

ENHANCEMENT OF MICROBIALLY INDUCED CARBONATE PRECIPITATION USING ORGANIC BIOPOLYMER

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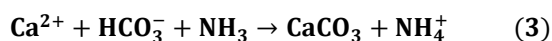
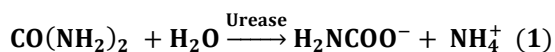
ABSTRACT: Microbially induced carbonate precipitation (MICP) by using ureolytic bacteria is a novel and environmentally friendly way to treat the un-cemented sand. It was implemented successfully in number of geotechnical applications. Use of organic additives to improve the MICP process is a novel and interesting approach. In this research, effect of the cationic biopolymer poly-L-lysine on the MICP process was investigated by using ureolytic bacteria *Pararhodobacter* sp. Urea hydrolysis by the bacteria in the presence of CaCl₂ was conducted with the addition of the polymer under different conditions. Morphology of the precipitate of CaCO₃ after oven dried was analyzed by using the scanning electron microscope. Bell-shaped curve was obtained for the variation between the amount of the precipitate with the increase of the amount of the poly-L-lysine. Poly-L-lysine gives higher amount of precipitate than conventional MICP process and morphology of the crystals changed drastically from well-developed rhombohedral crystals to ellipsoidal shaped aggregates.

Keywords: Bio-polymer, Crystals, Morphology, Ureolytic bacteria

1. INTRODUCTION

Today in the world, due to urbanization and rapid growth of population, lands available for the infrastructures and other developments have been decreasing. So people are moving to use loose problematic soils to construct earth structures by using proper ground improvement techniques. Chemical grouting is a most common and effective way to treat weak soils. Cement is the main material use for the chemical grouting, but cement production is more energy consuming and environmental unfriendly method, CO₂ emission from the cement production is 7% from the global CO₂ emissions [1]. So, today world is seeking for an environmentally friendly biological approach to treat the loose soils.

Microbial Induced Carbonate Precipitation (MICP) is a one of such kind of novel and environmental friendly ground improvement method. In MICP process, CaCO₃ is precipitated by hydrolysis of the Urea by using ureolytic bacteria and it includes biochemical reactions governed by urease enzyme produced by these ureolytic bacteria as given in the Eqs.(1-3) [2]-[7].



Use of organic additives such as biopolymers and protein to improve the weak soil is an alternative biological approach to the MICP process. Biopolymers are organic polymers synthesized by plant or microorganism and it is a natural, non-toxic and non- petroleum based product. They consist of monomeric units bonded covalently to form large molecules. Number of researches had been done previously to investigate the applicability of biopolymers to improve the weak soil and they concluded that bio-polymer have a better ability to improve the weak soils [8]-[12].

Combination of the above two biological processes such as use of biopolymer to the MICP process seems to be novel and innovative idea to improve the efficiency of the MICP process. In this research, effect of the cationic biopolymer on the MICP process was investigated by using ureolytic bacteria *Pararhodobacter* sp.

Poly-L-lysine was used as the cationic biopolymer and it is a kind of basic polypeptide. In neutral pH, its amino group side chains are positively charged [13]. MICP method is used as an environmentally friendly ground improvement method, mainly for solidify the sandy soils. Use of poly-L-lysine might be helpful to improve the efficiency of the sand solidification. Because, silica (SiO₂) is the main constituents of the sand and the silica particles have negative charge due to dissociated silanol group (-SiO⁻). Bacterial cells generally show negative charge. In sand solidification, bacterial cells should be bound to sand particles for efficient solidification. To increase

the adhesion of cells to sand particles, positively-charged poly-L-lysine can be used as a binder. So poly-L-lysine would help to improve the efficiency of the sand solidification by using MICP method [14]. Chemical structure of the poly-L-lysine is shown in Fig.1. In this research work, we examined the effect of poly-L-lysine on the MICP process.

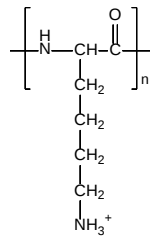


Fig. 1 Structure of the poly-L-lysine.

