11th Int. Conf. on Geotechnique, Construction Materials & Environment, Kyoto, Japan, 3-5 November 2021, ISBN: 978-4-909106063 C3051

EVALUATING THE EFFECTS OF POLYMERS ON MICROBIALLY INDUCED CARBONATE PERCIPITATION

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ABSTRACT

Microbially induced carbonate precipitation (MICP) is relatively a new environmentally friendly soil improvement method. In MICP process, the formation of calcium carbonate occurs within the soil matrix as the consequence of biochemical process mediated by ureolytic bacteria. The objective of this preliminary work is to evaluate the effects of adding polymers on MICP process. Basically, the polymers are organic macromolecules and have potential to offer several benefits such as increasing solution viscosity, extending supply time, localization of cementation reactions, reduction of harmful byproduct ammonium and enhancement of strength. For the above-mentioned objective, two different types of polymers are studied: (i) Polyvinyl alcohol (PVA) and (ii) Polyacrylic acid (PAA). *Lysinibacillus xylanilyticus* are cultivated in the standard medium with varying contents of polymers. The growth and urease activity of the bacteria are determined with the incubation time. In addition, set of test tube tests is performed to assess the precipitation characteristics of calcium carbonate at the presence of polymers. The outcomes indicated that the polymers do not have any adverse effects on the growth of bacteria. However, the PAA is found significantly enhance the urease activity compared with typical bacteria culture medium, whereas it is not evidenced in PVA. Moreover, compared to the typical MICP process, the addition of polymers produce more spherical-shaped calcium carbonate crystals, which highly differ from typical rhombohedral-shaped crystals. With the above findings, future works are recommended on soil to investigate the cementation efficiency and adsorption of ammonium by-products.

Keywords: Microbially induced carbonate precipitation (MICP), soil improvement, polymer, urease activity, crystal morphology

INTRODUCTION

In recent years, the biological method has attracted attention as an environmentally friendly method of ground improvement. Microbial Induced Carbonate Precipitation (MICP) is one of such kind of novel geotechniques. In MICP process, precipitation of $CaCO_3$ is induced by non-pathogenic bacteria that mediate the hydrolysis of the urea [1]. The sequential bio-chemical reactions that occur during the MICP process are listed in Eqs. (1-3).

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

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

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

Most of the MICP related research works attempt to involve *Sporosarcina pasteurii* which is a popular gram positive bacteria with high urease activity [2]–[4]. In due course, few researchers worked on identifying some other potential ureolytic bacteria for MICP purposes. Their outcomes indicate that the use of indigenous bacteria, instead of popular exogenous bacteria, offers numerous benefits in terms of reliability and bio-safety. *Lysinibacillus xylanilyticus* is one of such reliable species, and which was previously isolated from Hokkaido expressway slope, Japan [5]. Following the demonstration by Japan MICP researchers, the species were repeatedly used in many MICP related works [6]–[7]. Due to their consistence performance, the *Lysinibacillus xylanilyticus* is used in this study to evaluate the effects of polymers in MICP process.

Use of additives such as polymers to improve the weak soil is an alternative biological approach to the MICP process [8]. Number of researches has done previously to investigate the applicability of hydrophilic polymers. Hydrophilic polymer solution can restrain the desiccation of the cementation solution and extend the time window for MICP-related reactions following one-shot injection, which can be critical for certain applications where the treated surficial soils are exposed to air and sunlight. Owing to these favorable characteristics, hydrophilic polymers have increasingly been utilized for assisting mineral precipitation-based improvement and remediation approaches in the field of civil engineering [9].

Previously, the effect of polyvinyl alcohol (PVA) on strengthening was investigated. Using PVA to increase the surface erosion resistance of MICP treated soil also studied. Their outcomes indicats

that the polymers provide additional cohesive forces [10]. In another instance, polyacrylic acid (PAA) was applied to not only improving the mechanical strength of the loose soil but also mitigating excessive ammonium contamination [11]. It is worth mentioning, however, only a very few studies focused on the effect of polymer on biological and bio-chemical responses, providing insufficient information in the scientific literature.

