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Effects of Cationic Polypeptide on $CaCO_3$ Crystallization and Sand Solidification by Microbial-Induced Carbonate Precipitation

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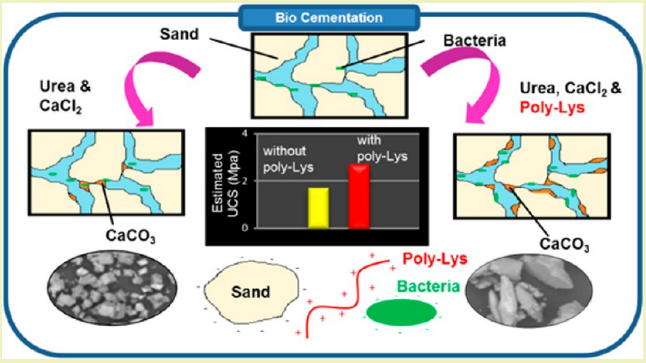
ABSTRACT: The use of organic additives to improve microbial-induced carbonate precipitation (MICP) is a novel and innovative idea. This study is the first to address the effects of the cationic biopolymer polylysine (poly-Lys) on $CaCO_3$ crystallization and sand solidification by MICP. $CaCO_3$ was precipitated with and without poly-Lys by hydrolysis of urea by using ureolytic bacteria, *Pararhodobacter* sp., in the presence of $CaCl_2$ under different experimental conditions. The morphologies and polymorphs of the oven-dried precipitates were investigated using scanning electron microscopy and X-ray diffraction. A larger amount of precipitate was obtained with poly-Lys than with the conventional MICP method. The curve for the relationship between the poly-Lys concentration and amount of precipitate was bell shaped. In the presence of poly-Lys, the morphology changed from rhombohedral crystals to twin spherical crystals. The effects of poly-Lys on sand solidification were also investigated by syringe solidification at different bacterial injection intervals with and without poly-Lys. The addition of poly-Lys gave strongly cemented sand specimens that were stronger than those obtained by the conventional method. The results confirm that poly-Lys addition is an effective and sustainable way to improve the MICP efficiency and production of green materials for engineering applications.



KEYWORDS: *Biocement, Biomineral, Calcium carbonate, Morphology, Polylysine*

INTRODUCTION

The amount of suitable land available for construction is decreasing because of rapid population growth and urbanization. Unsuitable ground is therefore being used for building constructions, and appropriate ground improvement techniques are needed to improve the properties of weak soils. The available traditional ground improvement techniques have various limitations, and most of these methods are environmentally unfriendly. Recently, biological approaches have attracted attention as environmentally friendly ground improvement methods.

One innovative idea is to use nonpathogenic microorganisms that are naturally present in the subsurface soil to improve the properties of uncemented soils.[1] These microorganisms help to provide the cementing materials through enzymatic reactions. $CaCO_3$ is a well-known cementing material and a common biomineral. The formation of artificial $CaCO_3$ as a cementing material via biological processes is an ecofriendly method for treating uncemented soils. This concept is called microbial-induced carbonate precipitation (MICP), and this biocement can be introduced as a sustainable construction material.[2] MICP is a biogeochemical process in which $CaCO_3$ precipitation in the soil matrix is achieved by urea hydrolysis using enzyme urease, which is produced by ureolytic bacteria. The overall process involves the biochemical reactions represented by eqs 1−3.[3−8] The precipitated $CaCO_3$ acts as a binding material, which binds soil grains together at the particle−particle interfaces, leading to increased soil stiffness and strength.

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

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

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

Previous research has shown that MICP can increase the soil strength and stiffness and maintain its permeability, with minimum disturbance to the soil.[1,8−11] $CaCO_3$ precipitation is governed by four major factors: calcium concentration, carbonate concentration, pH, and availability of nucleation sites.[11] The MICP efficiency can be improved by adjusting these parameters with suitable chemicals, but adding chemicals to improve the efficiency can damage the environment. It is therefore better to use naturally formed environmentally

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friendly additives. The use of naturally formed organic additives to improve the MICP performance is therefore attractive. In nature, biominerals are mainly deposited in organic matrixes that consist mainly of macromolecules such as proteins or polysaccharides, which act as templates for mineral nucleation and growth.[12−14] It has been proven that combinations of inorganic minerals and organic macromolecules provide good hybrid materials with well-controlled morphologies, structures, and unique properties.[15−18] In the present study, we mimicked this concept to improve the efficiency of MICP by producing new bioinspired materials.