2. METHOD

2.1 Preparation of Microbial Cell Culture

Ureolytic bacteria *Pararhodobacter* sp. was isolated from the beach sand in Sumuide, Nago, Okinawa, Japan [15] and cultivated using Zobell2216E medium (Polypeptone 5.0 g/L, Yeast extract 1.0 g/L and FePO₄ 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 pre-culture 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, bacterial culture was centrifuged to collect the cell pellets, which were dispersed again using distilled water to make bacterial suspension. Cell concentration of the bacterial suspension was measured by UV-vis spectrophotometer and then OD₆₀₀ value was adjusted to one.

2.2 Precipitation Test

2.2.1 Effect of the poly-l-lysine on the MICP process

Same concentration (0.3 mol/l) of CaCl₂ and urea solutions and the stock solution of the poly -L-lysine were used for the precipitation test. Two sets of samples were prepared without adding biopolymer and with biopolymer by varying the concentration of bacteria (OD₆₀₀) 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 24hours and dry weights were measured. Weight of the precipitate was calculated by subtracting the empty weight of the tube and the empty weight of the filter paper from the dry weight of the tube and the dry weight of the filter paper. FTIR analysis was conducted for the sample with and without poly-l-lysine and finally, samples were analyzed by using Scanning Electron Microscope (SEM) to identify the morphology of the precipitated calcium carbonate crystals.

2.2.2 Effect of the poly-L-lysine concentration

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

Table 1 Experimental condition for system with and without poly-L-lysine

Sample No	CaCl ₂ (M)	Urea (M)	Bacteria OD ₆₀₀	Poly-L-Lysine (mg/L)
A	0.3	0.3	0.01	0
B			0.1	
C			0.2	
D			0.3	
E			0.01	10
F			0.1	
G			0.2	
H			0.3	

Table 2 Experimental conditions to evaluate the effect of poly-L-lysine concentration

Sample No	CaCl ₂ (M)	Urea (M)	Bacteria OD ₆₀₀	Poly-L-Lysine (mg/L)
1	0.3	0.3	0.1	0
2				1
3				10
4				30
5				50

3. RESULTS AND DISCUSSION

3.1 Effect of the Poly-L-Lysine on the MICP Process

Variation of the amount of precipitated CaCO_3 with and without adding poly-L-lysine under different bacteria concentrations is given in the Fig.2.

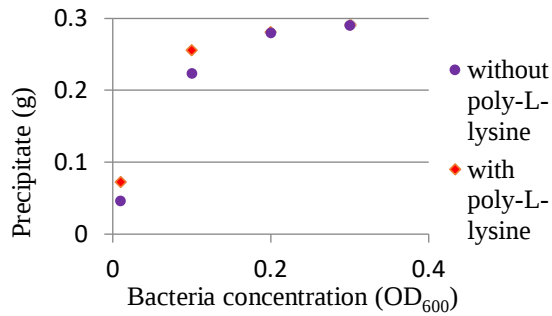


Fig. 2 Variation of the amount of carbonate precipitates with the bacteria concentration (OD₆₀₀)

According to the Fig. 2, it can be seen that in both of the cases, amount of the precipitated CaCO_3 increase with the bacteria concentration. However, considerable amount of increment can be seen only up to the OD₆₀₀=0.2 after that, rate of the precipitation of CaCO_3 was getting decrease and it is

almost become constant for further increase of the concentration of bacteria. So, higher concentration of bacteria inhibits the formation of calcium carbonate. It may be due to the lack of bacteria growth due to higher concentration of the bacteria.

System with the poly-L-lysine gave higher amount of precipitate compared with that without biopolymer. However, for higher bacteria concentration, amount of precipitated CaCO_3 with and without biopolymer were approximately similar. At higher bacteria concentration, significant influence from the biopolymer couldn't be identified. However, it can be seen that poly-L-lysine has positive effect on the MICP process.

Figure 3 shows the Scanning Electron Microscopy (SEM) images for the various bacteria concentrations.

