Effect of Organic Bio-polymer on Bio-mineralization of $CaCO_3$

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Abstract

Organic matrix in the biogenic $CaCO_3$ has a significant influence on the $CaCO_3$ crystal growth, its polymorphs and morphology. In this research, effect of the cationic and anionic organic bio-polymers on the crystallization of $CaCO_3$ was investigated in microbial induced carbonate precipitation (MICP) process. In the current study, poly-L-lysine and poly-glutamate were used as cationic and anionic biopolymers, respectively. Urea hydrolysis by ureolytic bacteria *Pararhodobacter* sp. led to $CaCO_3$ formation in the presence of Ca^{2+} ions. The reaction was conducted with the addition of the polymers under different conditions. After oven-drying precipitation, the amount of precipitate was measured and morphology of the precipitate was analyzed by using scanning electron microscope. Bell-shaped curve was obtained in the relationship between the amount of the precipitate and the poly-L-lysine concentration. However, amount of precipitate remained approximately constant with the increase of the poly-glutamate concentration. In the presence of poly-L-lysine, morphology of the crystals changed from well-developed rhombohedral crystals to ellipsoidal shaped aggregates. But in poly-glutamate addition, polyhedral and spherical crystals are predominant.

Keywords: Biopolymer, Calcite, Microbial induced carbonate precipitation, Morphology

1. Introduction

Bio mineralization is the depositions of minerals in biological systems, mainly within tissues, bones, teeth and shells. It provides a protection, structural support and mechanical strength for the organism. Among bio minerals calcium is the most common and the main constituent of skeletal structures. Generally, formations of bio minerals are associated with an organic matrix. In most of the cases, organic matrix is a polymeric frame work which consists with macromolecules such as protein and polysaccharides and it provides the structural framework for the nucleation of the bio minerals [1].

In this research, effect of the cationic and anionic organic biopolymers on the crystallization of the $CaCO_3$ was investigated by using microbial induced carbonate precipitation method (MICP). In MICP process, $CaCO_3$ is precipitated by hydrolysis of the urea by using ureolytic bacteria and it includes biochemical reactions as given in the following equations (1)-(3) [2].

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} H_2NCOO^- + NH_4^+ \quad (1)$$

$$H_2NCOO^- + H_2 \rightarrow HCO_3^- + NH_3 \quad (2)$$

$$Ca^{2+} + HCO_3^- + NH_3 \rightarrow CaCO_3 + NH_4^+ \quad (3)$$

Poly-L-lysine and poly-glutamate were used as the cationic and anionic bio polymers, respectively. Chemical structures of poly-l-lysine and poly-glutamate are given in Figure 1.

Poly-L-lysine is basic polypeptide and has a property of cell adhesion and amino acid side chains are positively charged at neutral pH. Poly-glutamate is an acidic polypeptide and neutral pH its amino acid side chains are negatively charged [3] [4].

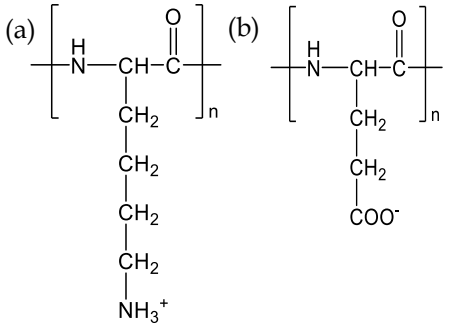

Figure 1 - Structure of (a) Poly-L-Lysine (b) Poly-glutamate

2. Methodology

2.1 Preparation of the microbial cell culture

Ureolytic bacteria *Pararhodobacter* sp. which was isolated from the beach sand in Sumuide, Nago, Okinawa, Japan [5] was cultivated using Zobell2216E medium (Polypeptone 5.0 g/L, Yeast extract 1.0 g/L and $FePO_4$ 0.1 g/L, prepared by using artificial sea water, pH 7.6-7.8). Bacteria cells were precultured using 5ml of Zobell2216E medium and kept in the shaking incubator at 30°C and speed of 160rpm for 24 hours. One ml of the preculture was inoculated into 100ml of fresh Zobell2216E medium and kept in the shaking incubator under the same condition as pre-culture for 48 hours. Finally cell pellets were obtained by centrifugation (10°C, 8000rpm, 5 min) of the bacterial culture. The cell pellets were dispersed again using distilled water to make bacterial suspension. By using UV–vis spectrophotometer, cell concentration of the bacteria suspension was measured and then OD_{600} value was adjusted to one.

