International Journal of GEOMATE, May, 2025 Vol.28, Issue 129, pp.55-65
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2025.129.4855
Geotechnique, Construction Materials and Environment

EVALUATING BIO-CEMENTATION INJECTION TECHNIQUES FOR SILICA SAND: DURATION, DISTRIBUTION, AND STRENGTH ENHANCEMENT

*Rangga Adiprima Sudisman[1], Ia.Mustika Ningrum[2], Suharti Sastroredjo[3],
Arlyn Aristo Cikmit[2], Aulia Afifatuz Zulfah[2], and Sifra Dovanka Trista Wewengkang[2]

[1]Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia;
[2]Civil Engineering Study Program, Faculty of Infrastructure Planning, Universitas Pertamina, Indonesia;
[3]Chemistry Study Program, Faculty of Science and Computer, Universitas Pertamina, Indonesia

*Corresponding Author, Received: 18 Dec. 2024, Revised: 31 Jan. 2025, Accepted: 12 Feb. 2025

ABSTRACT: Challenges frequently encountered in research on loose sand improvements through the use of microbial-induced calcite precipitation (MICP) include the non-uniformity of cemented products and blocking in the vicinity of the injection pipe. One of the most important factors influencing MICP's success is the injection method. In this study, three bio-cementation injection methods using *Sporosarcina pasteurii* bacteria were compared on silica sand samples in the laboratory. The aim was to assess the performance of each injection method in terms of time efficiency, cementation product distribution, and comparison in soil shear strength before and after cementation. The employed injection techniques encompassed direct mixing (DM) injection, two-phase (TP) injection, and modified stage (MS) injection. The findings indicated that none of the three injection methods yielded ideal outcomes in all aspects. The MS injection produced the most cemented volume by 57.5%. However, no strength increases were observed, as evidenced by the hybrid sample's internal friction angle (ϕ) of 31.72°, which was similar to that of uncemented sand. Conversely, the DM injection produced the largest improvement in shear strength with a 46.416° ϕ value, although cementation was limited to the upper half of the sample by 52.4% volume.

Keywords: Bio-cementation, Injection method, Microbial-induced calcite precipitation, Shear strength, Soil improvement

1. INTRODUCTION

Loose sand is made up of small particles that are loosely bound together and not well compacted, so there are a lot of voids between the grains. Due to the voids, it has high permeability, allowing water to flow through easily. The shear strength of loose sand comes mainly from the friction between particles, and the internal friction angle is lower than that of more compacted sands. Unlike clays or silts, the particles do not have cohesion, which makes loose sand unstable and easy to collapse under pressure. Moreover, in saturated conditions, loose sand is more prone to landslides and liquefaction under seismic loads. Therefore, efforts are required to improve soil conditions so that the loose soil becomes denser. One of the novel soil improvement techniques that can be done is to conduct a cementation process using Microbial Induced Calcite Precipitation (MICP). This process not only enhances the soil mechanical properties but also provides a sustainable and environmentally friendly solution to soil stabilization compared to conventional grouting. MICP is a soil improvement approach that makes use of bacteria's urease hydrolysis capacity to precipitate calcium carbonate ($CaCO_3$), which then binds soil grains together to strengthen the soil structure [1].

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2)$$

Eq. (1) depicts the urease hydrolysis process facilitated by bacteria. When urea ($CO(NH_2)_2$) combines with water, it emits carbonate ions (CO_3^{2-}) and ammonium (NH^{4+}) into the environment, raising the pH to an alkaline level. When calcium ions (Ca^{2+}) are present, they react with carbonate ions to produce calcium carbonate, as depicted by Eq. (2). Such conditions are favorable for calcium carbonate precipitation [2]. Therefore, the addition of a Ca^{2+} ion source, such as calcium chloride ($CaCl_2$), into the soil is required to provide bacteria with Ca^{2+} nourishment for calcium carbonate to be formed. Bacteria capable of hydrolyzing urease are typically found in the genera Bacillus, Sporosarcina, Sporolactobacillus, Clostridium, and Desulfotomaculum [3], while *Sporosarcina pasteurii* is the most widely used bacterium and is quite effective in the $CaCO_3$ precipitation process in studies related to MICP [4-5]. Several factors influence the efficacy of the MICP approach, including the type of bacteria, bacterial concentration, bacterial size and shape, soil particle size, nutrition, chemical solution, pH level, temperature, and injection method or cementation protocol [6].

One of the most common issues encountered when using MICP is non-uniformity in cementation results, which is heavily influenced by the injection method [6-7]. Single-phase injection, such as direct mixing injection [8], is prone to causing clogging near the injection pipe. This is because when the bacterial solution is mixed with cementation solution, immediate bio-flocculation occurs, and the holes around the injection pipe get blocked or clogged due to cementation. These clogs hamper bacterial movement in the subsurface [9-10]. Since the areas that can be cemented are areas that can be reached by bacteria, clogging reduces the volume of cemented soil to below the target, and soil strength does not improve considerably. Lim et al. found a similar difficulty in a laboratory-scale study, where the percentage of cemented soil reached only 36.1% of the total sample volume [11]. On the other hand, sequentially injecting the bacterial solution followed by the cementation solution, such as two-phase injection [12] and stage injection [13], increases the possibility that the bacteria will be washed away during the cementation injection solution, resulting in non-uniform bio-cementation. Therefore, further studies regarding the effectiveness of the MICP injection method are still required.

In this study, the authors conducted a laboratory-scale investigation to compare three injection methods: direct mixing, two-phase injection, and modified-stage injection using *Sporosarcina pasteurii* bacteria on silica sand samples. The aim of this study is to compare the effectiveness of each injection method in terms of the required time it takes for the sample to be cemented, the fraction of cemented soil, and the shear strength of the soil before and after cementation.

2. RESEARCH SIGNIFICANCE

This research addresses the critical issue of soil instability in loose sands, which are prone to landslides and liquefaction, especially under seismic loads. By exploring the effectiveness of different injection methods for MICP, the study aims to enhance soil strength and stability through bio-cementation. At present, the cost of MICP technology for soil improvement is still very high, thus hampering its application on a larger scale [7]. Discussion on the economic aspect of MICP is beyond the scope of this study; nonetheless, identifying a more effective injection method indirectly enhances the effectiveness of the injection process, hence influencing cost efficiency. The findings could lead to more reliable and efficient soil improvement techniques, reducing the risks associated with loose sand in construction and infrastructure projects. Additionally, understanding the optimal conditions for MICP can contribute to the development of sustainable and environmentally friendly soil stabilization methods, leveraging natural processes to achieve engineering goals.