According to the SEM images, lower concentration of bacteria gave well-developed rhombohedral calcite crystals. At higher bacteria concentration, agglomeration of rhombohedral crystals can be seen and size of the crystals reduced with the increase of the bacteria concentration. Higher concentration of bacteria inhibits the growth of CaCO_3 crystals. In the presence of the poly-L-lysine, morphology of the crystals change to ellipsoidal shape as shown in Fig. 4. At higher bacteria concentration, both of the rhombohedral and ellipsoidal crystals can be seen. This morphology changes may be due to the adsorption of the poly-L-

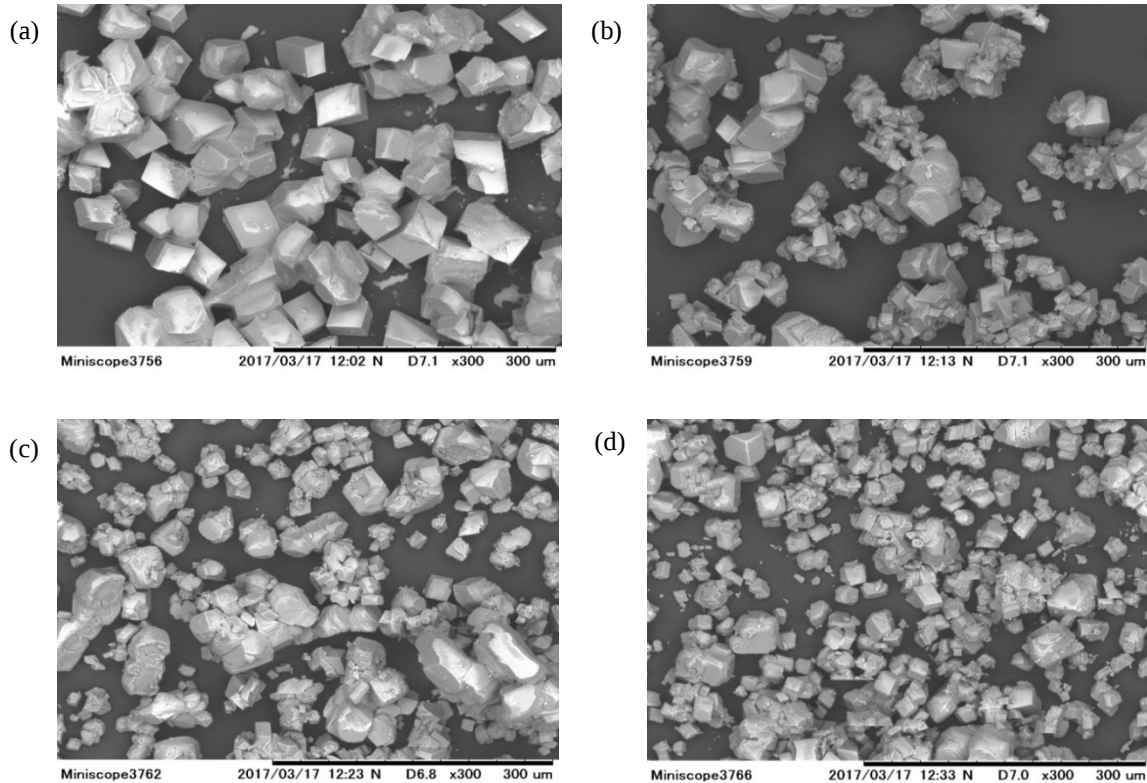


Fig. 3 SEM images of the CaCO_3 precipitate for different bacteria concentration without poly-L-lysine (a) OD₆₀₀ = 0.01 (b) OD₆₀₀ = 0.1 (c) OD₆₀₀ = 0.2 (d) OD₆₀₀ = 0.3