2.2 Effect of the poly-L-lysine and the poly glutamate on the $CaCO_3$ crystallization

Same concentrations (0.3 mol/l) of $CaCl_2$ and urea solutions were used for the precipitation test. Two sets of samples were prepared without adding biopolymer and with biopolymer by varying the concentration of bacteria (OD_{600}) as shown in Table 1. Total volume of the samples were adjusted to 10ml by using distilled water and after preparation, samples were kept in the shaking incubator under 30°C and 160 rpm speed for 24 hours. Then the reaction mixture was centrifuged to collect the precipitate, and supernatant of tubes were removed separately by using filter paper (11µm).

Both of the filter papers and the tubes with the precipitate were oven dried at 100°C for 24hours and dry weights were measured. Finally samples were analyzed by using Scanning Electron Microscope (SEM) to identify the morphology of the precipitated calcium carbonate crystals.

Table 1 - Experimental conditions for system with and without bio-polymer

$CaCl_2$ (M)	Urea (M)	Bacteria OD_{600}	Bio-polymer (mg/L)
0.3	0.3	0.01	0
		0.1	
		0.2	
		0.3	
		0.01	10
		0.1	
		0.2	
		0.3	

Another set of Samples were prepared by varying the concentration of bio-polymer as shown in the Table 2. Sample preparation is same as earlier and samples were analyzed using Scanning Electron Microscope (SEM).

Table 2 - Experimental conditions to evaluate the effect of bio-polymer concentration

$CaCl_2$ (M)	Urea (M)	Bacteria OD_{600}	Bio-Polymer (mg/L)
0.3	0.3	0.1	0
			1
			10
			30
			50

3. Results

3.1 Effect of the biopolymer on the $CaCO_3$ crystallization

Figure 2 shows the variation of the amount of $CaCO_3$ precipitation with poly-L-lysine and poly-glutamate and without biopolymer under different bacteria concentrations separately. Figure 3 shows the Scanning Electron Microscopy (SEM) images of the precipitate with different bacteria concentrations without bio-polymer. SEM images of the precipitate with different bacteria concentration in the presence of poly-L-lysine and poly-glutamate are given in Figure 4 and Figure 5, respectively.

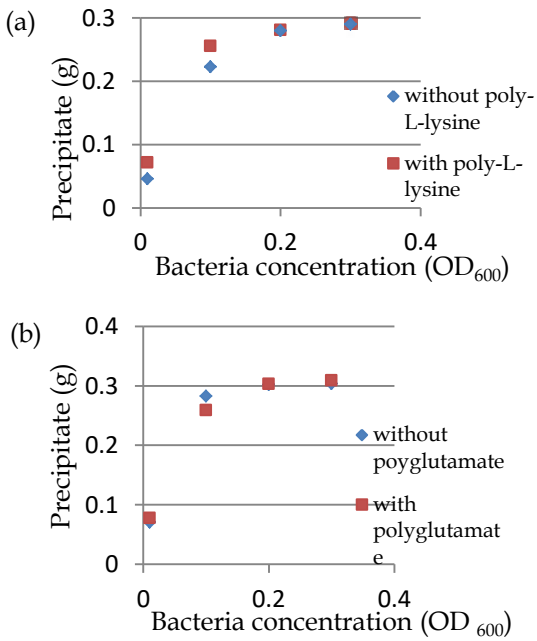

Figure 2 - Variation of the $CaCO_3$ precipitate with the bacteria concentartion in the presence of (a) Poly-L-Lysine and (b) Poly-glutamate

3.2 Effect of the biopolymer concentration

Figure 6 shows the variation of the amount of precipitate with the poly-L-lysine and poly-glutamate concentration. SEM images are given in Figure 7 and Figure 8.