3. MATERIAL AND METHOD

3.1 Sand Sample Preparation

This experimental investigation was conducted on silica sand. The sand sample preparation process involves sterilizing the sand and testing its physical properties. Sand sterilization was carried out by drying the sand at 110°C for 24 hours to remove native microorganisms that exist in the sand so that they would not affect the *Sporosarcina pasteurii* activities. Meanwhile, physical properties tests of sand were conducted based on the Indonesian National Standard (SNI), including sieve analysis (SNI 3423:2008), soil-specific gravity tests (SNI 1970-2008), and soil unit weight tests (SNI 03-3637-1994). The specific gravity (Gs) of sand is 2.688, while the dry unit weight (γ_d) is 15.958 kN/m^3. In accordance with ASTM D2334-19, the hydraulic conductivity of the sample acquired from the constant head test is 2.02 x 10^{-2} cm/s for the loose sample and 1.72 x 10^{-3} cm/s for the denser conditions. This value is within the range of 1.0 to 10^{-3} cm/s for typical well-graded or poorly-graded clean sands (SW/SP) with medium permeability [14].

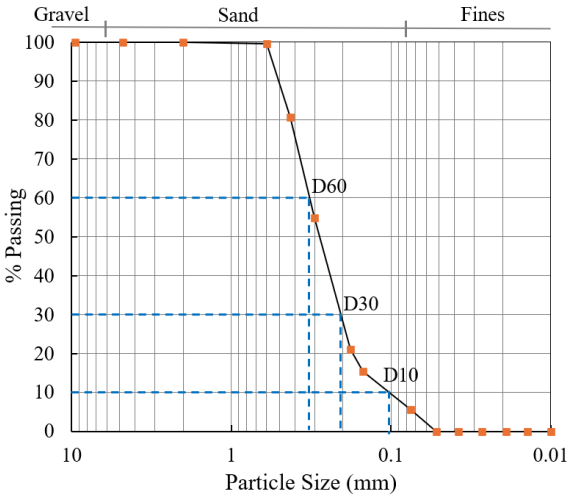
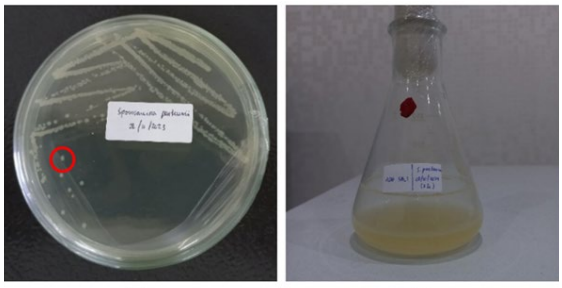


Fig. 1 Particle size distribution of the sand sample



(a) (b)

Fig. 2 Cultured bacteria at (a) Nutrient Agar and (b) urea-$CaCl_2$ solution

Fig. 1 shows the particle size distribution of the sand. It reveals that the most dominant grain size ranges from 0.180 to 0.425 mm, which is adequate for the MICP approach based on the previous report, which found that soil with a gradation of 0.05-0.4 mm is suitable for the MICP application [15]. The sample consists of 0% gravel, 94.34% sand, and 5.66% fines. The uniformity coefficient (Cu) and gradation coefficient (Cc) were also obtained from the graph, which are 3.00 and 1.27, respectively. Based on the Unified Soil Classification System (USCS), the sand was classified as poorly graded sand (SP).

3.2 Bacterial Culture and Cementation Reagents Preparation

Bacterial culture is the process of multiplying bacterial cells in new media under controlled laboratory conditions to produce a single colony. Bacteria were cultured from the *Sporosarcina pasteurii* isolate obtained from the National Research and Innovation Agency of the Republic Indonesia (BRIN). *Sporosarcina pasteurii* is preferred because it has high urease activity, the capability to thrive at pH levels exceeding 8.5 [16], and considerable resistance to ammonia effects [17].

The media used in this study were solid nutrient agar (NA) and liquid nutrient broth (NB), which were then gradually transferred to urea-$CaCl_2$ media. On solid media, the bacterial culture process was carried out using the quadrant streak method as shown by Fig. 2(a). Bacteria were cultured inside an incubator for 18-24 hours at a temperature of 30°C. For liquid media, bacteria were cultivated inside a shaker incubator with a stirring speed of 150 rpm. Fig. 2(b) depicts bacterial culture on urea-$CaCl_2$ media, which becomes turbid. Subsequently, the urea-$CaCl_2$ solution containing bacteria was then measured for its optical density (OD). The bacterial solution used in this study had optical density values in the range of 1.180-1.210.

A cementation reagent is a solution that serves as a liquid medium for bacterial growth and nourishment. The reagent was injected into the sample at regular intervals throughout the cementation process. Providing this cementation reagent is beneficial so that the bacteria introduced into the sand sample can survive and carry out the cementation process effectively. Every 20 mL of this solution consisted of 0.5 M urea-$CaCl_2$, 3 g of nutrient broth, 10 g of NH_4Cl, and 2.12 g of $NaHCO_3$. The interval for cementation regent injection in this experiment was 20 mL for each 12-hours cycle [18]. This solution was priorly filtered with a vacuum pump to remove any contaminants.

3.3 Injection Process

Fig. 3 depicts the sample configuration, which contains sand in a 120-mL syringe and a perforated injection pipe located in the center. The injection pipe was a modified IV tube with a 4 mm outside diameter that had been perforated on each side in four columns of 35 rows, while the end of the IV tube was closed. The bacterial solution and cementation reagent were injected into the sand via the injection pipe until all voids were filled (saturated state). A 0.45µm sterile microfilter was attached at one end of the IV tube which functions to filter bacteria and sand so that they are not carried away by the cementation reagent flow when the effluent was released via the bottom outlet. Every 12-hour cycle, pH was checked on the remaining effluent solution exiting the syringe outlet. The aim was to detect the existence of urease activity in the sample, indicated by an increase in pH to alkaline and a strong odor. The solution was injected into loose sand using three different injection methods: direct mixing (DM), two-phase injection (TP), and modified-stage injection (MS). The solution in the DM and TP injection procedures remained non-circulatory, but the solution in the MS injection method was circulated.

