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EFFECT OF ORGANIC BIOPOLYMER ON THE MICROBIALLY INDUCED CARBONATE PRECIPITATION AND ITS MORPHOLOGY

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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 $CaCl_2$ was conducted with the addition of the polymer under different conditions. Morphology of the precipitate of $CaCO_3$ 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, Calcite, Morphology, Ureolytic bacteria

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. CO_2 emission from the cement production is 7% from the global CO_2 emissions [1]. So today world is seeking for an environmentally friendly biological approach to treat the loose soils.

Microbially Induced Carbonate Precipitation (MICP) is a one of such kind of novel and environmental friendly ground improvement method. In MICP process, $CaCO_3$ 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][3][4][5].

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

$$H_2NCOO^- + H_2O \rightarrow HCO_3^- + NH_3 \quad (2)$$

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

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.

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 [6]. Generally, silica particles have negative charge due to the presence of the silanol group. If we use poly-L-lysine for the MICP process, negatively charged bacteria cell and silica particles could be bound with each other due to positively charged property of the poly-L-lysine. So it can be help to increase the efficiency of the MICP process [7]. 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.

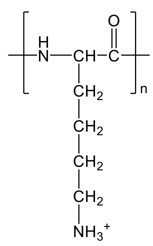


Fig. 1 Structure of the poly-L-lysine.

METHOD

Preparation of Microbial Cell Culture

Ureolytic bacteria *Pararhodobacter sp.* was isolated from the beach sand in Sumuide, Nago, Okinawa, Japan [8] and 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 30ºC and speed of 160 rpm 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 OD_{600} value was adjusted to one.

Precipitation Test

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

Same concentration (3 mol/l) of $CaCl_2$ and urea solutions and the stock solution of the poly -L-lysine (0.1g/L) 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 30ºC 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 100ºC 24 hours 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.

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

RESULTS AND DISCUSSION

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.

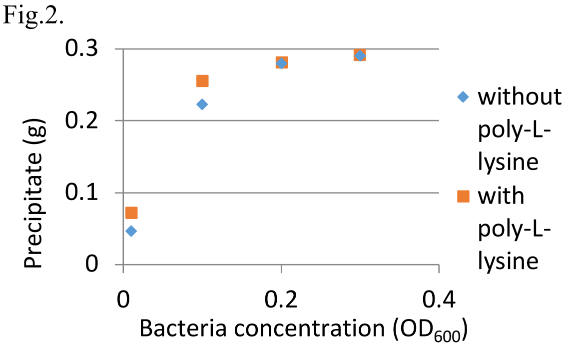


Fig. 2 Variation of the amount of carbonate precipitates with the bacteria concentration (OD_{600})

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 OD_{600}=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-lysine on the crystal surface and formation of new crystal faces.

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.