Thus, the objectives of this original work are to evaluate the (i) effects of polymers on the biological responses and (ii) efficacy of incorporating polymers in MICP process. For the assessment, two popular polymers: PVA and PAA, are chosen in this study. The experimental program mainly involves urease activity measurements and assessments of precipitation characteristics.

MATERIALS AND METHOD

2.1 Polymers used

The PVA used herein was obtained from Wako Pure Chemical Industries Ltd., Tokyo, Japan. The average degree of polymerization is between 400 to 600. The average molecular mass of PAA (Wako Pure Chemical Industries Ltd., Tokyo, Japan) used is 25000. Chemical structures of the PVA and PAA are shown in Fig. 1(a) and Fig. 1(b), respectively.

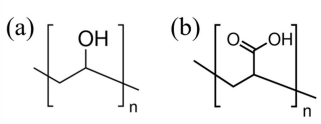


Fig. 1 Structures of the polymers (a) PVA (b) PAA

2.2 Preparation of bacteria culture

The gram-positive ureolytic bacteria *Lysinibacillus xylanilyticus*, previously identified from the Hokkaido slope were used in this research study. Firstly, to prepare the preculture, 5 mL of NH_4 -YE medium was inoculated by the stock bacteria (preserved at -80 ℃) and kept in the shaking incubator at 25 ℃ and 160 rpm for 24 hours. Secondly, 1 mL of the preculture was added into 100 mL of NH_4-YE medium with varying quantities of PVA or PAA (hereinafter referred to as main culture). The main culture was then kept in the shaking incubator under the same condition as preculture.

The culture mediums tested herein are all have 1.57 g of Tris buffer, 1 g of Ammonium sulfate and 2 g of Yeast extract per 100 mL. The additional adding polymers clearly presented in Table 1.

From case V1 to V6, the content of PVA increases. Similarly, from case A1 to A6, the content of PAA gradually increases. But for the case C, neither PVA nor PAA was added. It was used as a control group to compare the effects of additional polymers on microorganisms.

Table 1. The compositons of main culture

Case No.	PVA (g/L)	PAA (g/L)
V1	5	0
V2	10	
V3	20	
V4	40	
V5	60	
V6	80	
C	0	0
A1		2.5
A2		5
A3		7.5
A4		10
A5		12.5
A6		15

2.3 Urease activity test

Urease activity of the bacteria culture was measured using indophenol spectrophotometry method. 0.5 mL of bacterial culture was taken from main culture and added in 50 mL phosphate buffer solution with 0.1 mol/L urea. At the presence of hypochlorite, the ammonium ions produced from the urea hydrolyses react with phenol and produce the blue colour indophenol dye in the alkaline medium. The intensity of indophenol dye was measured via the wave length of 630 nm (OD_{630}) at every 5 min interval of catalyzation. Using a calibration curve established between ammonium ion concentration and intensity (OD_{630}), the rate of urea hydrolysis and urease activity can be obtained [12].

2.4 Precipitation test

After 1 ml bacteria samples separately took from each case in Day 3, centrifugated and removed supernatant and each sample tube was added 10 mL same concentration (0.3 mol/L) of $CaCl_2$ and Urea solution. Samples were kept in the shaking incubator under 25 ℃ 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 (11 μm).

Both of the filter papers and the tubes with the precipitate were oven dried at 60 ℃ for 48 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.