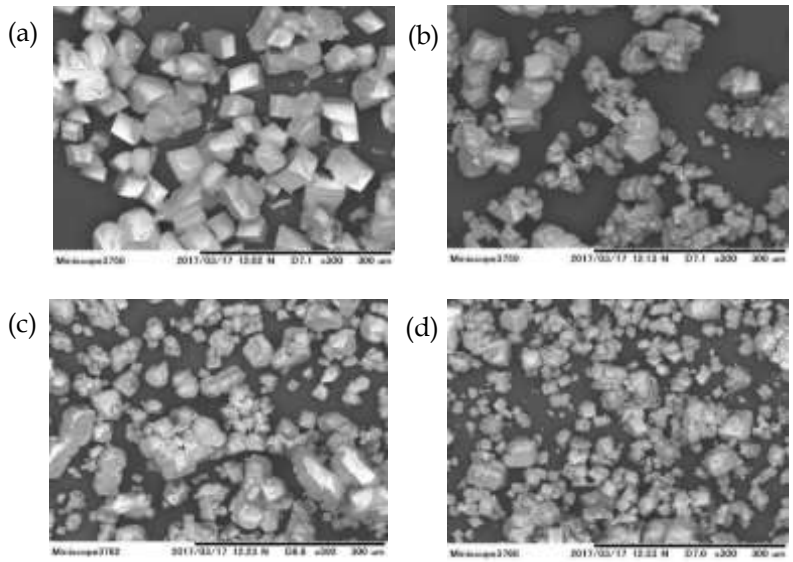

Figure 3 - SEM images of the $CaCO_3$ precipitate at different bacteria concentration without bio-polymer (a) OD_{600} = 0.01, (b) OD_{600} = 0.1,(c) OD_{600} = 0.2, (d) OD_{600} = 0.3

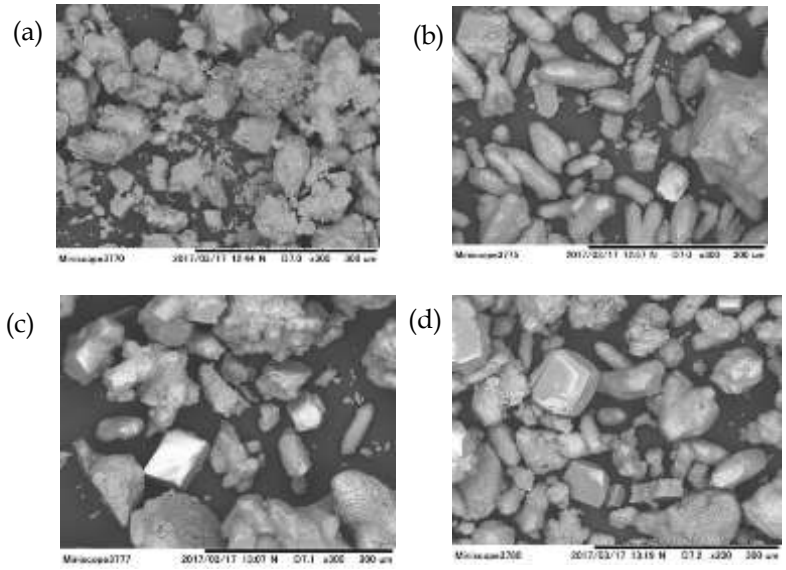

Figure 4 - SEM images of the $CaCO_3$ precipitate at different bacteria concentration with poly-l-lysine (a) OD_{600} = 0.01, (b) OD_{600} = 0.1, (c) OD_{600} = 0.2, (d) OD_{600} = 0.3