Previous studies have shown the effects of acidic polypeptides on $CaCO_3$ crystallization,[19−22] but there are few reports of the use of basic polypeptides to control $CaCO_3$ crystallization.[15,20] This study is the first to address the effects of a cationic polypeptide on $CaCO_3$ crystallization and the MICP process by using the ureolytic bacteria *Pararhodobacter* sp. Most of the MICP related research works were based on the *Sporosarcina pasteurii*, which is Gram positive bacteria with high urease activity.[11,23] Similar to S. *pasteurii,* Gram negative *Pararhodobacter sp* has been successfully applied in sand solidification[4,10,24] due to its high urease activity and long-term stability.[6] Polylysine (poly-Lys) was used as the cationic polypeptide; at neutral pH, its amino group side chains are positively charged.[20]

EXPERIMENTAL SECTION

Preparation of Bacterial Cell Culture. Ureolytic bacteria *Pararhodobacter* sp. isolated from beach sand in Sumuide, Nago, Okinawa, Japan, was used in this study.[25,26] Bacterial cells were precultured in Zobell 2216E medium (polypeptone (Nihon Seiyaku Co., Ltd., Tokyo, Japan) 5.0 g/L, yeast extract (BD Biosciences Advanced Bioprocessing, Miami, FL, USA) 1.0 g/L, and $FePO_4$ (Junsei Chemical Co., Ltd., Tokyo, Japan) 0.1 g/L in artificial seawater, pH 7.6−7.8; 5 mL) by shaking at 30 °C and 160 rpm for 24 h. The preculture (1 mL) was inoculated into fresh Zobell 2216E medium (100 mL). The mixture was kept in the shaking incubator under the same conditions as those used for preculturing for 48 h. The cells were collected by centrifugation of the bacterial culture. The cells were resuspended in sterilized water to adjust the cell concentration (OD_{600} = 1), which was determined by UV−visible spectroscopy (V-730, JASCO Corporation, Tokyo, Japan).

Precipitation of $CaCO_3$ by Ureolytic Bacteria. $CaCO_3$ precipitation was achieved by urea hydrolysis with bacteria in the presence of $CaCl_2$. The bacteria were added to a substrate solution containing urea (0.3 mol/L; Wako Pure Chemical Industries Ltd., Tokyo, Japan) and $CaCl_2$ (0.3 mol/L; Wako Pure Chemical Industries Ltd., Tokyo, Japan) in the presence or absence of poly-Lys (Wako Pure Chemical Industries Ltd., Tokyo, Japan). The reaction mixture (10 mL) was shaken at 30 °C and 160 rpm for 24 h. The samples were centrifuged to separate the $CaCO_3$ precipitate from the supernatant. The precipitates were dried in an oven at 100 °C for 24 h and then the dry weights of the precipitates were determined. The reactions were performed at various bacterial concentrations (OD_{600} = 0.01−0.2) in the presence (10 mg/L) or absence of poly-Lys. The same reactions were conducted at various poly-Lys concentrations (0−50 mg/L) with a bacterial concentration of OD_{600} = 0.1. All experiments were done in triplicate.

X-ray Diffraction and Scanning Electron Microscopy Analyses. The polymorphs of the $CaCO_3$ precipitate were identified by X-ray diffraction (XRD; MiniFlex, Rigaku Co., Ltd., Tokyo, Japan) analysis of ground samples under Ni-filtered Cu 1.5406 Å radiation. The crystalline phases were identified with MATCH 3.4 software. Scanning electron microscopy (SEM; Miniscope TM3000, Hitachi, Tokyo, Japan) was used to investigate the morphologies of the precipitated $CaCO_3$ crystals.

Sand Solidification in Syringe. Sand solidification in syringe was performed with or without poly-Lys to check the effects of the polymer on sand solidification under various conditions, as shown in Table 1. The method previously described by Danjo and Kawasaki was used for the experiments.[24]

Table 1. Experimental Conditions for Sand Solidification

	poly-Lys	bacteria injection
S1		once[b]
S2	100 mg/L[a]	once[b]
S3		twice[c]
S4	100 mg/L[a]	twice[c]

[a]Initial concentration of the poly-Lys solution applied to the column in solidification test. [b]Injection of bacteria culture only at the beginning of solidification test. [c]Injection of bacteria culture at the beginning and after 7 days of solidification test.

Mikawa sand (40 g, mean diameter D_{50} = 0.6 mm) was oven-dried at 110 °C for 2 days and placed in a 35 mL syringe (mean diameter D_{50} = 2.5 cm and height 7 cm) in three layers. Each layer was subjected to 20 hammer blows. Then first poly-Lys (5 mL, 100 mg/L) was injected to the syringe, and next, the bacterial solution (16 mL, OD600 = 1) was injected and maintained for 5−10 min to allow fixation of the bacteria to sand particles. After that, the solution was drained from the outlet, leaving 2 mL of the solution above the surface. After that the cementation solution (20 mL; 0.3 M urea, 0.3 M $CaCl_2$, 0.02 M sodium hydrogen carbonate, 0.2 M ammonium chloride, and 3 g/L nutrient broth (BD Biosciences Advanced Bioprocessing, Miami, FL, USA)) was injected into the syringe and drained, leaving 2 mL of the solution above the surface to maintain wet conditions. All solutions were injected through gravity. Ammonium chloride was added to the cementation solution as a nitrogen source of the bacteria. Experiments were done in triplicate in an incubator at 30 °C.

