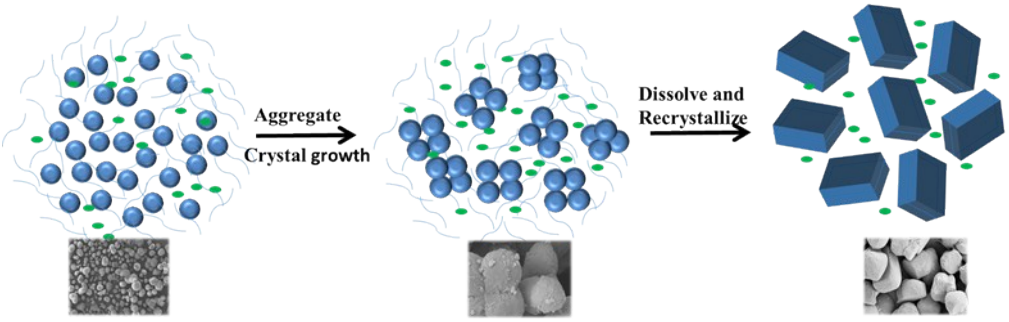


Fig. S1. Typical SEM images of mineralized samples in the absence of additives. a) mineralized samples were obtained at the mineralization time of 6 h; b) mineralized samples were obtained at the mineralization time of 12 h; c) mineralized samples were obtained at the mineralization time of 18 h.

Scheme S1 shows the formation of cube-like calcium carbonate crystals. The spherical particles attract each other by electrostatic interaction, and the four spherical

particles form a cube-like calcite by dissolution and recrystallization.



Scheme S1. Formation of cube-like calcium carbonate crystals in the absence of additives.

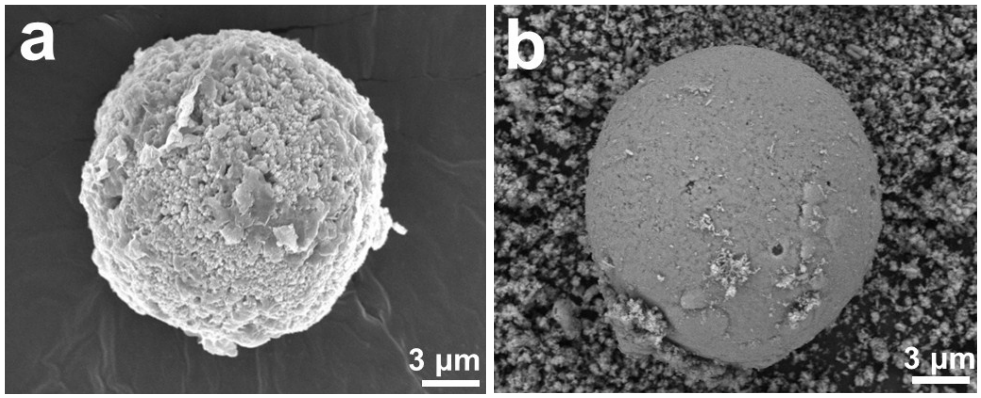


Fig. S2. Typical SEM images of mineralized samples in the absence of additives. a) rough spherical $CaCO_3$ obtained under mineralization time of 6 hours. b) smooth spherical CaCO3 obtained under mineralization time of 12 hours.