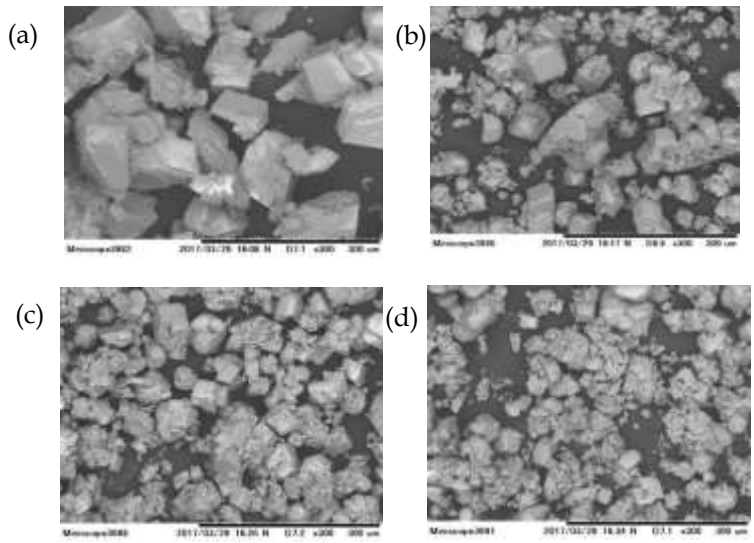

Figure 5 - SEM images of the $CaCO_3$ precipitate at different bacteria concentration with poly-glutamate (a) OD_{600} = 0.01, (b) OD_{600} = 0.1, (c) OD_{600} = 0.2, (d) OD_{600} = 0.3

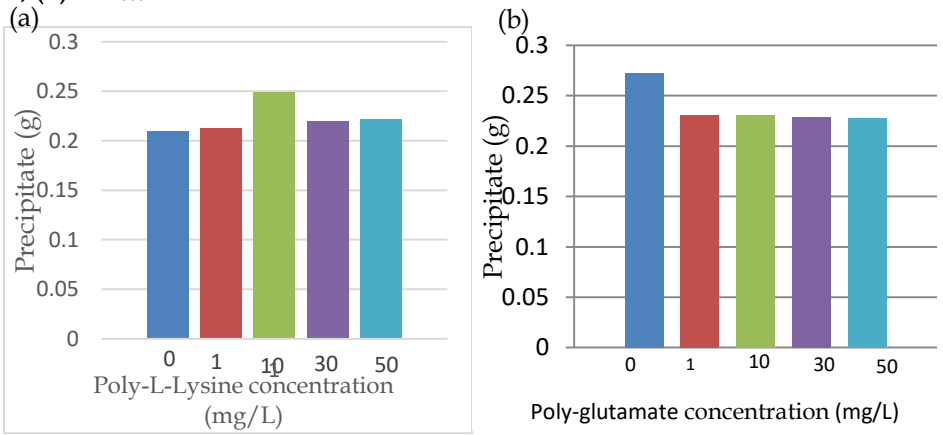

Figure 6 - Variation of the amount of carbonate precipitate with the bio-polymer concentration (a) poly-l-lysine (b) poly-glutamate

4. Discussion

4.1 Effect of the bio-polymer on the CaCO3 crystallization

According to the Figure 2 and Figure 3, it can be seen that amount of precipitate increase with bacteria concentration with and without bio-polymer. However, in both cases rate of precipitate formation seems to reach plateau around OD_{600} = 0.2 because the theoretical maximum amount of $CaCO_3$ formation is 0.3g. System with the poly-L-lysine gave higher amount of precipitate compared with that without poly-L-lysine. But for the poly-glutamate the amount of precipitate was similar or lower than the obtained without poly-glutamate.