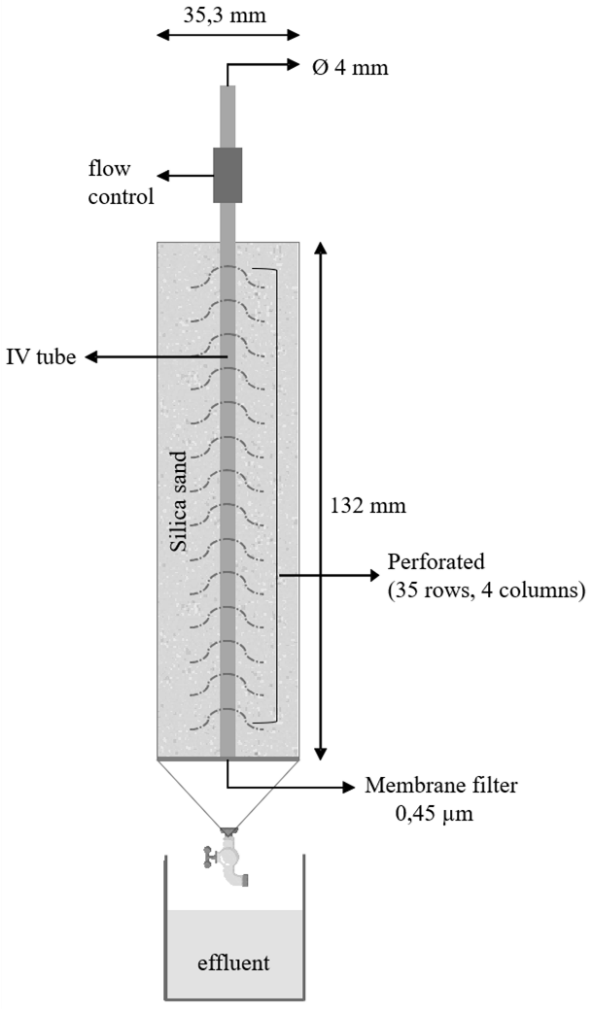


Fig. 3 Sample configuration

The direct mixing method involved first mixing the bacterial solution and cementation reagent before injecting them into the sand and leaving them for 12 hours. Next, the cementation reagent was injected every 12 hours until the soil was cemented. Sand was considered to have undergone cementation when blocking occurred and injection pressure became too heavy. The direct mixing process is illustrated in Fig. 4 (a). The two-phase injection method involved injecting the bacterial solution at the initial procedure and leaving it for 12 hours. Following that, the cementation reagent was administered every 12 hours (Fig. 4(b)). The third injection method, the modified stage injection, was a modification of the method proposed by Tobler et al. [13]. This method was carried out by (i) continuously circulating the bacterial solution with a peristaltic pump for 2 hours, (ii) leaving it for 12 hours, then (iii) injecting the cementation reagent and leaving it for the next 12 hours. The cycle was repeated until the sand was observed to be cemented (Fig. 4(c)).

In this experiment, the injection of the sample was divided into three batches, where sample group 1 (DM-1, TP-1) was given the injection cycles first, followed by sample group 2 (DM-2, TP-2) eight days later, and lastly sample group 3 (MS-1 and MS-2) four days later. These batch differences may result in varying environmental conditions, such as temperature difference, for the bacteria.

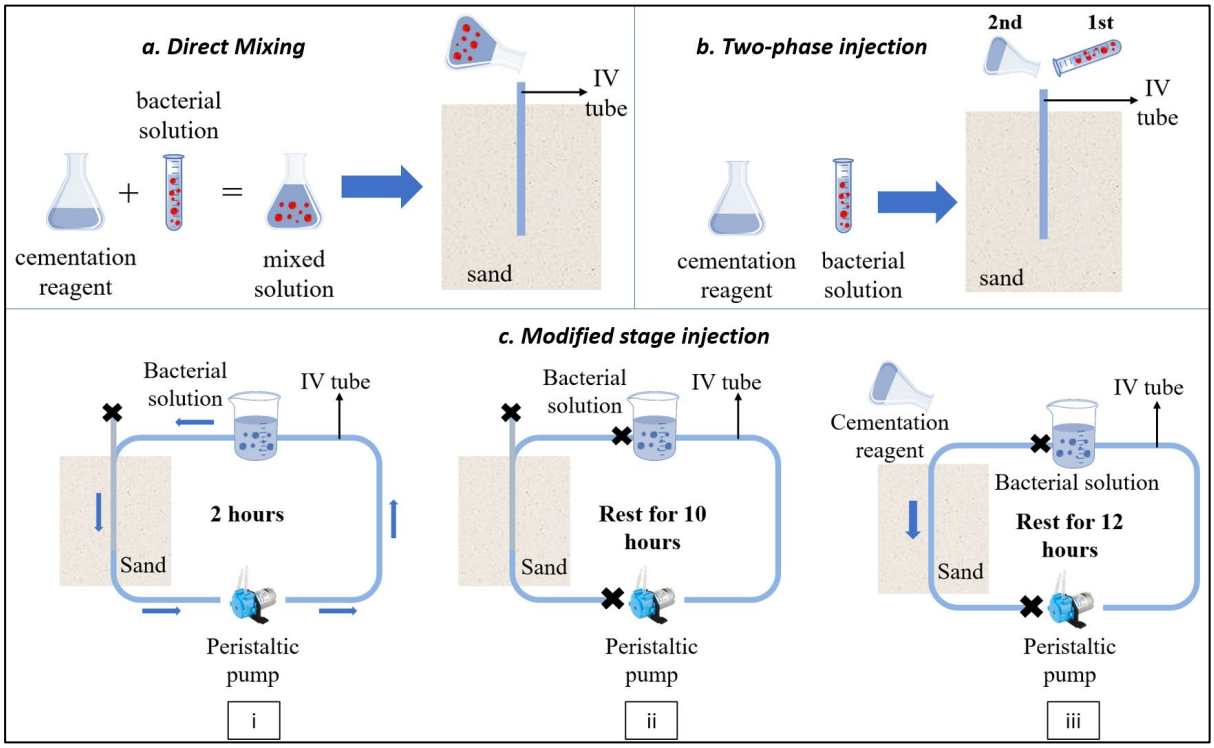


Fig. 4 Injection method: (a) Direct mixing (DM); (b) Two-phase injection (TP); (c) Modified stage injection (MS)

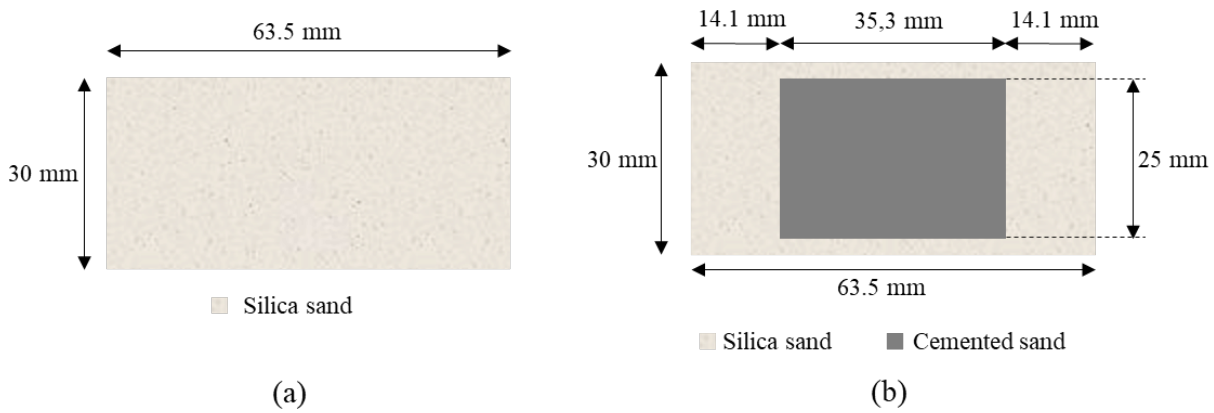


Fig. 5 Illustration of direct shear sample tests: (a) loose sample; (b) hybrid sample