2.5 Scanning Electron Microscopy (SEM) analysis

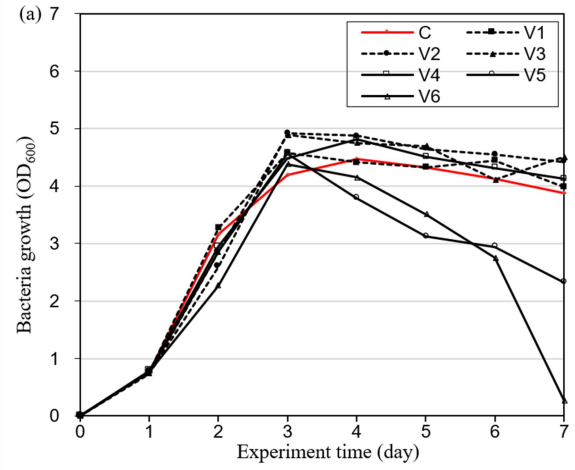
The shape of calcium carbonate crystals can be affected by many factors. In order to minimize interference from organic components in the culture medium and different urease activities from different cases, using 1 ml of bacteria sample in the same control case C in day 3. After centrifugation and supernatant removement, 3 groups both added with the 10 mL of same concentration (0.3 mol/L) of Urea and $CaCl_2$ solution. One group was added with 1 g/L of PVA, and the other group was added with 1 g/L of PAA, one group left as control group.

Samples were kept in the shaking incubator under 25 ℃ and 160 rpm speed for 24 hours. Then the reaction mixture was centrifuged to collect the precipitate. After 48 hours of 60 ℃ oven dry, scanning electron microscopy (SEM) was performed using Miniscope TM3000, Hitachi (Tokyo, Japan) to identify the morphology of the precipitated calcium carbonate crystals.

RESULTS

3.1 Growth of the bacteria

Variation of the population of samples under different polymers concentrations are given in the Fig. 2. It can be seen from the Fig. 2(a) that the PVA has no obvious inhibitory effect on the growth of bacteria, bacteria can still grow, even at a high concentration up to 80 g/L. In contrast, PAA strongly inhibits bacterial growth when the concentration is higher than 10 g/L (Fig. 2(b)).



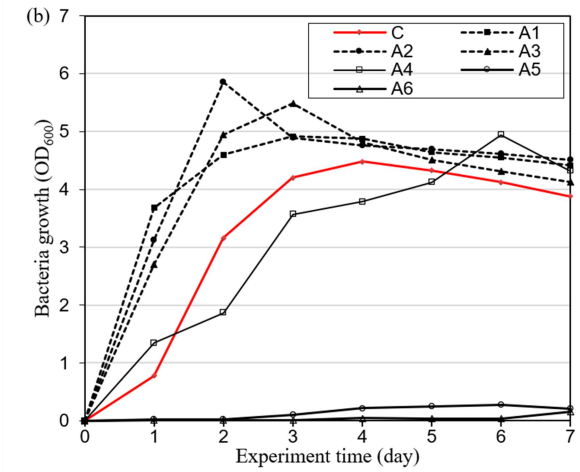
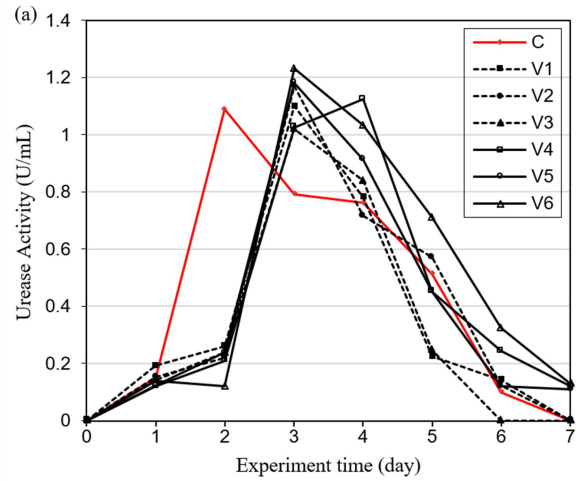