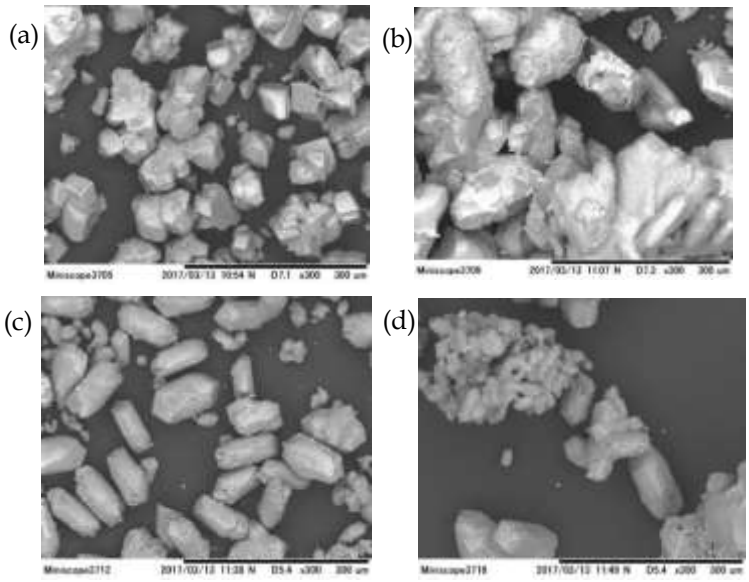

Figure 7 - SEM images of the $CaCO_3$ precipitate for different poly-L-lysine concentration (a) 1 mg/L, (b) 10 mg/L, (c) 30 mg/L, (d) 50 mg/L

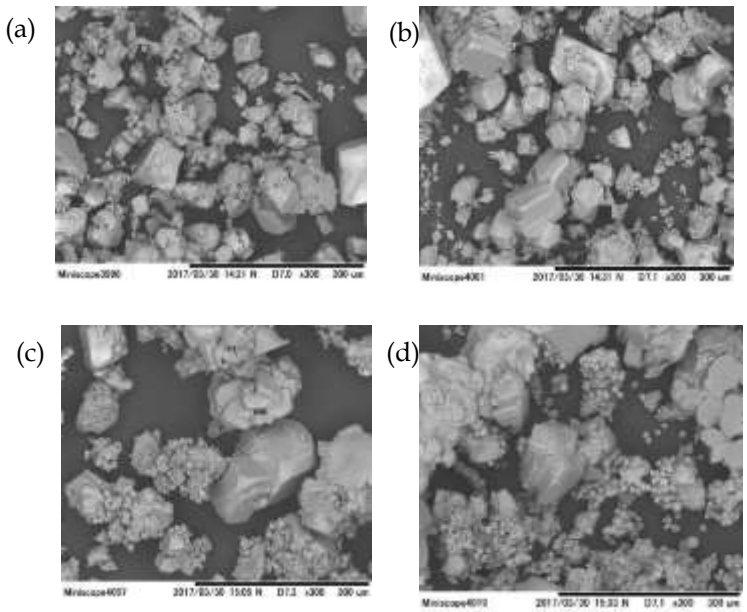

Figure 8 - SEM images of the $CaCO_3$ precipitate for different poly-glutamate concentration (a) 1 mg/L, (b) 10 mg/L, (c) 30 mg/L, (d) 50 mg/L

Amount of precipitate was approximately similar in the system with and without polymers at higher cell concentrations. Furthermore, poly-L-lysine has a positive effect on the $CaCO_3$ crystallization but poly-glutamate has a negligible effect on the $CaCO_3$ crystallization. According to the SEM images in Figure 3, low bacteria concentration gave well developed rhombohedral crystals while higher bacteria concentration resulted in agglomeration of small rhombohedral crystals. So, higher bacteria concentration gives higher precipitate but it also inhibits the growth of the carbonate crystals. In the presence of the poly-L-lysine as shown in the Figure 4, morphology of the crystals changed to the ellipsoidally shaped crystals and both of the ellipsoidal and rhombohedral crystals can be seen at higher bacteria concentrations. In the presence of the poly-glutamate (Figure 5) polyhedron type crystals are predominant and with the increase of the bacteria concentration agglomeration of polyhedron crystals can be seen. This morphology change may be due to the adsorption of the bio-polymer on the crystal surface and the formation of new crystal faces [3] [4].