Table 1. Required injection time

Sample	Time to achieve pH 9 (hours)	Time to stop injection (hours)	Date of Injection	
			Start	End
DM-1	108	600 (no blocking)	11 Jan 2024	5 Feb 2024
DM-2	36	600 (no blocking)	19 Jan 2024	13 Feb 2024
TP-1	144	600 (no blocking)	11 Jan 2024	5 Feb 2024
TP-2	48	600 (no blocking)	19 Jan 2024	13 Feb 2024
MS-1	132	600 (no blocking)	23 Jan 2024	17 Feb 2024
MS-2	84	384	23 Jan 2024	9 Feb 2024

3.4 Analysis of $CaCO_3$ Concentration

The concentration of $CaCO_3$ was quantified to ascertain the extent of $CaCO_3$ crystal development within the sand's pores. Multiple techniques exist for quantifying $CaCO_3$ content, including thermo-gravimetric analysis (TGA), which yields reliable results comparable to those obtained from X-ray diffraction and the ASTM method [19]. The TGA method determined the $CaCO_3$ content by assessing the weight loss of a cemented sand sample subjected to high-temperature combustion up to 1000 °C, utilizing the SDT 650 and TGA instrument Trios V5.0.0.44616. The initial weight (W_{init}), weight at weight loss started (W_{start}), and weight at weight loss ended (W_{end}) were determined, respectively. The $CaCO_3$ content was calculated using Eq. (3), in which 2.274 represents the constant ratio of the atomic weight of $CaCO_3$ to CO_2 resulting from the combustion process. Further, the $CaCO_3$ content in the sample can be correlated with its shear strength.

$$CaCO_3\% = \frac{W_{start}-W_{end}}{W_{init}\times 2.274} = \frac{\Delta W_{loss}}{W_{init}\times 2.274} \quad (3)$$

3.5 Direct Shear Test

The shear strength was evaluated in accordance with ASTM D3080-04 Direct Shear Test, both before and after cementation, under dry unsaturated conditions. Fig. 5(a) illustrates the sample condition of loose silica prior to cementation. Due to the limited size of the cemented sample, a hybrid sample (consisting of cemented sand sample in middle and surrounded by loose silica) was evaluated to determine the shear strength of the hardened sample, which consists of a cemented sample in the middle of the mold surrounded by loose silica (Fig. 5(b)). This scenario did not yield the actual strength of the cemented sample, but it demonstrated how strength increases in soil reinforced by cemented sand columns. The soil strength parameters were determined from Eq. (4) by setting the cohesion value of sandy soil to zero.

$$\tau = c + \sigma \tan \emptyset \quad (4)$$

4. RESULT AND DISCUSSION

4.1 Effluent pH Control (Cementation Process)

According to Kim et al. [20], the optimal pH for *Sporosarcina pasteurii* bacteria to perform urease activity was in the range of 8.7-9.5. The initial pH of the sample before injection was relatively neutral (pH 7). The pH increased following the injection, showing that urease activity occurred in each sample. The length of time required for the effluent from each sample to reach pH 9 is shown in Table 1.

Injection was performed at different periods: in batch one, on DM-1 and TP-1; in batch two, on DM-2 and TP-2; and in batch three, on MS samples. Since the room temperature could not be kept constant and was influenced by the ambient temperature, the difference in injection period allowed for different experimental temperature settings. As a result, each sample had a varied duration to attain alkaline conditions. DM-1, TP-1, and MS-1 samples required longer durations than DM-2, TP-2, and MS-2 samples to achieve pH 9. These findings are likely to be influenced by a variety of factors, including changes in bacterial quality and uncontrolled fluctuations in experimental temperature, which result in differences in the pace of bacteria carrying out the urease process. Moreover, the initial OD value of the bacterial solution for each sample slightly varied. However, after the pH value in all samples' effluent reached 9, it remained steady until the injection was terminated.

4.2 Proportion of Cemented Sample

In this study, the injection cycle was intended to be carried out continuously until the injection pipe became blocked. As the injection process progresses, $CaCO_3$ is progressively generated within the pores, resulting in a decreasing capacity of the sample to facilitate the flow of the solution [21], hence increasing the pressure required for syringe injection. The time required for the sample to be blocked during injection also varies as shown in Table 1. In the DM, TP, and MS-1 samples, blocking only occurred at the top part of the sample. As a result, the sample will continue to allow the injected cementation reagent to seep for an extended period of time. In fact, previous

research found that bacterial urease activity decreases after 16 to 32 days [15]. Therefore, in this study, the injection duration was limited to 25 days (600 hours), despite the fact that no blockage or only partial blockage occurred. Meanwhile, the MS-2 samples had the shortest injection duration; the injection pipe was clogged after 384 hours. This is assumed to be owing to the repeated bacterial injection process every 24 hours, which circulated for two hours, resulting in a higher quantity of bacteria inside the MS-2 sample than other samples. Therefore, the more bacteria in the silica sand, the faster $CaCO_3$ formation occurs. Despite receiving the same treatment in the same batch, MS-1 and MS-2 have different injection durations. This result may be caused by variables other than the environment and injection method that affect the quality of bacteria. One of the hypothesized factors is the effect of bacterial density in the solution with a slightly different initial OD [9, 22]. Changes in the sample's density may also have an effect on the permeability [23]. In this case, the density of the sample was measured solely by the weight of the soil volume that filled the syringe in a loose condition. However, the density can change due to impact or vibration to the sample, as well as inconsistency of injection pressure. These factors are challenges to be clarified in further research.