3. $CaCO_3$ Crystals Growing in the Presence of Magnesium ions

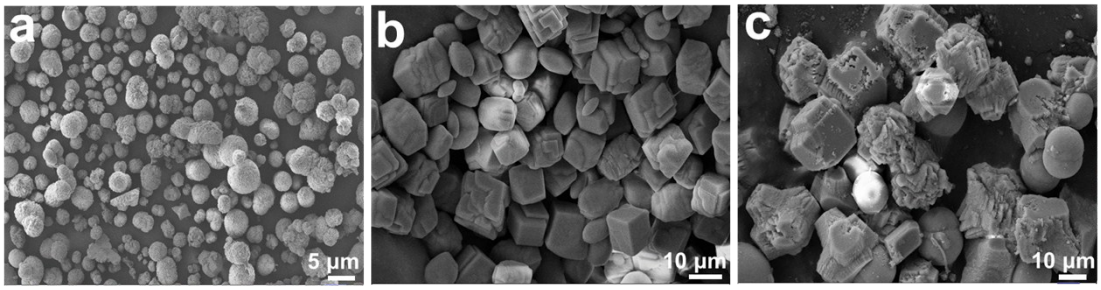


Fig. S3. Typical SEM images of mineralized samples in the presence of magnesium ions. a) mineralized samples were obtained at the mineralization time of 6 h; b) mineralized samples were obtained at the mineralization time of 12 h; c) mineralized samples were obtained at the mineralization time of 18h.

4. $CaCO_3$ Crystals Growing in the Presence of SF

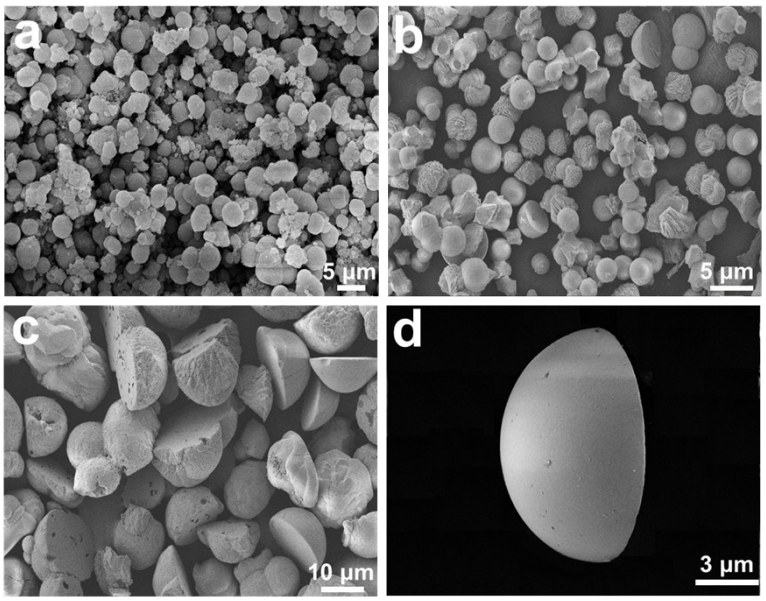


Fig. S4. Typical SEM images of mineralized samples in the presence of SF. a) mineralized samples were obtained at the mineralization time of 6 h; b) mineralized samples were obtained at the mineralization time of 12 h; c-d) mineralized samples were obtained at the mineralization time of 18h.

5. $CaCO_3$ Crystals Growing in the Presence of SF/Magnesium ions

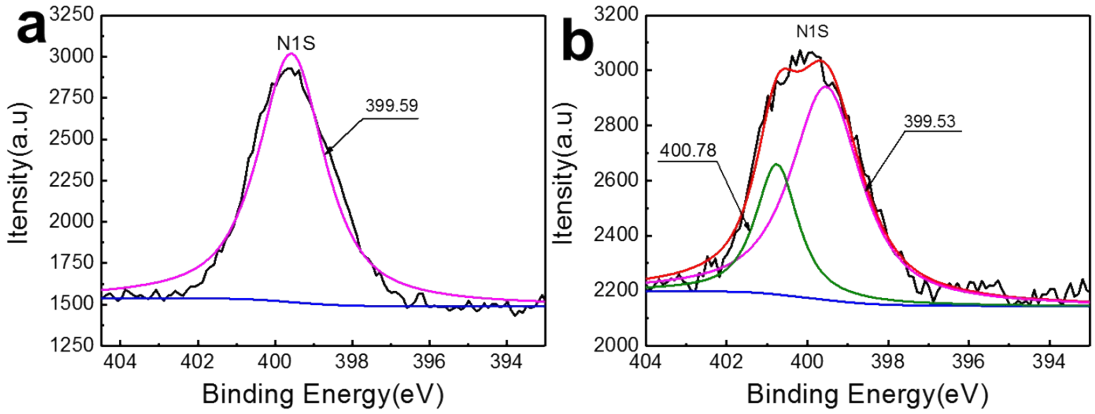


Fig. S5. (a) XPS spectrum of N1s in the absence of additives, (b) XPS spectrum of N1s in the presence of SF/Magnesium ions when concentration of magnesium ions is 2 g/L.