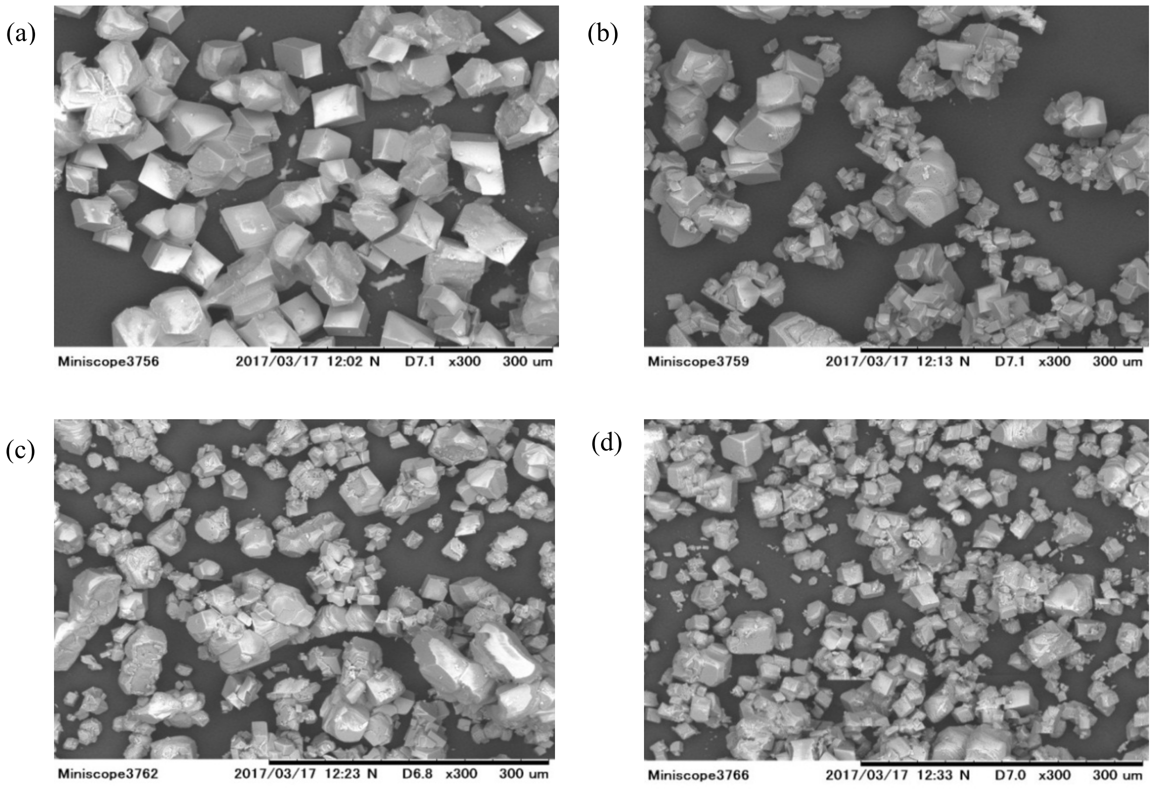


Fig. 3 SEM images of the $CaCO_3$ precipitate for different bacteria concentration without poly-L-lysine (a) OD_{600} = 0.01 (b) OD_{600} = 0.1(c) OD_{600} = 0.2 (d) OD_{600} = 0.3