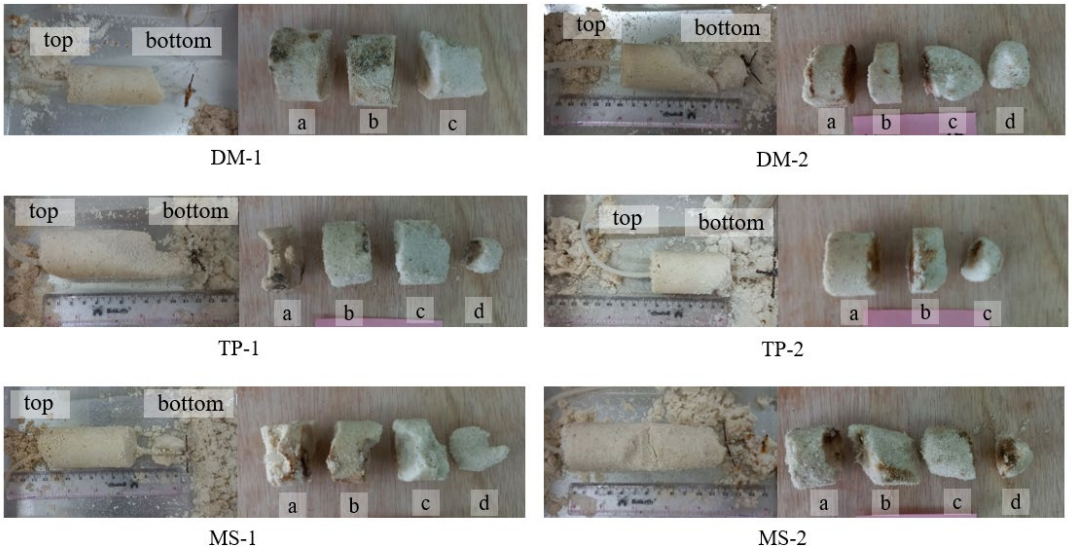


Fig.6 Results of cementation with injection method variations: Direct Mixing (DM), Two Phase (TP), and Modified Stage (MS)

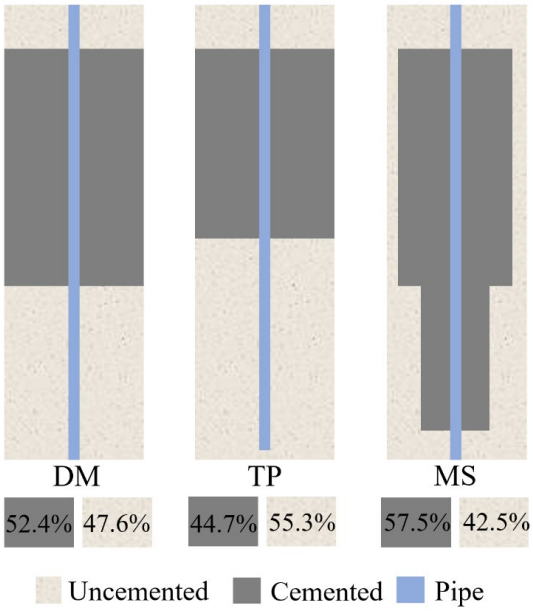
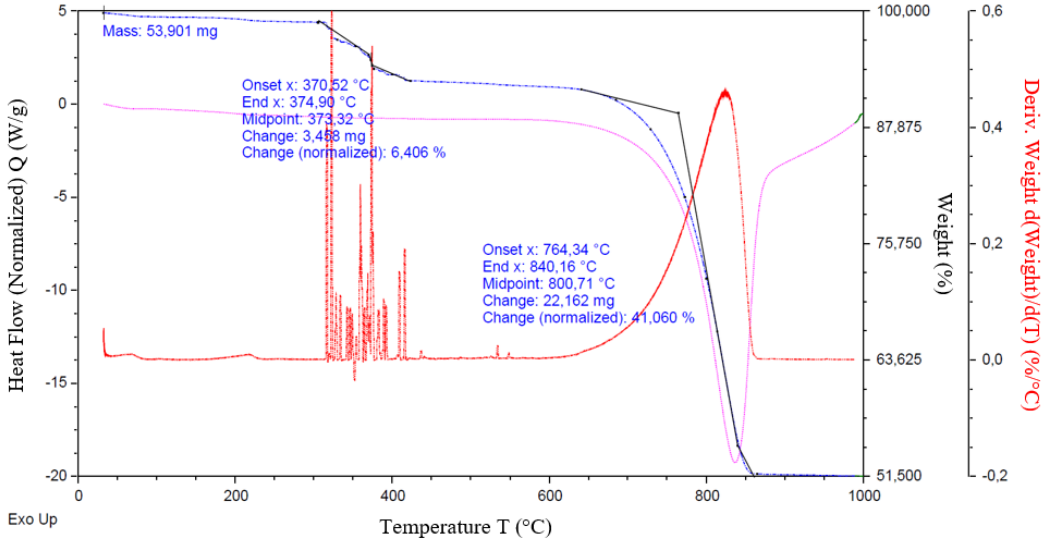


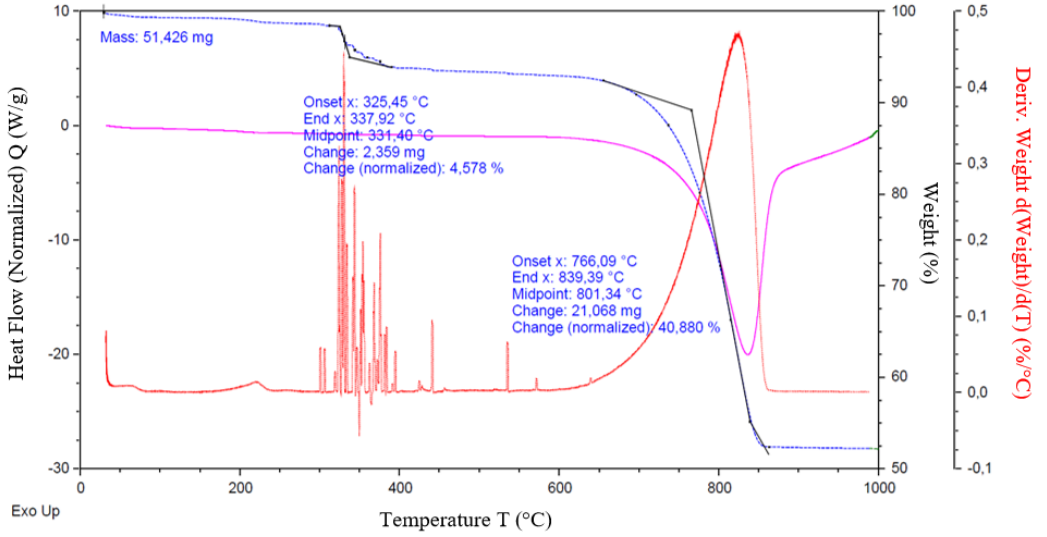
Fig.7 Combined and simplified illustration of cementation result

Fig. 6 presents the documentation of the cemented sample, which is further combined and simplified into an image as shown in Fig. 7. Approximately 1 cm of the uppermost part of all the samples did not undergo cementation. The absence of radial holes at the upper part of the injection pipe results in the minimum accumulation of bacterial solution and cementation reagents at the top. The majority of cementation occurs at a depth of 1 cm to approximately half the length of the syringe. For the non-circulatory injection samples (DM and TP), the cementation results at the half top were as wide as the syringe diameter, but the bottom part of the sample remained loose and uncemented. This condition is thought to be due to the shortage of oxygen at the bottom of the syringe, which prevents the facultative anaerobic *Sporosarcina pasteurii* bacteria from producing urease optimally under anaerobic conditions [24]. As a result, the $CaCO_3$ precipitation process slows as depth increases [25].