Fig. 2 Variation of the bacteria population with adding polymer (a) PVA (b) PAA



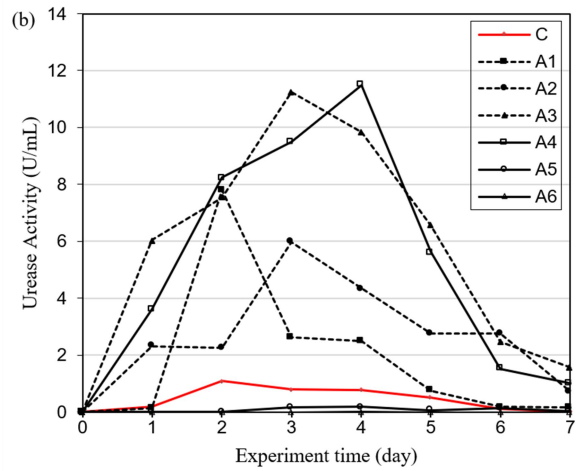


Fig. 3 Variation of the urease activity with adding polymer (a) PVA (b) PAA

3.2 Urease activity

Figure 3 shows that different concentrations of PVA have no obvious effect on the activity of bacteria. The activities were almost similar to that observed of the control. On the other hand, the low concentrations of PAA have a very obvious promotion effect on urease activity. For instance, at the concentration of 10 g/L, the urease activity is found to be relatively optimum, that exceeds of a value over 11 U/mL. However, when the PAA concentration further increases, the urease activity dramatically diminishes.

3.3 Amount of $CaCO_3$ precipitation

Figure 4 shows the variation of the amount of $CaCO_3$ for the different considered cases. In the cases of adding PVA do not show any evident trend (no significant changes). But, for the PAA cases, when the concentration increases, the amount of precipitation shows an increasing tendency. However, when the concentration increases above 10 g/L, it suddenly drops very close to 0 g.

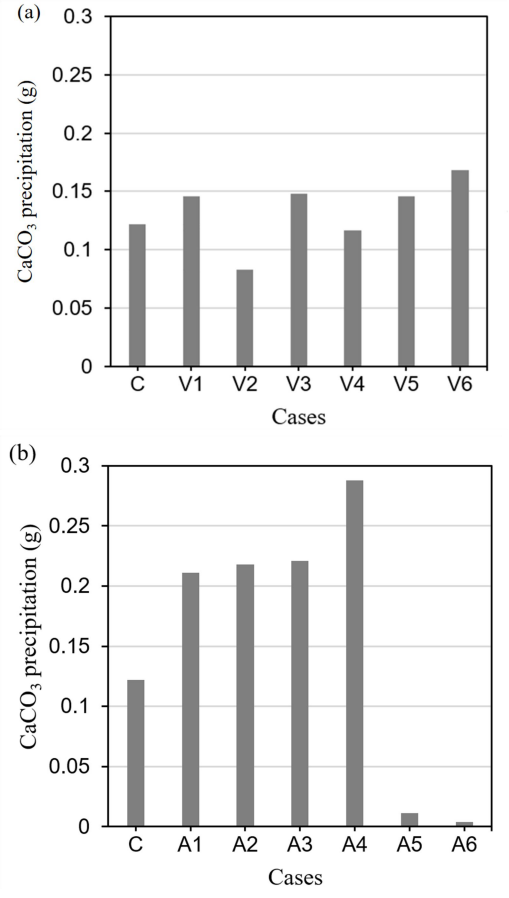


Fig. 4 Variation of the $CaCO_3$ amount with different polymer cases (a) PVA (b) PAA.

3.4 Morphology of the precipitated crystals

According to Fig. 5, scanning electron microscope (SEM) images of various morphologies are shown at the same scale.