Every 3 days, the pH and Ca^{2+} concentration of the drainage were measured. The experiments were conducted with various bacterial injection intervals. In one set, bacteria were injected only once, on the first day. In another set, bacteria were injected twice, i.e., on the first day and again after 7 days. During the experiment, poly-Lys was injected twice, on the first day and again after 7 days. After curing for 14 days, the unconfined compressive strength (UCS) of the samples was determined with a needle penetration device (SH70, Maruto Testing Machine Company, Tokyo, Japan). First, the needle was injected into the sand specimen, and the penetration force (N) and distance (mm) were recorded. Unconfined compressive strengths (UCS) of the samples were determined by using the calibration equation given in eq 4 which was provided by the manufacturer. In the instrument manual it is mentioned that this calibration equation was developed by considering 114 natural rock samples and 50 improved soils with cements.

$$\log(y) = 0.978 \log(x) + 2.621 \quad (4)$$

x = penetration gradient (N/mm); y = unconfined compressive strength.

RESULTS AND DISCUSSION

Effects of Poly-Lys on $CaCO_3$ Precipitation. Figure 1 shows the amounts of $CaCO_3$ formed by ureolytic bacteria at different concentrations in the presence or absence of poly-Lys. In both cases, the amount of $CaCO_3$ precipitate increased with increasing bacterial concentration. The amount of precipitate formed is directly related to the urease activity of the relevant bacteria.[27] The urease activity of *Pararhodobacter* sp. increases with increasing cell concentration.[6] The increase in urease activity with increasing cell concentration leads to increased precipitation and causes rapid nucleation and growth of

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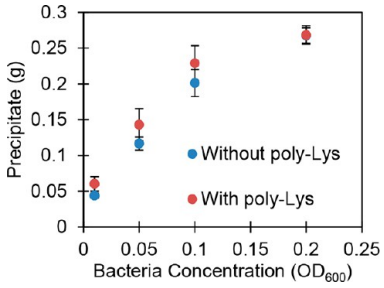

Figure 1. Amounts of precipitate formed at various bacterial concentrations (OD_{600}) with or without addition of poly-Lys. Error bars show the standard deviation of the values in three independent experiments.

$CaCO_3$ at high bacterial cell concentrations. Previous reports have stated that the rate of $CaCO_3$ precipitation with S *porosarcina pasteurii* depends on the rate of urea hydrolysis and to increase the rate of urea hydrolysis the ureolytic biomass must be increased.[8] A higher concentration of urease also increases the $CaCO_3$ formation rate.[14]

The amount of precipitate formed in the system with poly-Lys was higher than that formed in the absence of poly-Lys. The positive effect of poly-Lys on $CaCO_3$ precipitation is clearer at low bacterial concentrations. A significant effect of poly-Lys was not detected at higher bacterial concentrations; this indicates that the bacterial activity may be the dominant factor rather than the effects of poly-Lys. Acidic polypeptides clearly affect $CaCO_3$ crystallization because the negative charge on the carboxylic group is strongly attracted to Ca^{2+}, and in the presence of CO_3^{2-}, a $CaCO_3$ nucleus can be formed.[19,21,22,28] The effects of basic polypeptides on $CaCO_3$ crystallization are unclear and difficult to explain. One research group reported chemical precipitation of $CaCO_3$ from $Ca(NO_3)_2$ and Na_2CO_3 in the presence of L-Lys. They suggested that the positive charge on L-Lys strongly attracts CO_3^{2-} ions, resulting in CO_3^{2-} ion enrichment and higher super saturation of CO_3^{2-} ions in local regions. This is favorable for $CaCO_3$ crystal nucleation and growth.[21] The positive effect of poly-Lys on $CaCO_3$ precipitation can be similarly explained. Most acidic polypeptides inhibit $CaCO_3$ nucleation and growth.[12,21] However, the addition of poly-Lys does not inhibit nucleation and growth of $CaCO_3$. The interactions between poly-Lys and calcite are purely electrostatic interactions between the positively charged polypeptide and the negatively charged calcite surface.[20,28] Poly-Lys therefore has a positive effect on $CaCO_3$ crystallization and can be used to improve the MICP efficiency.

The crystal morphologies of the samples prepared at various bacterial concentrations were examined by SEM (Figure 2). Figure 2a shows that well-developed rhombohedral crystals were obtained at low bacterial concentrations. In contrast, at higher bacterial concentrations, individual rhombohedral crystals were not be observed and rhombohedral crystal agglomeration occurred. Another important point is that the crystal size decreased with increasing bacterial concentration. This may be because the number of nucleation sites increased with increasing bacterial concentration. Urease from *Para-rhodobacter* sp. is not secreted into the extracellular medium but accumulates in or on the cell as a membrane enzyme.[6] The bacterial cells can therefore act as nucleation sites for $CaCO_3$ crystals. Because of the lack of nucleation sites at low bacterial concentrations, after the initial calcite precipitation on the nucleation sites, calcite crystals can grow continuously and produce larger crystals.[29] However, at higher bacterial concentrations, each cell acts as a nucleation site and a large number of small crystals are produced, without growth of the individual crystals.