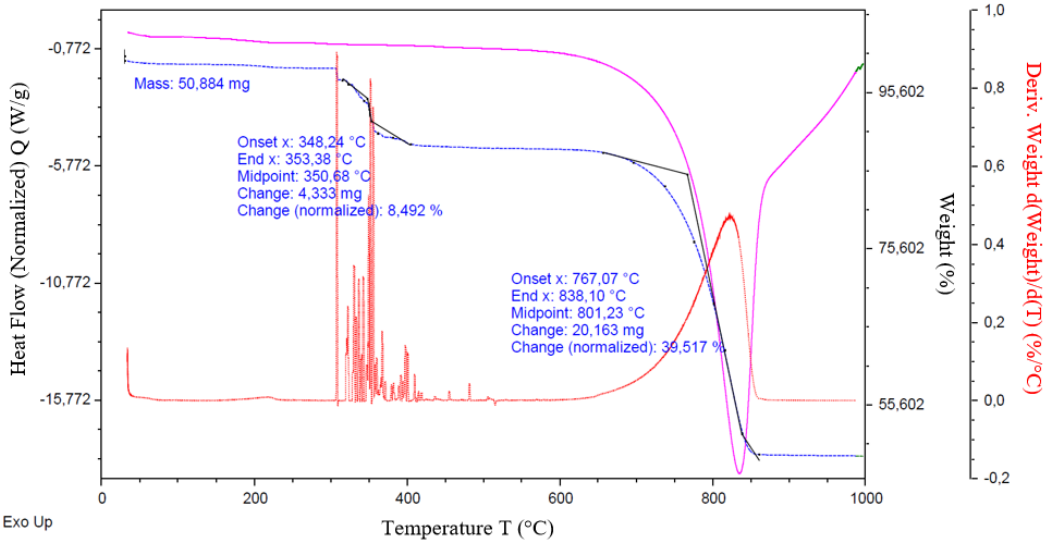
.



(a)



(b)



(c)

Fig.8 Thermogravimetric analysis results; (a) DM sample, (b) TP sample, (c) MS sample

In contrast to the results of the non-circulatory injection, the MS sample had a longer cemented section with a smaller diameter. Moreover, the diameter of the cemented parts tends to reduce as the depth increases. The MS samples exhibit a broader cementation range, resulting in a higher figure for the fraction of cemented sand derived from volume measurements compared to the TP and DM samples. The MS sample is cemented at 57.5%, followed by the DM sample at 52.4%, and the TP sample has the smallest cementation proportion of 44.7%. This finding is assumed to be the result of two hypotheses. First, a substantially larger number of bacteria circulated into the MS sample. The increased number of bacteria enables a more even distribution of bacteria to reach the bottom of the syringe. Another suggestion is that air was inadvertently introduced into the sample while circulating the bacterial solution; nevertheless, air can enter the syringe because the pump mechanism utilized to circulate is open flow. Several investigations have found that the microbial growth rate and ureolytic activities of *Sporosarcina pasteurii* are influenced by aerobic or anaerobic conditions, which are determined by the availability of oxygen [24, 26, 27]. To clarify this suggestion, further research requires comparative analysis of samples that were injected with additional air and those that were not

4.3 $CaCO_3$ Concentration Analysis

Fig. 8 depicts the TGA results of the cemented samples. In all three samples, there were two significant slope declines that indicated weight loss, particularly in the temperature range of 300-400 °C and 750-840 °C. The $CaCO_3$ concentration was determined by analyzing weight loss within the temperature range of around 750-840 °C [28], while the slope observed between 300-400 °C suggested water evaporation, organic material degradation, or the presence of hydroxides. The results indicated that the $CaCO_3$ concentrations in the DM and TP samples were comparable at 18.06% and 18.02%, respectively. However, the MS sample exhibited a reduced $CaCO_3$ concentration of 17.44%. The solution circulation mechanism in the MS sample enables bacteria to reach deeper regions and stay active, yet it lacks sufficient time for $CaCO_3$ accumulation due to the continuous disturbance caused by the flow. The amount of $CaCO_3$ may indicate the sample's density, which may subsequently influence its strength. Higher $CaCO_3$ content implies reduced pore space between sand grains and enhanced binding among them [29].

4.4 Shear Strength of Sand and Hybrid Sample

Table 2 compiles the shear strength of loose silica and hybrid samples (cemented sand column surrounded by loose sand) for each injection method. Due to the limited number of cemented samples, hybrid samples were only tested at two normal stress variations. Furthermore, the high shear strength of the cemented samples limited the direct shear apparatus' ability to perform tests at higher normal stress values. The direct shear stress results from samples DM-hybrid-2 and TP-hybrid-2, presented in Fig. 9, depict (a) shear stress (τ) against horizontal displacement and (b) vertical strain against horizontal displacement. The recorded data exhibits a consistent pattern with other samples tested under similar normal stresses. All samples demonstrated a contraction during the test, with no observed dilation. The shear stress did not exhibit a distinct peak value. The author attributes the absence of a visible peak shear stress to the relatively low normal stress applied during the direct shear stress. This suggests that, compared with the strength of cemented samples, the normal stress was still too low to accurately model the Mohr-Coulomb failure criterion for the samples. Therefore, further research is recommended using more samples subjected to higher normal stress to better understand and validate the Mohr-Coulomb criteria.

The cemented column positioned in the center of the DM-hybrid and TP-hybrid samples has a targeted diameter of 35.3 mm, which accounts for 29% of the overall sample volume. The direct shear test results, presented in Fig. 10, indicated a notable enhancement in the shear strength of the DM-hybrid and TP-hybrid specimens in comparison to the shear strength of uncemented silica. Under uncemented conditions, loose silica exhibits an internal friction angle of 31.94°, which is considered rather acceptable for loose fine sand. The DM-hybrid and TP-hybrid samples exhibited an increased shear strength, as evidenced by the generation of high internal friction angles, specifically 46.42° and 45.09°, which is considered very dense sand [14]. This result corresponds with previous studies suggesting that soil strength parameter improved in fine sand [21, 30] and sand with fines [31] treated with MICP, as well as clay and silt [32-34], which are correlated with the existence of $CaCO_3$ between soil pores.