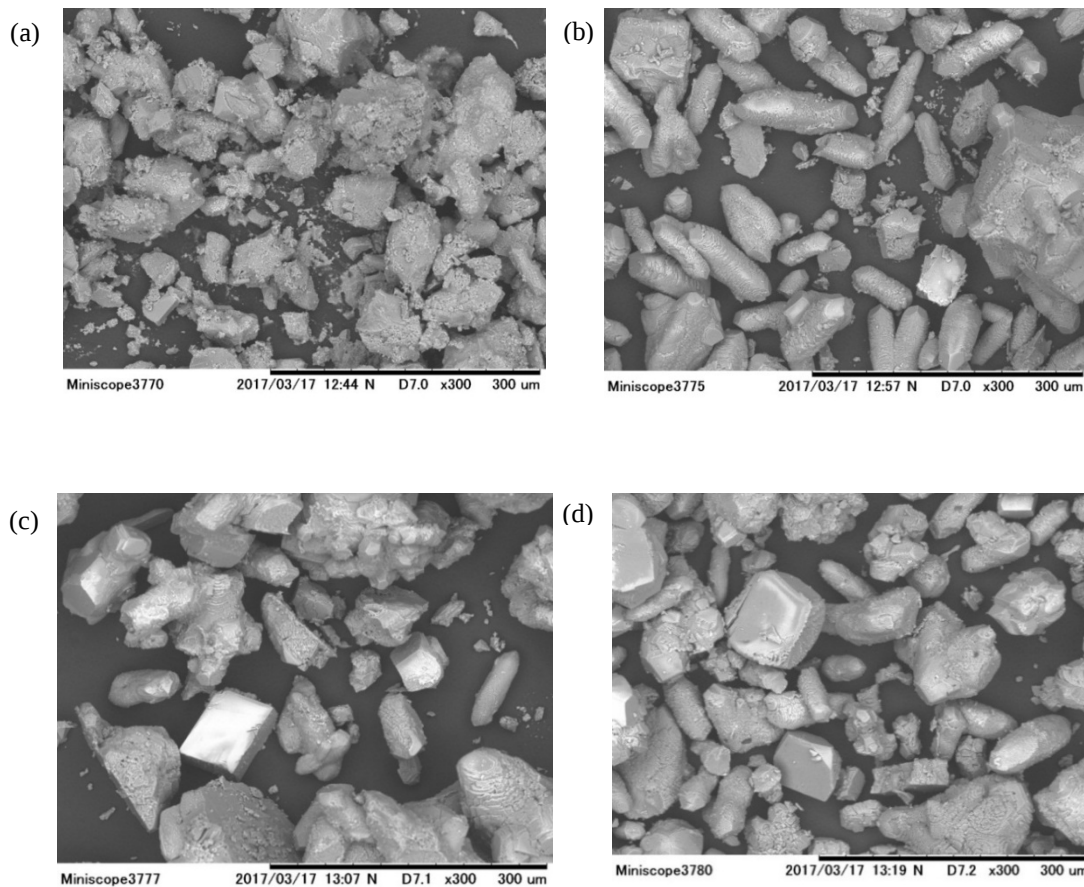


Fig. 4 SEM images of the CaCO_3 precipitate for different bacteria concentration in the presence of poly-L-lysine (a) $\text{OD}_{600} = 0.01$ (b) $\text{OD}_{600} = 0.1$ (c) $\text{OD}_{600} = 0.2$ (d) $\text{OD}_{600} = 0.3$

lysine on the crystal surface and formation of new crystal faces.

3.2 Effect of the Amount of Poly-L-Lysine

Variation of the amount of the CaCO_3 precipitate with the increase of the amount of the poly-L-lysine is given in the Fig. 5.

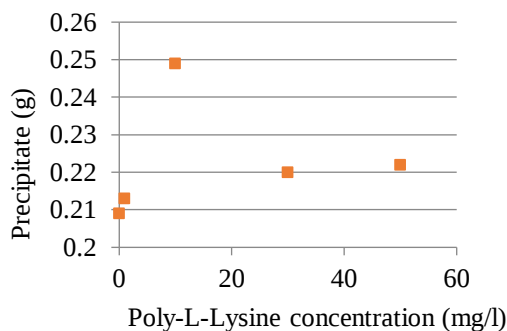


Fig. 5 Variation of the amount of carbonate precipitate with the poly-L-lysine concentration

The amount of precipitate increase with the poly-L-lysine concentration and further increase of the poly-L-lysine concentration leads to reduce the amount of precipitate. This may be due to the excessive amount of poly-L-lysine would reduce the activity of the bacteria. Highest amount of precipitate was obtained at the poly-L-lysine concentration of 10 mg/L. According to the SEM images given in Fig. 6, crystal size was larger compared with the other concentrations when the concentration of the poly-L-lysine was 10mg/L. At lower concentration of poly-L-lysine, combinations of polyhedral crystals were predominant. With the increase of the concentration, morphology of the crystals changed to ellipsoidal shape aggregates. At higher concentration of poly-L-lysine, agglomeration of ellipsoidal shaped crystals were obtained.

