Indonesian Geotechnical Journal, December 2025, Vol 4 (3). Page 177 - 188
DOI: https://doi.org/10.56144/igj.v4i3.137
EISSN: 2829-7520
https://indonesian-geotechnical-journal.org/index.php/JGI/issue/archive

Microbial Enzyme Extract Evaluation for Calcium Carbonate Biocementation: Implications for Ground Improvement

Lutfian Rusdi Daryono[1,*], Muhammad Akmal Putera[1], Tomohiko Abe[1], Aoi Ichinohe[2], Kazunori Nakashima[3], Satoru Kawasaki[3]
[1]Engineering and Development Divisions, NITTOC Construction Co., Ltd., Japan
[2]Division of Sustainable Resources Engineering, Graduate School of Engineering, Hokkaido University, Japan
[3]Division of Sustainable Resources Engineering, Faculty of Engineering, Hokkaido University, Japan
*Correspondence: luthfian.daryono@gmail.com; lutfian.rusdi.daryono@nittoc.co.jp

SUBMITTED 17 December 2025 **REVISED** 27 December 2025 **ACCEPTED** 31 December 2025

ABSTRACT Microbially Induced Carbonate Precipitation (MICP) has emerged as a promising technique for slope surface stabilization or other ground improvements. However, its effectiveness in soil finer than fine sand is limited due to challenges associated with bacterial cell transport. This study explores the feasibility of an alternative approach, Enzyme Induced Carbonate Precipitation (EICP). EICP uses enzymes extracted from microbial bacteria to produce carbonate. In this study, urease was extracted from whole-cell cultures of *Lysinibacillus xylanilyticus (LyXy)* which was developed by Hokkaido University and using cyclic sonication. Precipitation test was then conducted by applying the extracted enzyme solution to sand specimen with more than 30% fine sand content. The performance of EICP was evaluated against traditional MICP with a focus on the capability to precipitate carbonate in fine sand soil. Experiments involving temperature and addition frequency of solidification solutions were also conducted for the EICP. Results indicated that the EICP exhibited higher enzymatic activity than the MICP for the given soil specimen. Furthermore, it was also found that EICP performs the best at lower temperature of 15°C (the dependencies from 15°~35°C) and higher carbonate content was obtained when the solidification solutions were added at lower volume but higher frequency. The unconfined compressive strength obtained for EICP at optimum conditions was 4 MPa, while the least optimal was 1 MPa.

KEYWORDS Microbial Induced Carbonate Precipitation (MICP); Enzyme Induced Carbonate Precipitation (EICP), Enzyme Extract, Soil Stabilization, Fine-grained

1 INTRODUCTION

Bio-cementation is an emerging soil stabilization technology that relies on environmentally friendly biogeochemical processes. Microbial induced carbonate precipitation (MICP) and Enzyme induced carbonate precipitation (EICP) are the two most common and widely investigated bio-cementation techniques. It should be noted that there are already several hundred articles published on the application of MICP and EICP for various geotechnical purposes (Ivanov and Chu, 2008; Liu et al., 2020; Omoregie et al., 2020; Sharma et al., 2022). The MICP technology utilizes living bacteria cells that contain intra-cellular urease (Dejong et al., 2022; Sun et al., 2021). On the other hand, in the EICP technology, free urease enzymes are used in place of ureolytic bacteria (Hamdan and Kavazanjian, 2016; Almajed et al., 2018). In both technologies, bacteria or enzymes introduced are used to catalyze the hydrolysis of supplied urea, producing ammonium and carbonate ions. When the hydrolysis happens in a media containing calcium ions (e.g., $CaCl_2$), calcium carbonate ($CaCO_3$) precipitates inside the pores of the soil, binding the soil particles together at particle-particle contact points and enhances the engineering properties of soil (Montoya and de Jong, 2015; Lin et al., 2016). This process is also often called solidification of soil. Equation 1 and 2 show the equation of the MICP and EICP process.