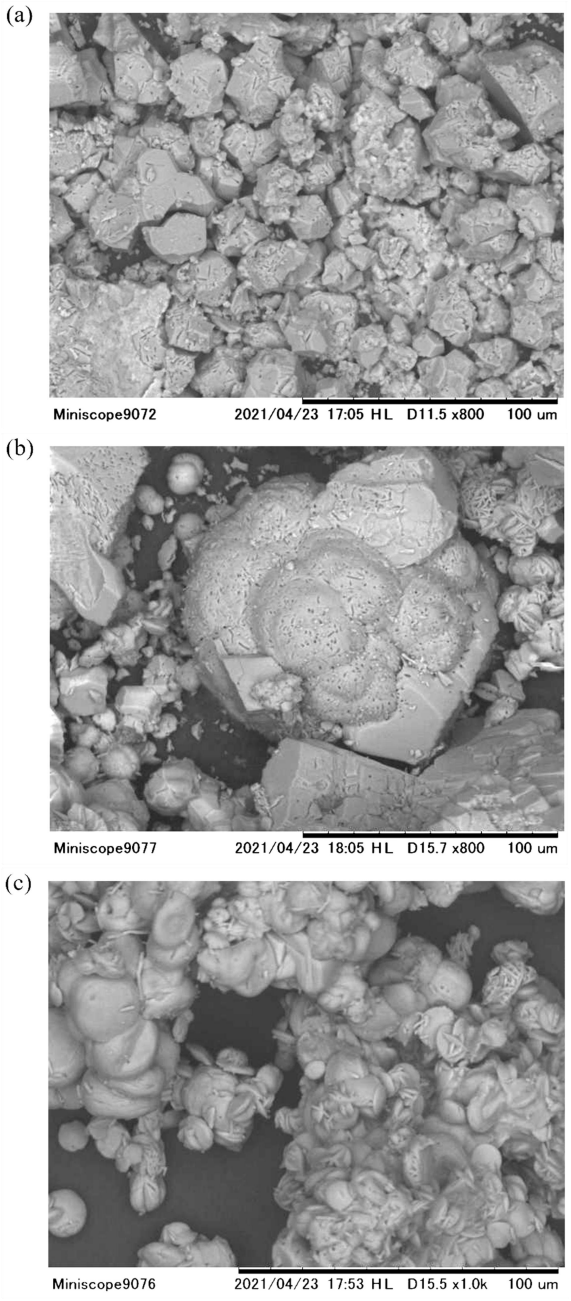


Fig. 5 Variation of the $CaCO_3$ morphology under different condition (a) no polymer adding (b) PVA (c) PAA.

The calcium carbonate crystal morphology without any polymers is shown in Fig. 5(a) (control), indicating the formation of typical rhombohedral-shape crystals. When the PVA is added to the medium, relatively spherical-shaped crystals are precipitated. At the same time, an accumulation of

pallets-like calcium carbonate crystals could be seen in the PAA added cases. It should be noted that the morphology of the crystals seen in Fig. 5(a) significantly different from that observed in Fig. 5(b). This suggests that the crystallization effects highly rely on the intrinsic characteristics of polymers.

DISCUSSION

From the outcomes, it is found that the PVA does not inhibit both the growth and urease activity of bacteria, regardless of its concentration in the reaction medium. In contrast, the addition of PAA was found to favor the urease activity of the bacteria, which showed an increase over more than two-folds. However, the concentration over 10 g/L strongly inhibited the bacteria growth, urease activity and precipitation efficiency. This could probably be attributed to the characteristics of the polymer. The PVA is a neutral polymer, while the PAA is an acidic one; therefore, the low pH conditions caused by high concentration of PAA (above 10 g/L) were extremely unfavorable and harsh for bacterial growth.

In consideration of both Fig. 3 and Fig. 4, there were no considerable positive or adverse influences observed from the PVA on the MICP process. Interestingly, the lower concentrations of the PAA (below 10 g/L) promoted the ureolytic performance of the bacteria, hence the mineralization efficiency. One possible explanation could be that the preferential consumption of PAA by the bacteria that aided to produce more amino acids. Nevertheless, the clear mechanism for the incredible performance is still unclear, needing further investigation (left for the future work).

It was evident (from the SEM analysis) that with the addition of polymer, the morphology of the crystals transitioned from rhombohedral to round/ spherical-shaped crystals with more aggregation. The morphology changes could possibly be due to the adsorption of the polymers on the crystal surface, which led to the formation of new crystal faces.

CONCLUSIONS

According to the obtained results, a moderate amount of PAA has a significant positive effect on urease activity, which has great significance to the practical application of engineering and can be used to achieve high strength cementation in the future research. On the contrary, PVA does not promote or inhibit MICP, even at high concentrations. It also has potential application value which can be used to adjust the viscosity of the cementation solution to control the occurrence position of the MICP.

Morphology of the crystals changes from the polyhedral crystals to agglomeration of round crystals with the attendance of polymer. The relationship between the crystal morphology and ground improvement is still not clear. However, this is a 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.

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