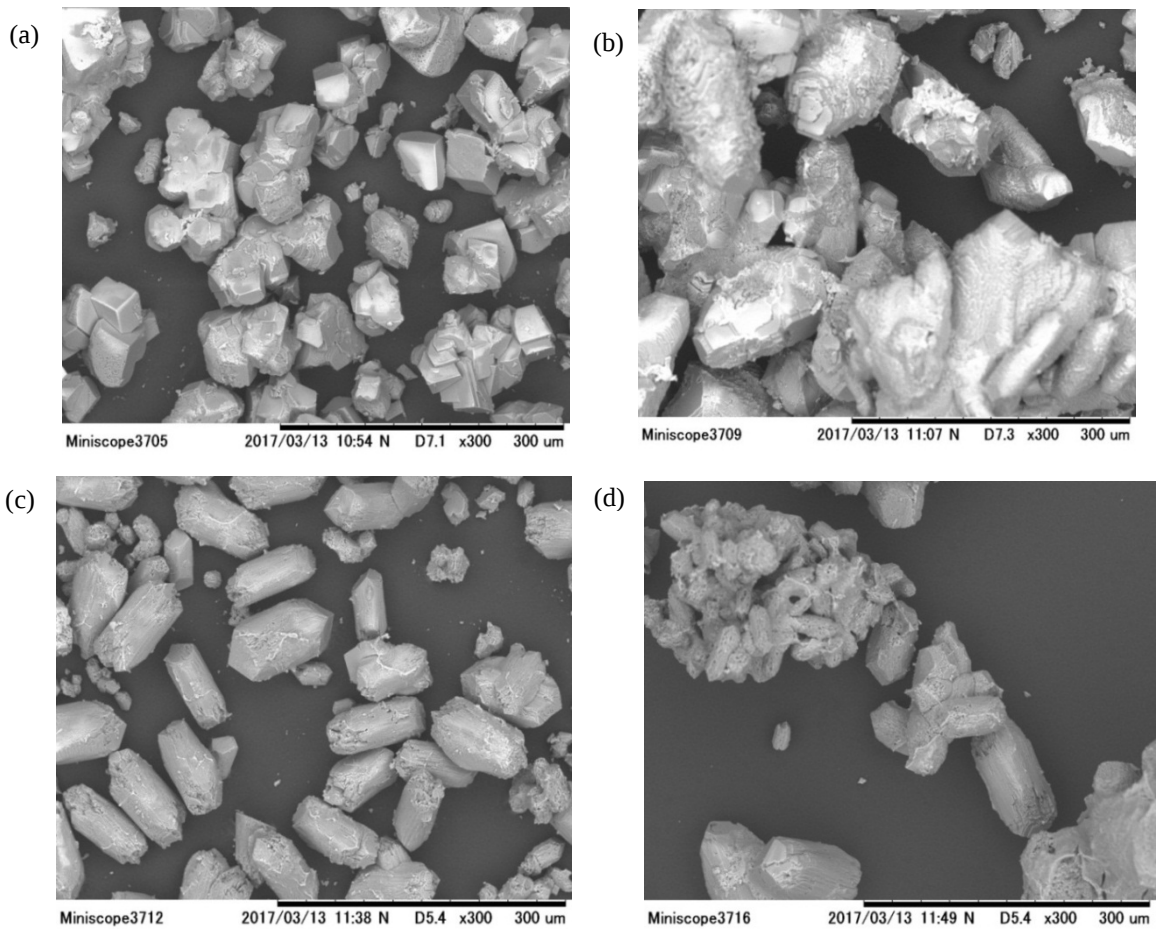


Fig. 6 SEM images of the CaCO_3 precipitate for different poly-L-lysine concentration (a) 1 mg/L (b) 10 mg/L (c) 30 mg/L (d) 50 mg/L