Among the numerous studies on bio-cementation, sustained and systematic investigations by specific research groups have played a critical role in advancing MICP applications. One such example is the

research group at Hokkaido University, which has focused on MICP-based ground solidification for more than a decade. Some research outputs include Danjo and Kawasaki (2014), Kubo et al. (2015), Amarakoon and Kawasaki (2018), Dilrukshi and Kawasaki (2018), Gowthaman et al. (2019), Daryono et al. (2020), Imran et al. (2021), and Chen et al. (2024).

$$H_2N - CO - NH_2 + 2H_2O \xrightarrow{ureolytic\ bacteria/urease} CO_3^{2-} + 2NH_4^+ \quad (1)$$

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

Both technologies are expected to be applicable for ground improvement, such as for slope protection (Gowthaman et al., 2023), mitigation of liquefaction, etc. In comparison to concrete reinforcement, (e.g., soil nail) MICP and EICP provide alternatives that have less negative impact on the environment. However, the MICP method has some limitations in its application, i.e., some types of soil are difficult to solidify with MICP. For example, the bacteria have difficulty in propagating in fine sand and fine-grained soil, hence uneven improvement would be achieved with MICP. In contrast, EICP which directly uses urease enzyme extracted from microorganisms, plants, etc., does not have this weakness.

Free urease enzymes used in EICP can be produced with high purity through industrial means. However, it is very expensive, and is used in food industry, drug industry or bio-medical applications. Their cost was once reported as 10 million JPY per kilogram (Gowthaman et al., 2021). It is therefore manifest that the use of commercial enzymes is not affordable in real-scale applications in terms of the cost. Fortunately, the urease enzymes can also be obtained in a crude form from soybeans, jack beans, watermelon seeds, etc. Jack bean is the most commonly used urease source in EICP studies; however, the activity of the extract might vary depending on the state and conditions that the extraction was carried out (Almajed et al., 2020, 2018, 2021). Imran et al. (2021) disclosed that the urease derived from germinated watermelon seeds consists of higher activity compared to that from non-germinated seeds.

Keeping the limitations of both MICP and EICP methods in mind, this study was performed with the purpose of evaluating the feasibility of bacterial enzyme induced carbonate precipitation for improving soil strength with calcium carbonatization. For this study, the urease enzyme was obtained from sonication process, rather than the expensive commercially available enzymes or plant-based enzyme sources. For that, the culture of *Lysinibacillus xylanilyticus* was subjected to cyclic sonication to extract urease suspension. The enzyme was then applied to the fine sand samples through injection method to ensure well distributed improvement within the soil.

2 MATERIALS AND METHODS

2.1 Bacteria Culturing, Sonication, and Enzyme Extraction

The ureolytic bacterium used in this research work was *Lysinibacillus xylanilyticus (LyXy)*. The bacterium was earlier isolated by Gowthaman et al. (2019) from Hokkaido expressway slope, Japan. Pre-sterilized Beer Yeast (BY) was used as the culture medium for cultivating *Lysinibacillus xylanilyticus*. For the preparation of BY medium, 4 grams of NICHIGA beer yeast powder was mixed in 100 ml distilled water and stirred at 600 rpm for 24 hours. The solution was then placed in a centrifuge at 8000 rpm to remove the undissolved solid particles. The solution was then autoclaved to reduce the risk of culture contamination. The bacteria were precultured in 5 ml Beer Yeast (BY) medium under shaking incubation at 25 ℃ and 160 rpm for 24 hours. 1 ml of the preculture was inoculated into 100 ml of fresh BY medium and incubated at 25 ℃ and shake into incubator stirrer with the 160 rpm speed. Sufficient growth of the bacteria was ensured by measuring the intensity of the culture under the wavelength of 630 nm (OD_{630}). It should be noted that the optimal growth of *LyXy* is typically attained after 48 hours of incubation. Prior to the sonication, the bacteria culture was centrifuged at 25 ℃ and 160 rpm for 5 minutes, and the supernatant solution was replaced with lysis buffer of pH 8.0. The solution is then placed in a sonication chamber and a series of cyclic sonication steps (i.e., 1 min “on” followed by 1 min “off” - for a 5-min period) was performed to the

bacteria culture. Following the sonication process, to separate residual cellular debris (i.e., solids) from the extracted urease, the solution was placed in centrifuge at 11,900 rpm for 10 minutes. The extracted enzyme solution was then diluted using the lysis buffer equivalent to the initial volume of the bacteria culture (100 mL of culture). Following the sonication, the urease extracts were stored at different temperatures (15–35°C)The activity of the extracted urease was determined using the method described in the next section.