Figure 3 shows the morphologies of the crystals in the presence of poly-Lys at various bacterial concentrations. In the presence of poly-Lys, the crystal morphology became ellipsoidal; this was especially clear at low bacterial

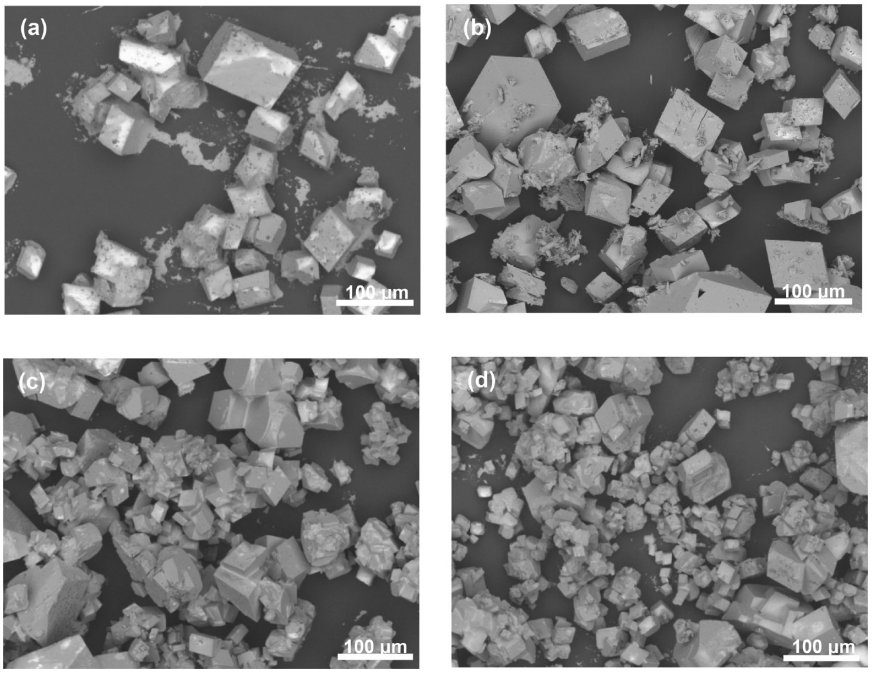

Figure 2. SEM images of $CaCO_3$ precipitates at various bacterial concentrations without poly-Lys: (a) OD_{600} = 0.01, (b) OD_{600} = 0.05, (c) OD_{600} = 0.1, and (d) OD_{600} = 0.2.

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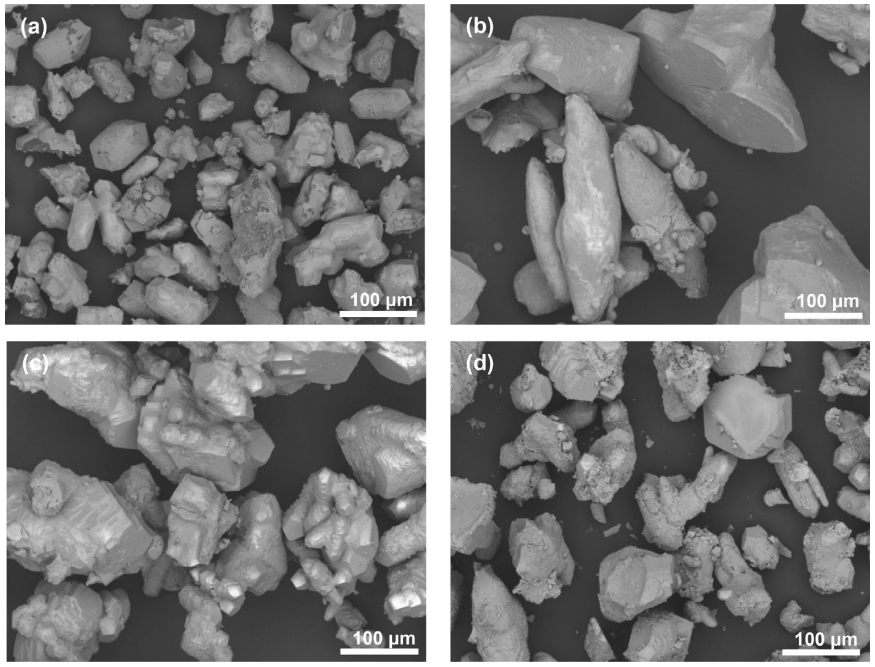

Figure 3. SEM images of $CaCO_3$ precipitates at various bacterial concentrations with poly-Lys (10 mg/L): (a) OD_{600} = 0.01, (b) OD_{600} = 0.05, (c) OD_{600} = 0.1, and (d) OD_{600} = 0.2.

concentrations. At higher bacterial concentrations, a combination of ellipsoidal and rhombohedral crystals was obtained. However, the XRD patterns in Figure 4 show that the main component of the obtained crystals, with and without poly-Lys, was calcite.

Formation of larger number of smaller crystals in higher bacteria cell concentration would increase the efficiency of the MICP process in the solution due to produce of larger amount of $CaCO_3$ precipitate.