Table 2. Shear strengths from direct shear test

Normal Stress (kPa)	Average Shear Stress (kPa)			
	Loose Silica	DM-hybrid	TP-hybrid	MS-hybrid
31.592	17.149	33,719	32,163	23,481
63.185	36.234	66,125	63,163	33,442
126.370	81.007	N/A	N/A	N/A
ϕ (degree)	31.944	46.416	45.094	31.724

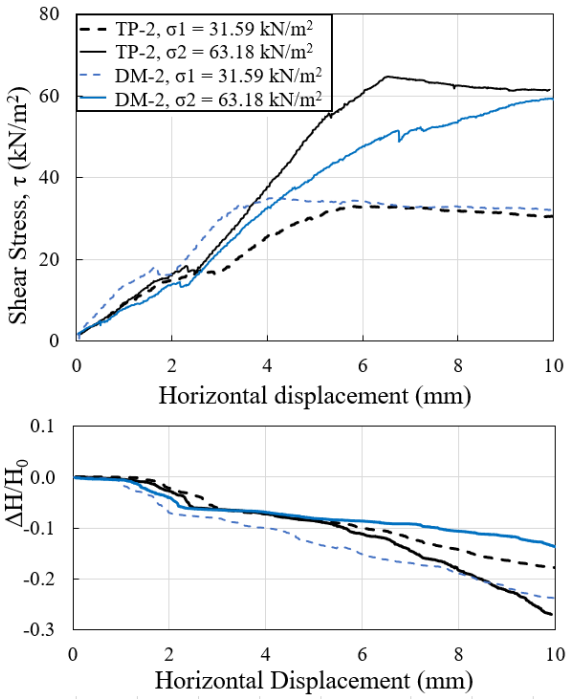


Fig. 9 Direct shear stress result: (top) shear stress to horizontal displacement, (bottom) vertical strain to horizontal displacement

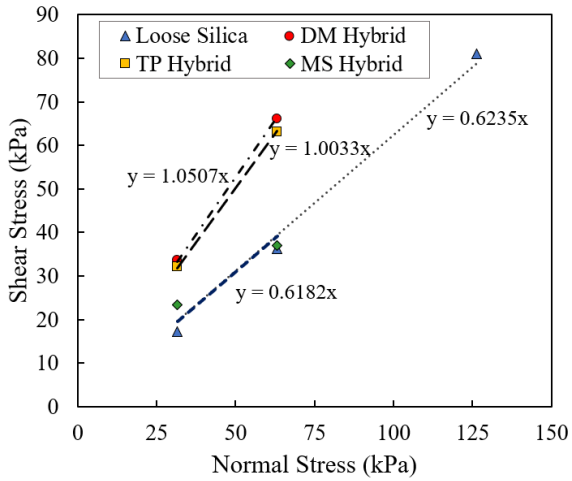


Fig. 10 Normal-shear stress relationship based on Mohr-Coulomb failure criteria

The cemented sample size in the MS-hybrid sample was less than the intended size, accounting for only approximately 16-28 mm (6-18% of the total sample volume). Concurrently, the MS-hybrid sample did not exhibit any strength increase in comparison to the shear strength of uncemented silica, and in fact, it was slightly weaker with an internal friction angle of 31.72°. This result occurred because the cemented sample's reduced diameter did not contribute additional strength to the hybrid sample system. The volume of the cemented column in the MS-hybrid sample accounted for just 6-18% of the total sample volume in the direct shear test. In addition, this may also be caused by weak cementation bonds, as evidenced by the $CaCO_3$ concentration analysis, which indicated that the MS sample exhibited the lowest concentration relative to the DM and TP samples.

5. CONCLUSION

This study demonstrates that none of the three injection methods implemented may generate an ideal cementation product that satisfies all aspects considered. The required time for cementation should not be determined solely based on the injection method, as it appears to be substantially influenced by uncontrollable fluctuations in ambient temperature.

Non-circulatory injections (DM and TP) may generate the maximum diameter of the cemented column sample; however, cementation occurs only in the top half of the sample, leaving the bottom uncemented. Moreover, the DM and TP methods create higher $CaCO_3$ concentrations of 18.06% and 18.02%, respectively, compared to the 17.44% $CaCO_3$ in the circulating injection (MS). The combination of greater diameter and higher $CaCO_3$ content contributes significantly to the hybrid sample's shear strength. The cemented column strengthens the hybrid sample, increasing the internal friction angle to 46.42° for the DM-hybrid sample and 45.09° for the TP-hybrid sample, which is significantly higher than the unreinforced sand of 31.94°.

In contrast, circulating injection (MS) results in a larger volume of cemented sample spread along its length but with a smaller diameter. Additionally, circulation of the solution inhibits the accumulation of $CaCO_3$. The smaller diameter of the cementation column and its lower $CaCO_3$ concentration did not affect the hybrid sample's improvement in shear strength, as determined by the internal friction angle, which was nearly identical to the unreinforced condition at 31.7°.

To clarify the factors diminishing the effectiveness of the MICP injection process, further research is warranted. This research should consider additional variables influencing $CaCO_3$ accumulation, including environmental temperature regulation, injection-circulation pressure control, and the control of oxygen availability within the sample. Furthermore, increasing the sample size will facilitate the identification of mechanisms that may arise under more realistic field conditions.

6. ACKNOWLEDGMENTS

The Directorate of Research, Technology, and Community Service, Ministry of Education, Culture, Research, and Technology of the Republic of

Indonesia, financed this research project in 2024, and it was supported by Institut Teknologi Bandung's P2MI-FTSL 2025 initiative.

7. REFERENCES

[1] Fujita, Y., Ferris, F. G., Lawson, R. D., Colwell, F. S., and Smith, R. W. Calcium Carbonate Precipitation by Ureolytic Subsurface Bacteria, Geomicrobiol J, Vol. 17, No. 4, 2000, pp. 305–318.

[2] Soon, N. W., Lee, L. M., Khun, T. C., and Ling, H. S. Improvements in Engineering Properties of Soils through Microbial-induced Calcite Precipitation, KSCE Journal of Civil Engineering, Vol. 17, No. 4, 2013, pp. 718–728.

[3] Kucharski, E. S., Ruwisch, R. C., Whiffin, V., and Al-thawadi, S. M. J. Microbial Biocementation, US Patent US8182604B2.

[4] Siddique, R. and Chahal, N. K. Effect of Ureolytic Bacteria on Concrete Properties, Const Build Mat, Vol. 25, No. 10, 2011, pp. 3791-3801,

[5] Chuo, S. C., Mohamed, S. F., Setapar, S. H. M., Ahmad, A., Jawaid, M., Wani, W. A., Yaqoob, Y. A., and Ibrahim, M. N. M. Insights Into the Current Trends in the Utilization of Bacteria for Microbially Induced Calcium Carbonate Precipitation, Materials, Vol. 13, No. 21, 2020, 4993.

[6] Erdmann N. and Strieth D. Influencing Factors on Ureolytic Microbiologically Induced Calcium Carbonate Precipitation for Biocementation, World J Microbiol Biotechnol, Vol. 39, No. 61, 2023, pp. 1-18.

[7] Zhang J., Shi X., Chen X., Huo X., and Yu Z. Microbial-Induced Carbonate Precipitation: A Review on Influencing Factors and Applications, Advances in Civil Engineering. Vol. 2021, No. 1, 2021, pp. 1-16.