4.2 Effect of the Bio polymer Concentration

According to the results given in the Figure 6, amount of the precipitate increase with the poly-l-lysine concentration but further increase of the polymer concentration leads to reduce the amount of precipitate. So higher precipitate was obtained at the intermediate concentration. Higher concentration of poly-L-lysine would reduce the bacterial activity and hence reduce the amount of precipitate. On the other hand, the addition of poly- glutamate resulted in the decline in precipitation. In further addition of polymer approximately similar amount of precipitate was obtained at any concentration. So, effect of poly-glutamate concentration on the $CaCO_3$ precipitation can be neglected.

According to Figure 7, combination of the polyhedron crystals are predominant at lower concentration of poly-L-lysine and with increase of the concentration morphology of the crystals changed to the ellipsoidal shape crystals for higher concentration agglomeration of ellipsoidal crystals can be seen. As seen in the Figure 8 with the increase of the poly-glutamate concentration, morphology of the crystals changed drastically from agglomerations of polyhedron crystals to spherical crystals.

Dzakula et al (2009) had conducted similar research in precipitation of calcium carbonate using chemical reaction of $CaCl_2$ and Na_2CO_3 in the presence of calcite seed, poly-L-lysine and poly-glutamate. They concluded that the interaction between poly-L-lysine and calcite is pure electrostatic interaction between the positively charged polymer and negatively charged calcite surface. However interaction between the poly-glutamate and the calcite is a coordinative interaction between side chain of carboxylic group and the Ca^{2+} ions [3].

5. Conclusion

According to the results obtained, poly-L-lysine has positive effect on the $CaCO_3$ crystallization but the effect from poly-glutamate can be negligible. Amount of the $CaCO_3$ precipitate is directly proportional to the bacteria

concentration. However, rate of precipitation decreases with the bacteria concentration. Relationship between the poly-L-lysine concentration and the amount of precipitate is bell shaped but concentration of the poly-glutamate has a negligible effect on the $CaCO_3$ precipitation.

Morphology of the crystals changed from the well-developed rhombohedral crystals to agglomeration of the rhombohedral crystals with the increase of the bacteria concentration. But in the presence of the poly-L-lysine morphology changed to ellipsoidal shape crystals and with the presence of poly glutamate morphology changed drastically with the increase of the concentration from polyhedron crystals to spherical crystals.

Acknowledgement

The work was partly supported by a JSPS KAKENHI Grant Number JP16H04404; this support is gratefully acknowledged.

References

[1] Mann, S., (2001). Biomineralization, Oxford university press, Oxford, P.89-122.

[2] Fujita, M., Nakashima, K., Achal, V., & Kawasaki, S., (2017). Whole-cell evaluation of urease activity of *Pararhodobacter* sp. isolated from peripheral beachrock, *Biochemical engineering journal*, 124, pp. 1-5.

[3] Dzakula, B. N., Brecevic, L., Falini, G., & Kralj, D.,(2009). Calcite crystal growth kinetics in the presence of charged synthetic polypeptide, *Journal of crystal growth and design,* 9, pp.2425-2434.

[4] Dzakula, B. N., Falini, G., Brecevic, L., Skoko, Z., & Kralj, D., (2009). Effects of initial supersaturation on spontaneous precipitation of calcium carbonate in the presence of charged poly-$_L$-amino acids, *Journal of colloid and interface science*, 343, pp.553-563.

[5] Mitsuyama, S., Nakashima, K., Kawasaki, S., (2017). Evaluation of porosity in biogrouted sand using microfocus X-ray CT", *International journal of GEOMATE*, 12(31), pp. 71-76.