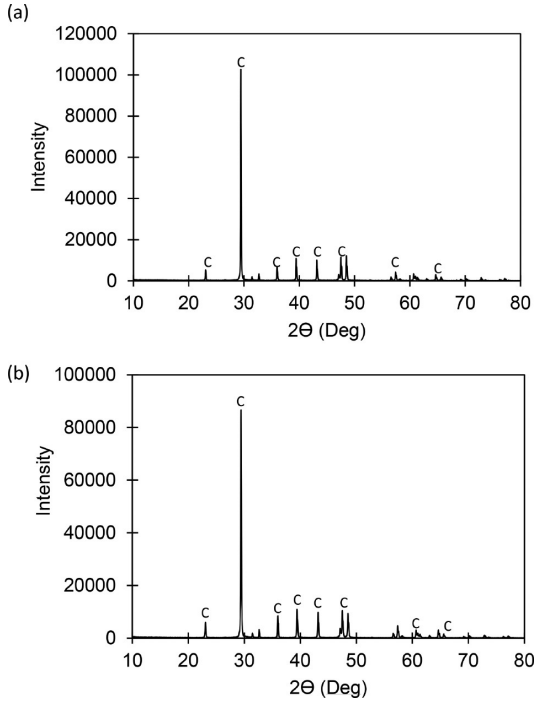

Figure 4. XRD patterns of $CaCO_3$ precipitates (a) without poly-Lys and (b) with poly-Lys.

Effects of poly-Lys Concentration on $CaCO_3$ Crystallization. Figure 5 shows the amounts of precipitate formed at

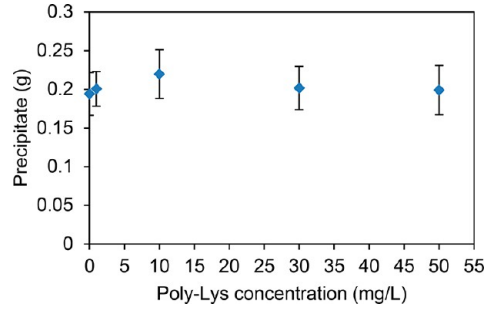

Figure 5. Variations in the amount of carbonate precipitated with changes in poly-Lys concentration. Error bars show the standard deviation of the values in three independent experiments.

different concentrations of poly-Lys. A moderate bell-shaped relationship was observed between the amount of precipitate and the poly-Lys concentration. The amounts of precipitate formed at intermediate poly-Lys concentrations were higher than those formed at lower and higher concentrations. Visual observations indicated that when the poly-Lys concentration was high, the precipitate was a gel rather than a solid. Before oven drying, the volumes of precipitates formed at high poly-Lys concentrations were higher than those of the other samples, but the dry weights, i.e., after oven drying, were lower than those of the other samples. A similar relationship between the amount of precipitate and the poly-Lys concentration was previously reported by another research group.[20] In their experiments, $CaCO_3$ was precipitated by a chemical reaction between $CaCl_2$ and Na_2CO_3 solutions in the presence of poly-Lys. They reported that increased calcite growth at low concentrations of poly-Lys and decreased growth at higher concentrations is typical of additives that are weakly, nonselectively bonded to the crystal surfaces.

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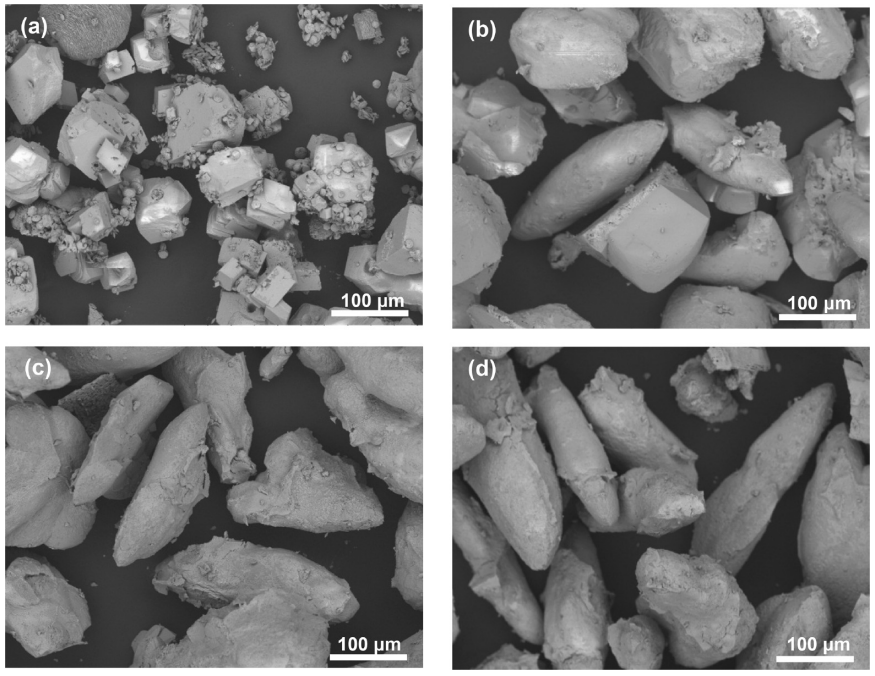

Figure 6. SEM images of $CaCO_3$ precipitated by bacteria (OD_{600} = 0.1) at different poly-Lys concentrations: (a) 1, (b) 10, (c) 30, and (d) 50 mg/L.