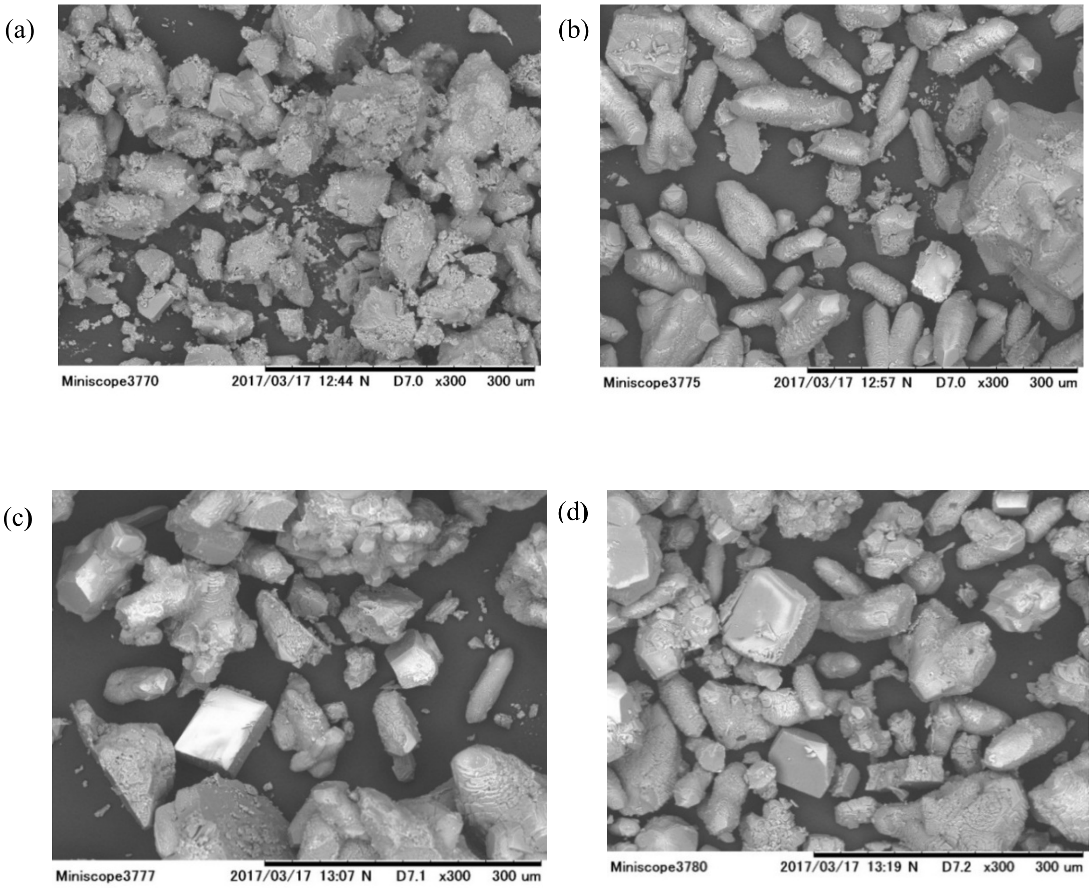


Fig. 4 SEM images of the $CaCO_3$ precipitate for different bacteria concentration in the presence of poly-L-lysine (a) OD_{600} = 0.01 (b) OD_{600} = 0.1(c) OD_{600} = 0.2 (d) OD_{600} = 0.3

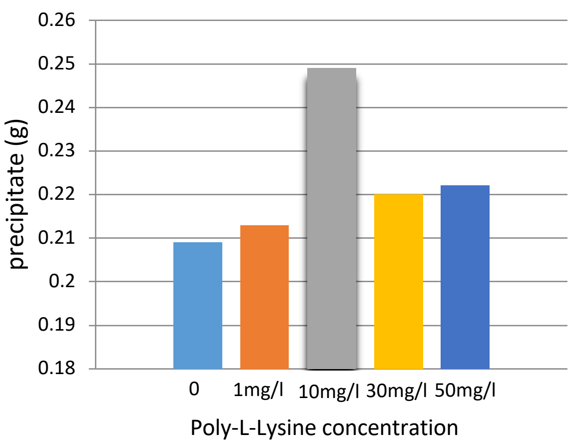


Fig. 5 Variation of the amount of carbonate precipitate with the poly-L-lysine concentration

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.