Similar relationship between the amount of precipitate and the poly-L-lysine concentration was found from the previous researches, which reported the precipitation of calcium carbonate using chemical reaction of CaCl_2 and Na_2CO_3 solutions in the presence of calcite seed and poly-L-lysine. They have explained that the increase of the calcite growth at low concentration of poly-L-lysine and growth reduction at higher concentration of poly-L-lysine is a typical behavior of the additives that were weakly, non-selectively, bonded to the surface. This explanation is identical to our research work. Furthermore, they have concluded that the interaction between the poly-L-lysine and the calcite is pure electrostatic and it is between the positively charged polypeptide and the negatively charged calcite surface [13], [16].

3.3 FTIR Analysis

Results of the FTIR analysis with and without poly-L-lysine are shown in Fig 7. According to the FTIR results, we could find an additional peak at 1200 cm^{-1} in the poly-L-lysine results. The peak is

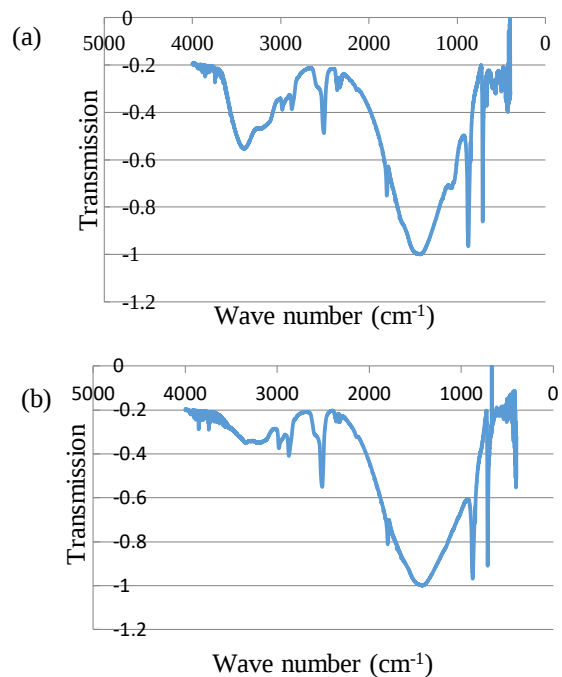


Fig. 7 FTIR spectra for samples (a) with poly-L-lysine (b) without poly-L-lysine

attributed to C-N bond. So we can conclude that some amount of poly-L-lysine is incorporated in CaCO_3 crystal or sometime poly-L-lysine act as a template of nucleation. Peaks at 713 cm^{-1} and 1420 cm^{-1} were attributed to calcite crystal.

4. CONCLUSION

According to the results obtained, poly-L-lysine has a positive effect on the MICP process. However with the presence of higher concentration of bacteria significant influence from the poly-L-lysine on the MICP process couldn't be seen. With the increase of the bacteria concentration, rate of the formation of calcium carbonate decrease. So, higher bacteria concentration will reduce the crystal growth.

Relationship between the amount of precipitate and the poly-L-lysine concentration is bell shaped and higher and lower concentrations of the poly-L-lysine inhibit the calcium carbonate precipitation but intermediate concentration give higher amount of precipitate.

Morphology of the crystals change from well-developed rhombohedral crystals to agglomeration of the rhombohedral crystals with the increase of the bacteria concentration and the size of the crystals reduces with the increase of the bacteria concentration. Presence of the poly-L-lysine produces ellipsoidal shape crystals. Morphology of the crystals change from the polyhedral crystals to agglomeration of ellipsoidal crystals with the increase of the poly-L-lysine concentration. Relationship between the crystal morphology and ground improvement is still not clear. However, this is quite important point in MICP. It thus should be revealed in the future work by comparing the crystal polymorph and the strength of cured soil.

5. ACKNOWLEDGEMENTS

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