The SEM images in Figure 6 show that the crystal morphology changed greatly with changes in the poly-Lys concentration. At low poly-Lys concentrations, polyhedral crystals were obtained, as shown in the Figure 6a. With increasing poly-Lys concentration, the morphology changed to peanut-like twin spheres, and at high poly-Lys concentrations, twin spheres were dominant, as shown in Figure 6d. These morphology changes depend on the conformation of the poly-Lys chain. During urea hydrolysis, the pH of the medium increases, and under alkaline conditions, poly-Lys has an α-helix confirmation.[30] This α-helix confirmation leads to tangentially oriented growth on the sphere surface and vertically oriented growth on the equatorial zone, to form peanut-like twin spheres, as shown in Figure 7.[15] Twin spheres are probably formed because of crystal growth on both sides of the nuclear plate.

Sand Solidification in Syringe. Four sets of experiment (S1−S4) were performed with/without poly-Lys addition and with various bacterial injection times. Figure 8 shows the Ca^{2+} concentrations and pH values of the effluents every 3 days.

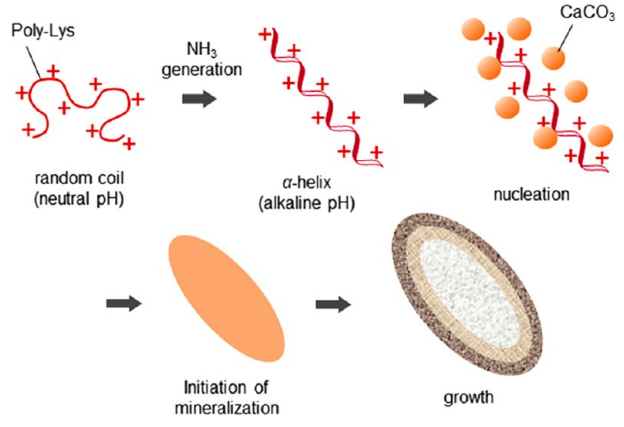

Figure 7. Formation of twin-sphere crystals in the presence of poly-Lys.

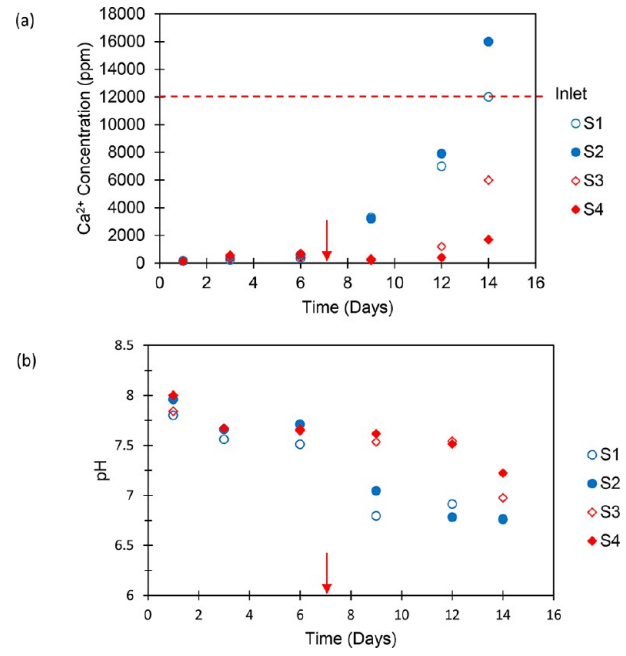

Figure 8. Time courses of (a) Ca^{2+} concentration and (b) pH of effluent in sand solidification tests. (Arrows indicate the time of bacteria reinjection to the sand specimens.)

Initially, a negligible amount of Ca^{2+} was detected in the effluents of all four samples. After 7 days, the effluent Ca^{2+} concentrations for S1 and S2 gradually increased with time, but the S3 and S4 effluent Ca^{2+} concentrations remained low for up to 12 days. Lower Ca^{2+} concentrations in the effluent show that more Ca^{2+} was deposited as $CaCO_3$ in the sand column, resulting in small amounts being washed out with the effluent.

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The initial low Ca^{2+} concentrations in the effluents of all the samples provide evidence of initially high precipitation of $CaCO_3$ in the sand column. However, the Ca^{2+} concentrations in the effluents in the systems without reinjection of bacteria (S1 and S2) increased gradually after 6 days, indicating insufficient consumption of Ca^{2+} ions and low precipitation of $CaCO_3$. In contrast, the Ca^{2+} concentrations in the systems that were reinjected with bacteria (S3 and S4) remained low, indicating efficient $CaCO_3$ precipitation in the column.