[8] Whiffin V. S., van Paassen L. A., and Harkes M. P. Microbial Carbonate Precipitation as a Soil Improvement Technique, Geomicrobiol J, Vol. 24, No. 5, 2007, pp. 417–423,.

[9] Stocks-Fisher S., Galinat J. K., and Bang S. S. Microbiological Precipitation of CaCO3, Soil Biol Biochem, Vol. 31, No. 11, 1999, pp. 1563–1571.

[10] Shahroki-Shahraki R., Zomorodian S. M. A., Niazi A., and O'Kelly B. C. Improving Sand with Microbial-induced Carbonate Precipitation, in Proc. of the Institution of Civil Engineers-Ground Improvement, Vol. 168 No. 3, 2015, 217–230.

[11] Lim A., Muhammad D. A., and Lestari A. S. Experimental Study of Biocementation Ability of Local Bacteria on Loose Sandy Soil, Jurnal Teknik Sipil, Vol. 26, No. 2, 2019, pp. 129-138, (in Indonesian).

[12] Le Métayer-Levrel G., Castanier S., Orial G., Loubiè J.-F., and Perthuisot J.-P. Applications of Bacterial Carbonatogenesis to the Protection and Regeneration of Limestones in Buildings and Historic Patrimony, Sediment Geol, Vo. 126, No. 1-4, 1999, pp. 25-34,

[13] Tobler D. J., Maclachlan E., and Phoenix V. R. Microbially Mediated Plugging of Porous Media and the Impact of Differing Injection Strategies, Ecol Engineering, Vol. 42, 2012, pp. 270-278.

[14] Das B. M. and Sobhan K. Principles of Geotechnical Engineering 9th Edition, Cengage Learning, 2018, pp. 1-819.

[15] Rebata-Landa V. Microbial Activity in Sediments: Effect on Soil Behavior (Doctoral Thesis), Georgia Institute of Technology, 2007, pp. 1-162.

[16] Ivanov V, and Stabnikov V. Construction Biotechnology: Biogeochemistry, Microbiology and Biotechnology of Construction Material and Processes, Green Energy and Technology, Springer, 2017, pp. 1-317.

[17] Acuna S. P. C., Jimenez M. L. B. Zambrano, J. J. M., and Sarmiento H. A. R. Soil Bacteria that Precipitate Calcium Carbonate: Mechanism and Application of the Process, Acta Agron, Vol. 67, No. 2, pp. 277-288, 2018.

[18] Lee L. M., Soon N. W., Khun T. C., and Ling H. S. Bio-mediated Soil Improvement Under Various Concentrations of Cementation Reagent, Applied Mechanics and Materials, Vol. 204–208, 2012, pp. 326–329.

[19] Choi S. G., Park S. S., Wu S., and Chu J. Methods for Calcium Carbonate Content Measurement of Biocemented Soils, J Mater Civ Eng ASCE, Vol. 29, No. 11, 2017, pp. 06017015-1-4.

[20] Kim G., Kim J, and Youn H. Effect of Temperature, pH, and Reaction Duration on Microbially Induced Calcite Precipitation, Appl Sci, Vol. 8, No. 8, 1277, 2018, pp. 1-10.

[21] Mwandira W., Nakashima K., and Kawasaki S. Bioremediation of lead-contaminated mine waste by Pararhodobacter sp. Based on the Microbially Induced Calcium Carbonate Precipitation Technique and Its Effect on Strength of Coarse and Fine Grained Sand, Ecol Eng, Vol. 109, 2017, pp. 57-64.

[22] Chou C. W., Seagren E. A., Aydilek A. H., and Lai M. Biocalcification of Sand through Ureolysis, J Geotech Geoenviron Eng ASCE, Vol. 137, No. 12, 2011, pp. 1179-1189.

[23] DeJong J. T., Mortensen B. M., Martinez B. C., and Nelson D. C. Bio-mediated Soil Improvement, Ecol Eng, Vol. 36, 2010, pp. 197-210.

[24] Martin D., Dodds K., Ngwenya B. T., Butler I. B., and Elphick S. C. Inhibition of Sporosarcina pasteurii under Anoxic Conditions: Implications for Subsurface Carbonate Precipitation and

Remediation via Ureolysis, Environ Sci Technol, Vol. 46, 2012, pp. 8351-8355.

[25] Ma L., Pang A. P., Luo Y., Lu X., and Lin F. Beneficial Factors of Biomineralization by Ureolytic Bacterium Sporosarcina pasteurii. Microb Cell Fact, Vol. 19, No. 12, 2020, pp. 1-12.

[26] Li M., Wen K., Li Y., and Zhu L. Impact of Oxygen Availability on Microbially Induced Calcite Precipitation (MICP) Treatment, Geomicrobiol J, Vol. 35, No. 1, 2018, pp. 15-22.

[27] Jain S., and Arnepalli D. N. Biochemically Induced Carbonate Precipitation in Aerobic and Anaerobic Environments by Sporosarcina pasteurii, Geomicrobiol J, Vol. 36, No. 5, 2019, pp.443–451.

[28] Karunadasa K. S. P, Manoratne C. H., Pitawala H. M. T. G. A., and Rajapakse R. M. G., Thermal Decomposition of Calcium Carbonate (Calcite Polymorph) as Examined by In-situ High-temperature X-ray Powder Difraction, J Phys Chem Solids, Vol. 134, 2019, pp. 21-28.

[29] Cheng L., Cord-Ruwisch R., and Shahin M. Cementation of Sand Soil by Microbially Induced Calcite Precipitation at Various Degree of Saturation, Canadian Geotechnical Journal, Vol. 50, No. 1, 2013, pp. 81-90.

[30] Mujah D., Cheng L., and Shahin M. Microstructural and Geomechanical Study on Biocemented Sand for Optimization of MICP Process, J Mater Civ Eng ASCE, Vol. 31, No. 4, 2019, pp. 04019025-1-10

[31] Moqsud M. A. Slope Soil Stabilization through Biocementation by Native Bacteria in Chugoku Region, Japan, International Journal of GEOMATE, Vol. 21, No. 83, 2021, pp. 36-42.

[32] Harianto T., Walenna M. A., and Torano C. P. A. Shear Strength Characteristics of Stabilized Sedimentary Soil with Bacillus Subtilis Bacteria, IOP Conf. Ser.: Earth Environ. Sci. 1117 012022, 2022, pp. 1-9.

[33] Lamuse M., Putra H., Erizal, and Ihsani Z. M. Improvement of Shear Strength Parameters on Clay Shale Using Calcite Precipitation Method, IOP Conf. Ser.: Earth Environ. Sci. 1416 012007, 2024, pp. 1-12.

[34] Alkadri, Djamaluddin A. R., Harianto T., and Arsyad A. Soil Mechanical Characteristics Improvement with Bacterial Biocementation Technology, International Journal of GEOMATE, Vol. 26, No. 115, 2024, pp. 27-33.