6. $CaCO_3$ Crystals Growing Using Chemical Methods

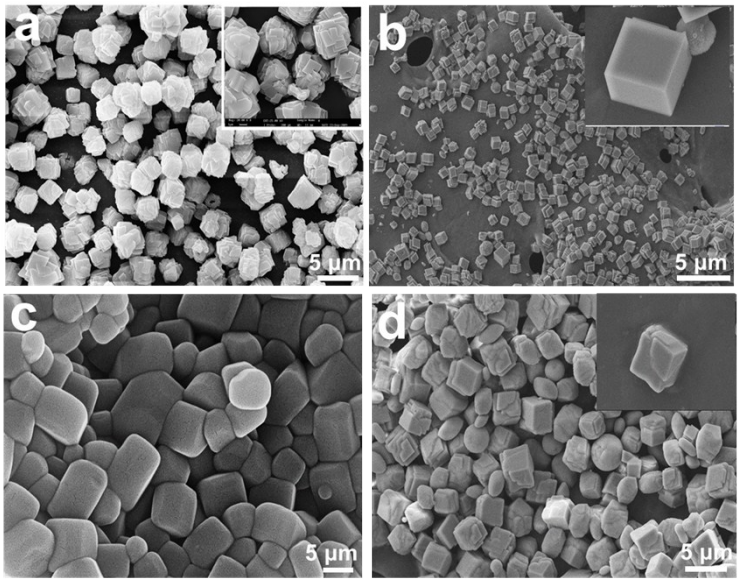


Fig. S6. Typical SEM images of mineralized samples were obtained by chemical method. a) mineralized samples were obtained in the pure water, b) mineralized samples were obtained in the presence of magnesium ions, c) mineralized samples were obtained in the presence of SF, d) mineralized samples were obtained in the presence of SF/Magnesium ions.

Fig.S7 shows the TGA data of calcium carbonate. The whole decomposition process was accompanied by three weight loss stages[1]. Weight lost slowly should be caused by the loss of crystal water under 335 °C. Obvious weight loss appeared in the curve during 335-650 °C, which should be caused by thermal decomposition of bacteria metabolites and SF in the mineralized samples. The mineralized samples in the presence of SF/Magnesium ions have a weight loss rate of 25.1%, the mineralized samples in the absence of additives have a weight loss rate of 23.4%. Obviously, the weight loss rate of the mineralized samples containing SF/Magnesium ions was 1.7% greater than that of the mineralized samples in the absence of additives, which should be attributed to the pyrolysis of SF in mineralized samples. The large thermal decomposition between 650-800 °C is due to the decomposition of calcium carbonate. From the analysis above it is safe to conclude that the SF is involved in the crystallization process of calcium carbonate.

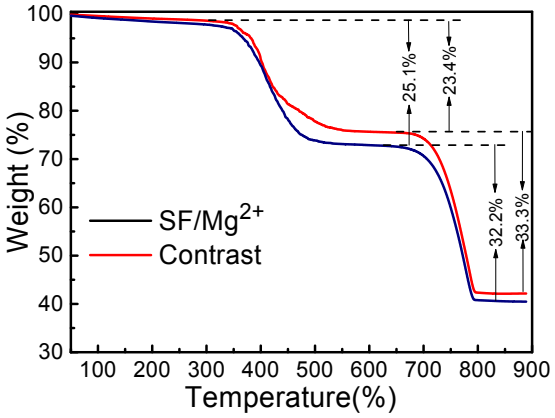


Fig. S7. TGA spectra of mineralized samples in the absence of additives and mineralized samples collected from SF and magnesium ions as a multi-additives microbial mineralization system.