The pH values for S1 and S2 decreased after 6 days, whereas the values for S3 and S4 were almost constant up to 12 days and then began to decline. We found a close relationship between the Ca^{2+} concentration and effluent pH. In urea hydrolysis by urease, the pH of the solution increases because of ammonia formation. The pH of the effluent therefore remained high (alkaline) when the urease activity was high. However, the urea hydrolysis rate decreased with time due to the deactivation of the bacteria. Bacterial cells themselves could act as nucleation sites for $CaCO_3$ deposition around the cell wall, leading to the gradual reduction of the bacterial urease activity.[31] On the other hand, reinjection of the bacteria helped to maintain the active cell density hence could maintain high pH value. At high urease activity, $CaCO_3$ is efficiently formed in the column, and the Ca^{2+} concentration in the effluent remains low. For *Pararhodobacter* sp., a significant loss of activity is seen under acidic (pH < 5) and alkaline (pH > 10) conditions, and the activity is maximum at around pH 8.[6] To increase $CaCO_3$ precipitation, it is therefore important to maintain the pH at around 7−8. The increase in the Ca^{2+} concentration and the decrease in the effluent pH are therefore attributed to decreased urease activity in the column. The urease activity was recovered by reinjection of the bacterial culture, to maintain a low Ca^{2+} concentration and high pH value throughout the test period.

Solidified sand specimens after 14 days before and after UCS measurements are given in Figure 9. The estimated UCS values for the syringe solidification samples are shown graphically in Figure 10. All the sand specimens were strongly cemented, according to the classification system proposed by Shafii and Clough,[32] in which cemented soils with UCS values less than 1 MPa are classified as moderately cemented soil, strongly cemented soil is defined as having a UCS between 1 and 3 MPa, and samples with UCS values greater than 3 MPa are classified as soft rock.

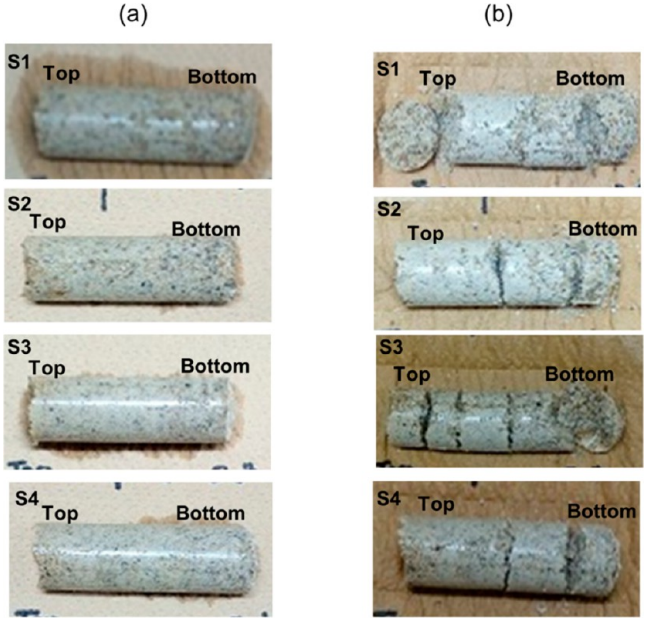

Figure 9. Solidified sand specimens after 14 days (a) before UCS measurements and (b) after UCS measurements.

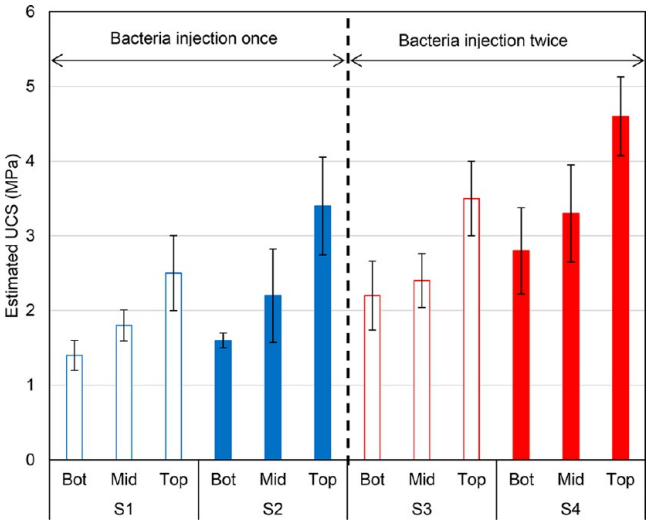

Figure 10. Estimated UCS values of solidified sand specimens. Error bars show the standard deviation of the values in three independent experiments. S1 and S3 (open) without addition of poly-Lys; S2 and S4 (closed) with addition of poly-Lys.

One interesting point is that the strength of the sand column decreased from top to bottom. This may be the result of high urease activity near the inlet, caused by accumulation of bacteria near the injection point. *Pararhodobacter* sp. are aerobic bacteria; therefore, the oxygen concentration plays a significant role in the bacterial activity. Lack of oxygen in the bottom of the specimen would inhibit the bacterial activity, and the strength of the specimen would therefore decrease from top to bottom.[4] It is important to achieve an even distribution of bacterial activity throughout the sand column to obtain a homogeneous sample. Controling the rate of $CaCO_3$ precipitation is also important. The injection rate of the cementation fluid plays a significant role in achieving a uniform distribution of bacteria through the sand column. At a low injection rate, immediate cementation can occur near the injection point. If the flow rate increases beyond the rate of urea consumption, and precipitation will lead to a uniform distribution of chemicals along the pathway.[8] However, this area has not yet been well studied, and it is difficult to give an exact explanation; therefore, further investigation is needed.