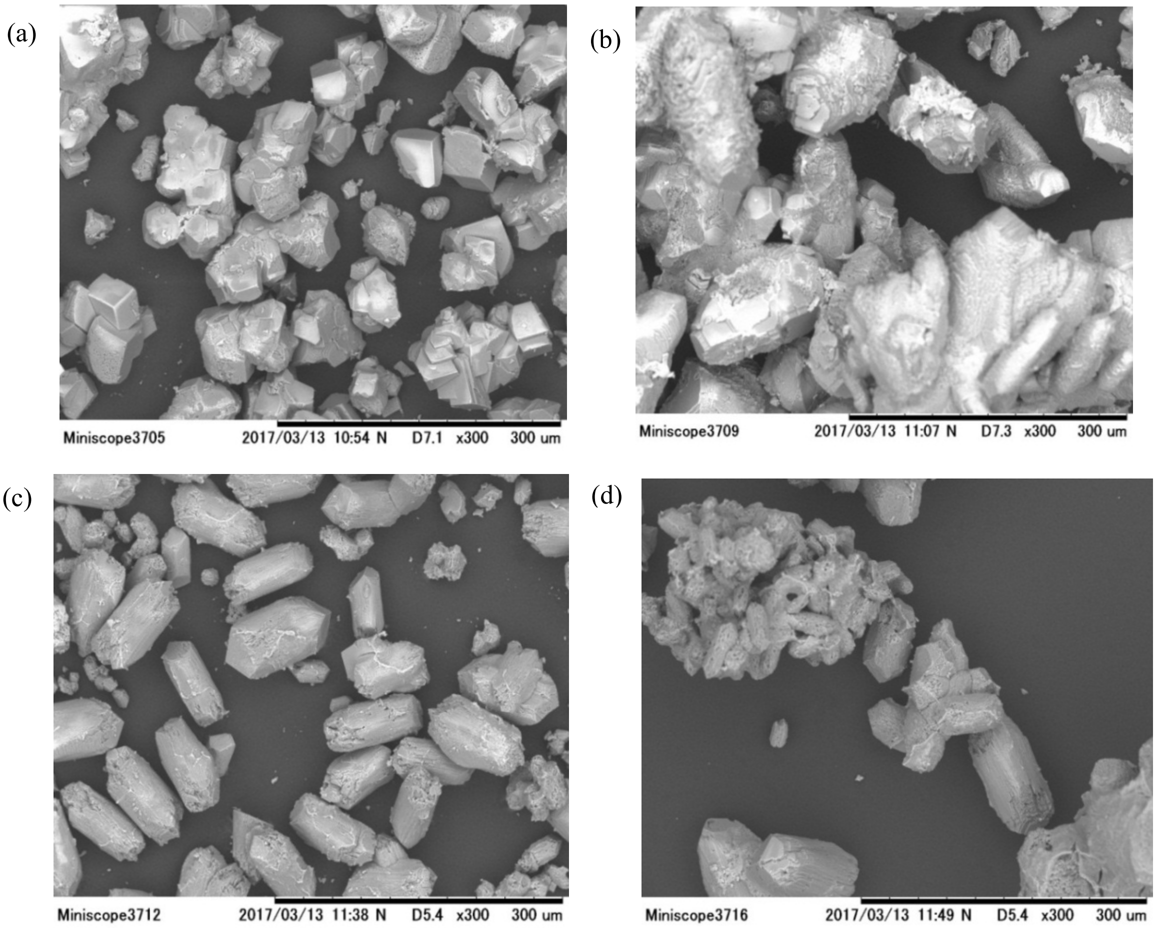


Fig. 6 SEM images of the $CaCO_3$ precipitate for different poly-L-lysine concentration (a) 1 mg/L (b) 10 mg/L (c) 30 mg/L (d) 50 mg/L

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 [6] [9].

FTIR Analysis

Results of the FTIR analysis with and without poly-L-lysine are shown in Fig 7. Both graphs look like similar. However, small peak can be seen at 1200 cm^{-1} in the graph with poly-L-lysine. This peak represents the C-N bond and we can conclude that some amount of poly-L-lysine present in the $CaCO_3$ precipitate.

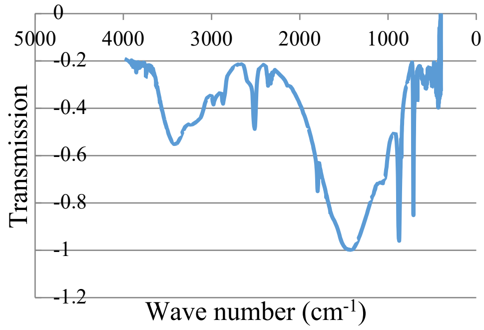
CONCLUSION

Effect of the cationic organic biopolymers on the MICP process was investigated by using poly-l-lysine. The results shows that,

- 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, the rate of the formation of calcium carbonate decrease. So, higher bacteria concentration will reduce the crystal growth.

(a)



(b)

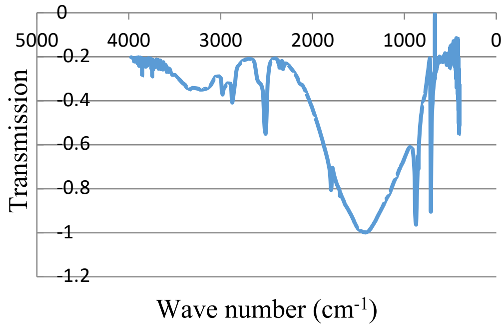


Fig. 7 FTIR spectra for samples (a) with poly-L-lysine (b) without poly-L-lysine

- 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 concentration inhibit the calcium carbonate precipitation but intermediate concentration give higher amount of precipitate.
- With the increase of the bacteria concentration morphology of the crystals change from well-developed rhombohedral crystals to agglomeration of the rhombohedral crystals and size of the crystals reduces.
- Presence of the poly-L-lysine produces ellipsoidal shape crystals. Morphology of the crystals changes from the polyhedral crystals to agglomeration of ellipsoidal crystals with the increase of the poly-L-lysine concentration.

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