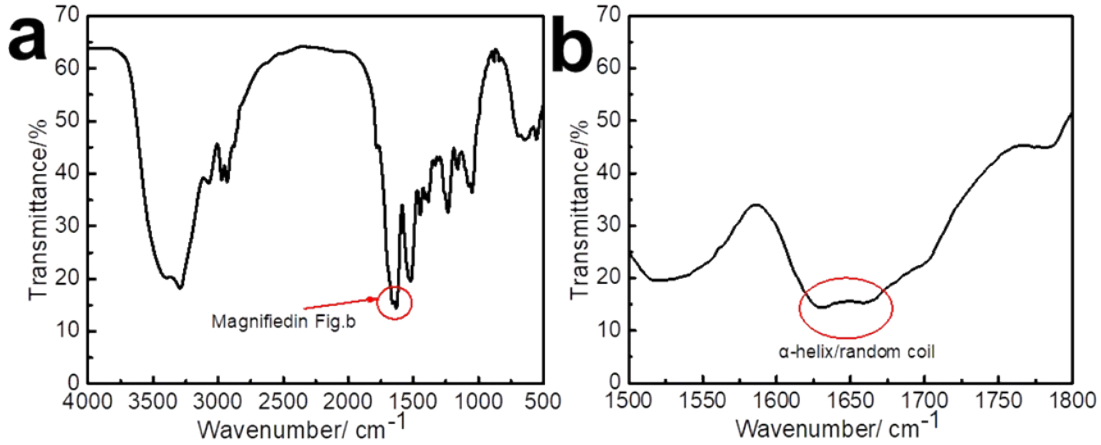


Fig. S8. FT-IR spectroscopy shows that SF exists as α-helix/random coil in aqueous solution.[2]

The colony of Bacillus pasteurii on LB agar medium is characterized by smooth colonies, homogeneous edges, clear, elevated, viscous (Fig. S9 (a)). Bacillus pasteurii is a spherical bacterium with a smooth surface and a size of 0.8-1.5 μm (Fig. S9 (b)). The morphology of Bacillus pasteurii is distinctly different from that of calcite rods.

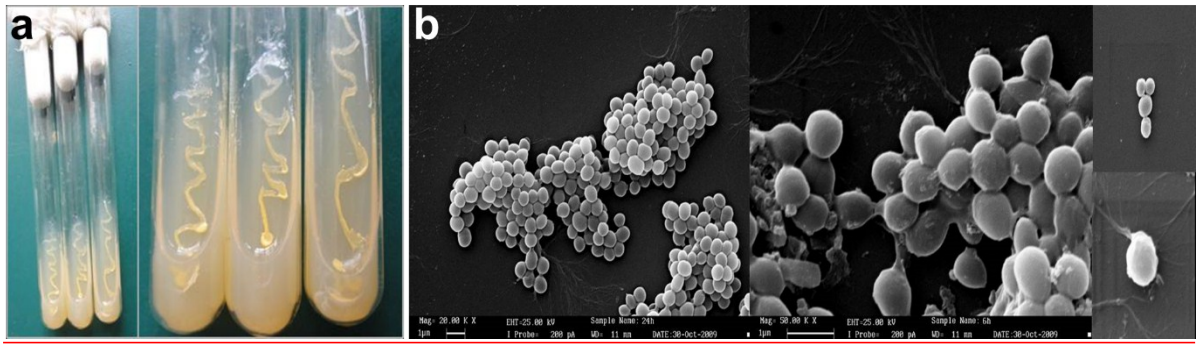


Fig. S9. (a) Bacillus pasteurii colony, (b)SEM of bacillus pasteurii mycelium.