In this work, sand solidification was strengthened by reinjection of bacteria after 7 days. Reinjection of bacteria after 7 days effectively maintained a high urea hydrolysis rate, which led to higher $CaCO_3$ precipitation and solidified sand with high UCS values. A number of other factors can cause a decrease in urease activity over long time periods, e.g., encapsulation of bacteria as a result of precipitation or being trapped inside pores, a reduction in chemical transport (nutrients) through pore spaces because of precipitation, and space limitations caused by saturation of pore fluids and accumulation of metabolic wastes.[27,29,33] Bacterial cell concentration also significantly affected the strength of the

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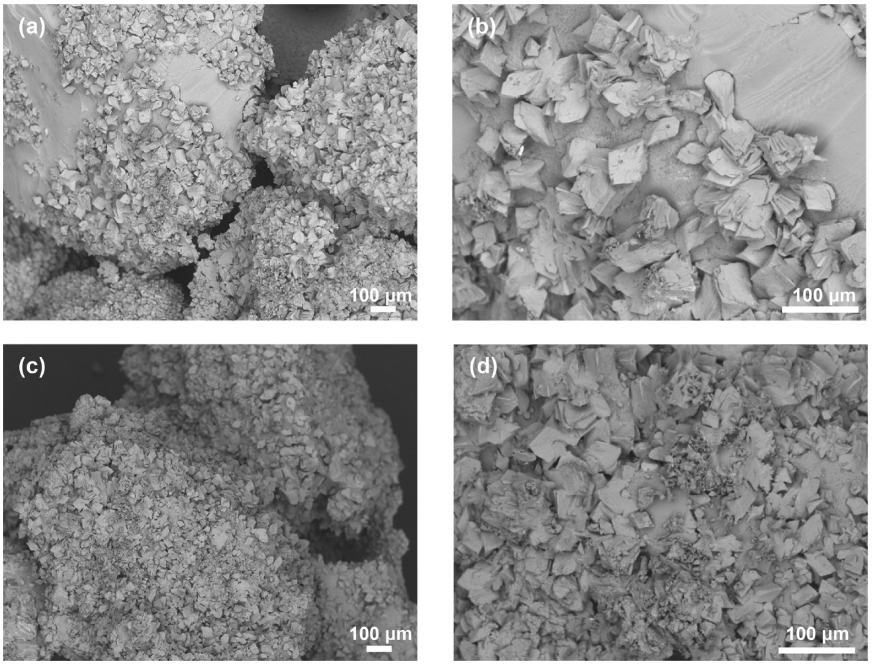

Figure 11. SEM images of cemented sand samples: without poly-Lys (a, b) and with poly-Lys (c, d).

sample. As shown in the Figure 3, a larger number of small crystals produced by higher cell concentration can create a favorable bridge between sand particles by filling the pore spaces more efficiently than a small amount of larger crystals which were produced by lower cell concentration.

The most important finding of this research is the positive effect of poly-Lys on sand solidification compared with the conventional method. Poly-Lys addition gave strongly cemented samples with better mechanical strength. The cell adhesion properties of poly-Lys could be one of the reasons for its positive effect on sand solidification. In sand solidification, the pore spaces between the sand particles should be filled effectively to achieve good strength. Bridge formation between sand grains is also important for preventing movement of sand grains and improving the strength and stiffness of the specimen.[34] SiO_2 is the main constituent of sand, and it has a negative charge because of dissociated silanol groups ($-SiO^-$). Most bacteria have negatively charged cell surfaces; therefore, there is a repulsive force between sand particles and bacterial cells. Positively charged poly-Lys could act as a binder and attach bacterial cells to sand particles, which would promote efficient cementation. The ionic strength of the solution and the salinity of the bacterial suspension also affect adsorption of bacteria on sand particles. The collision efficiency increases with increasing ionic strength of the solution, and adsorption of bacteria can be increased by increasing the salinity of the bacterial suspension.[34−36] The addition of poly-Lys helps to increase the ionic strength of the solution and improves adsorption of bacteria on sand particles; therefore, better cementation is achieved.

And also, as shown in Figures 3 and 6, ellipsoidal shaped crystals formed in the presence of the poly-Lys could fill the pore spaces more efficiently and hence better cementation could be achieved.

The SEM images in Figure 11 show that, in the absence of poly-Lys, cementation mainly occurs at the contact points between sand particles. This is the result of low adsorption of bacteria on the sand surface. The carbonate crystals formed in the absence of poly-Lys all have similar sizes and shapes, as shown in Figure 11b. Figure 11c and d shows SEM images of sand specimens solidified in the presence of poly-Lys. The sand particles are fully covered with carbonate, and strong bonds are formed as bridges between sand particles. These SEM images indicate that poly-Lys acts as a binder and facilitates bacterial cell adsorption on the sand particles. The irregular shapes and sizes of the crystals also assist good cementation.

According to the experimental results, the addition of poly-Lys was found to improve the efficiency of the MICP process. Therefore, naturally available cationic polypeptides/polysaccharides such as lysozyme and chitosan can be used for real field applications to improve the efficiency, more sustainably, in place of poly-Lys, supporting the concept of green engineering.

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Notes

The authors declare no competing financial interest.

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