In the process of microbial mineralization of calcium carbonate, microorganisms play two core roles: one is to provide urease for urea hydrolysis, and the other is to provide crystal nuclei for the formation of calcium carbonate crystals[3, 4]. It can be clearly seen from Fig.S10 that the calcium carbonate crystal surface has traces of bacteria. Therefore, we believe that bacteria exhibit synergistic effects with SF and magnesium cations.

$$NH_2\text{-}CO\text{-}NH_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (1)$$

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \quad (2)$$

$$Cl^- + HCO^{3-} + NH_3 \rightarrow NH_4Cl + CO_3^{2-} \quad (3)$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow \quad (4)$$

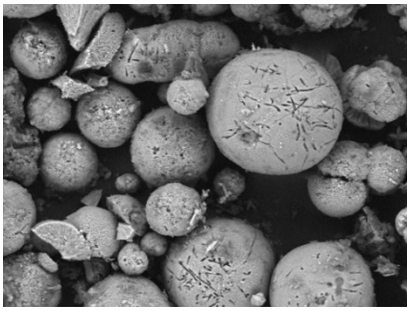


Fig. S10. Calcium carbonate crystals formed through microorganisms as nucleation sites.

Urease activity is found in a wide range of microorganisms and plants, and some produce large amounts of enzymes[5, 6]. Urease consists of three different subunits and has two nickel atoms at different active sites[7, 8]. Bacillus pasteurii has the ability to produce urease. Whether Bacillus pasteurii urease is an extracellular enzyme or an intracellular enzyme and whether it is an urea-inducing enzyme needs further

investigation.

As shown in Fig. 2,

(1) A is a blank LB medium, urea was added to A, and Ca^{2+} was added to A and reacted in a water bath at 37°C for 30 min. The results showed that no precipitation occurred.

(2) B was a sterile LB medium, and Bacillus pasteurii was inoculated in B. B was shake-cultured at 30°C and 150 r/min for 24 h, and the supernatant was taken by centrifuging the solution at 5000 r/min for 10 min. Urea and Ca^{2+} were added to the supernatant and reacted in a 37°C water bath for 30 min. The results showed that no precipitation occurred.

(3) C was sterilized LB medium, and Bacillus pasteurii was inoculated in C. C was shake-cultured at 30°C and 150 r/min for 24 h. The bacteria were taken by centrifuging the solution at 5000 r/min for 10 min. The bacteria were broken by sonication for 10 min. Urea and Ca^{2+} were added to the broken bacteria and reacted in a 37°C water bath for 30 min. The results showed that no precipitation occurred.

(4) D was sterilized LB medium, and Bacillus pasteurii was inoculated in D. D was shake-cultured at 30°C and 150 r/min for 24 h. Ca^{2+} were added to the supernatant and reacted in a 37°C water bath for 30 min. The results showed that no precipitation occurred.

(5) E was sterile LB medium, and urea was added to the LB medium through microfiltration membrane sterilization. Then, Bacillus pasteurii was introduced into the LB medium and shake cultured at 30° C. and 150 r/min for 24 h. The bacteria were taken by centrifuging the solution at 5000 r/min for 10 min. The bacteria were broken by sonication for 10 min. Urea and Ca^{2+} were added to the broken bacteria and reacted in a 37°C water bath for 30 min. The results showed that no precipitation occurred.

(6) F was sterile LB medium, and urea was added to the LB medium through microfiltration membrane sterilization. Then, Bacillus pasteurii was introduced into the LB medium and shake cultured at 30° C. and 150 r/min for 24 h. The supernatant was taken by centrifuging the solution at 5000 r/min for 10 min. Ca^{2+} were added to

the supernatant and reacted in a 37°C water bath for 30 min. The results showed that a white precipitate was produced.

From the above test results, Bacillus pasteurii can produce urease, which is produced under urea induction and is mainly an extracellular enzyme.

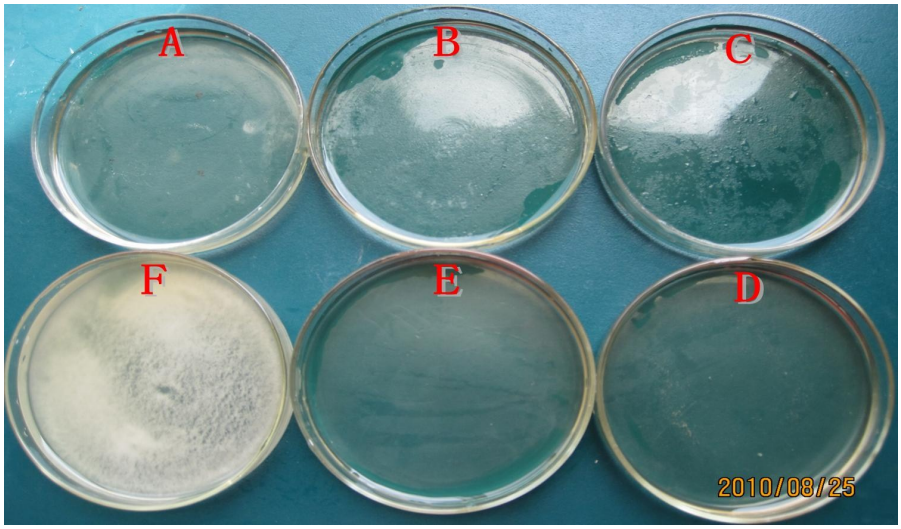


Fig. S11. Comparison for $CaCO_3$ precipitation induced by bacillus pasteurii.

Fig. S12, Fig. S13, and Fig. S14 are SEM images of $CaCO_3$ prepared at 25°C for cultures of bacteria, bacterial secretion, and bacterial body solution as a $CaCO_3$ deposition environment. Fig. S12 shows that spherical or spherical aggregates of various sizes and a certain proportion of regular oblique hexagonal $CaCO_3$ crystals were used to use the bacterial solution as the $CaCO_3$ crystallizing environment. Bacterial secretion act as crystal growth environments, and uniform, square-like $CaCO_3$ crystals are formed (Fig. S13). It shows that the culture medium and bacterial metabolites have a great influence on the crystal morphology. The bacterial body has almost no effect on the morphology of the crystals. The bacterial body has almost no influence on the crystal morphology. The morphology of the $CaCO_3$ crystal formed under the bacterial system is basically similar to the pure water system. The morphology is a block oblique hexahedral structure (Fig. S14). In addition, the pores on the surface of the crystal and the bacteria that remain on the crystal can be clearly seen from Fig. S14. The above results indicate that the main effect of the morphology of $CaCO_3$ crystals is bacterial secretion, while the bacteria themselves have little effect on the crystal morphology.

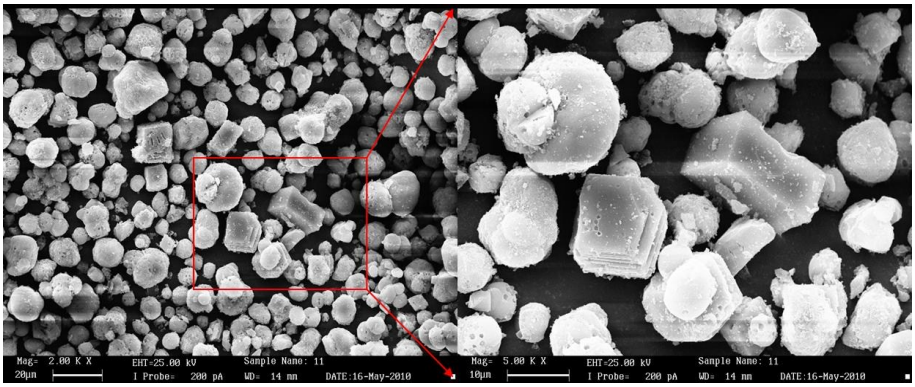


Fig. S12 SEM of CaCO3 crystals formed in bacteria solution.

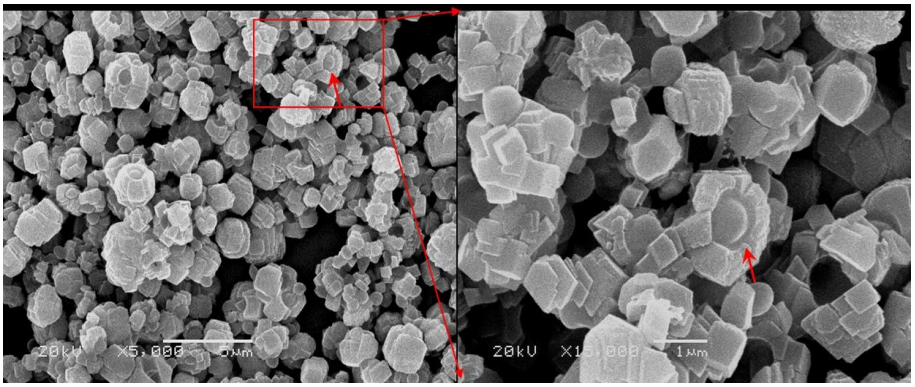


Fig. S13 SEM of CaCO3 crystals formed in bacterial secretion solution.

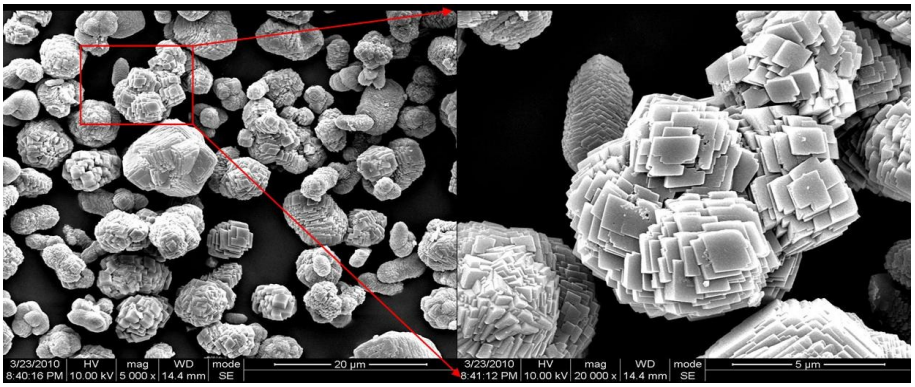


Fig. S14 SEM of CaCO3 crystals formed in bacterial body solution.

From the Fig. S15 we can clearly see that the specific surface area of the rough particles is slightly larger than the smooth particles.

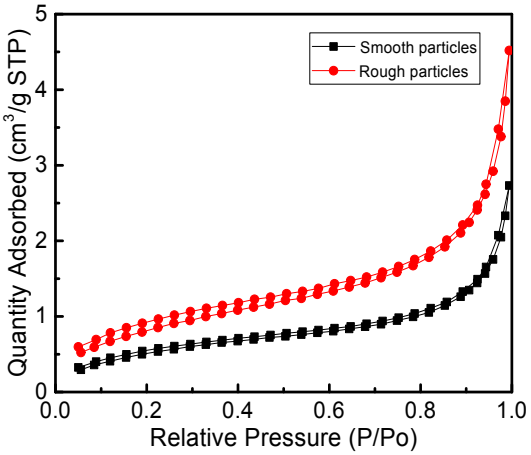


Fig. S15. N_2 adsorption/desorption isotherms curves

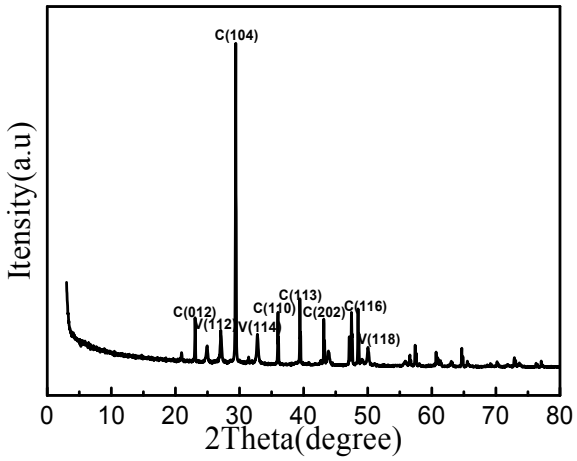


Fig. S16. (a) XRD patterns of mineralized samples were obtained in the presence of SF by chemical method.

References

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