2.2 Measuring the Activity of Extracted Bacterial Enzyme

The activity of extracted urease was measured using spectrophotometric determination of ammonia as indophenol (Bolleter et al., 1961). 1 ml of phenol and hypochlorite was mixed with 0.1 mol/L urea prepared in phosphate buffer solution. The additional chemical solutions, phenol, in the presence of hypochlorite, reacted with ammonium ions produced from urea hydrolysis, producing a blue color dye (also referred to as indophenol dye) were required to conduct the urea hydrolysis rate experiment. The dye intensity indicated the concentration of ammonium ions produced during the reaction. At every 5 minutes interval, the intensity of the dye was measured using spectrophotometry at the wavelength of 630 nm, and the rate of urea hydrolysis was estimated using the calibration curve developed between the concentration of ammonium ions and intensity. The activity of extracted urease is expressed in terms of urea hydrolysis rate.

2.3 Soil Specimens Used

The soil specimen used for this study was obtained from Wattsu, Hokkaido, Japan. The grain size distribution of Wattsu soil was obtained from previous study conducted by Gowthaman et al. (2023) for slope improvement with standard MICP method. The same grain size distribution of the Wattsu soil is shown in Figure 1. As shown in the figure, more than 50% belongs to the course sand grain size (0.2 mm to 2 mm), while around 30% belongs to the fine sand grain size (0.02 mm to 0.2 mm). Less than 20% of the soil is finer than 0.06 mm.

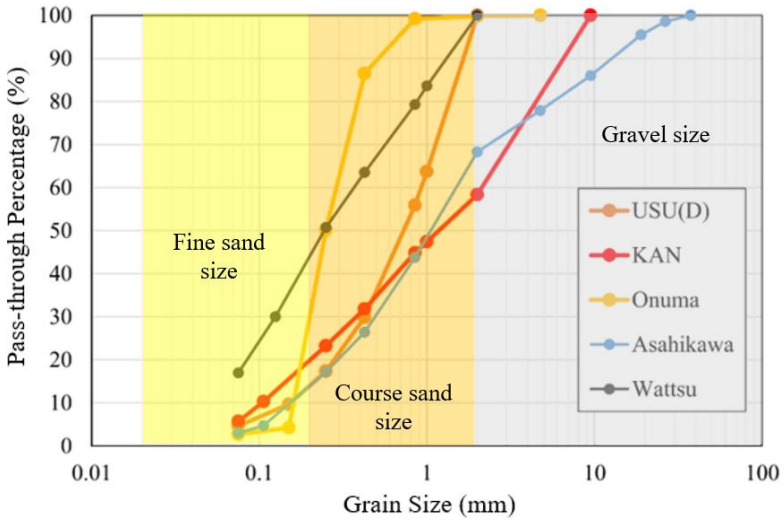

Figure 1. Grain size distribution modified from Gowthaman et al., 2023. Soil used in this study is from Wattsu.

2.4 Solidification Test Program and Strength Test

The test program are shown in Table 1. Six test cases, one MICP, 4 EICP and a control, were set up. As the main focus of this study was to determine the optimum conditions for EICP method, the bio-cementation processes were varied for the EICP cases, with variation in temperature (25 ºC for case 2 and 4; 15 ºC for case 3 and 5) and the rate of solidification solution (insert the solution name here) was added. For all tests, the initial bacteria solution (for MICP) and enzyme solutions (for EICP) is kept constant at 20 ml, with curing time of 7 days. Thereafter, for case 2 and 3, 20 ml of solidification solution with 1 M concentration was dropped once into the soil every 24 hours for a total of 14 days.

While for cases 4 and 5, 10 ml of solidification solution with 1 M concentration was dropped into the soil every 12 hours for a total of 14 days. Therefore, for all cases, the total amount of solidification solution added was 480 ml. Direct comparison between MICP and EICP methods was done through case 1 and case 2, no difference in the temperature nor the rate of solidification solution, just the difference in initial solution. For the control, no bacteria solution or enzyme solution was added, but the solidification solution was still added at the same rate as case 2 and 3.

The cementation strength of the specimens was examined using a needle penetration device/soft rock penetrometer (SH-70, Maruto Testing Machine Company, Tokyo, Japan). The needle penetration apparatus is a portable testing device developed in Japan for predicting the UCS of soft, weak to very weak rocks and cemented soil specimens. The specimen was horizontally positioned, and the needle of the device penetrated the cylindrical surface of the specimen at three locations (at the distance of 1 cm (top), 3 cm (middle) and 5 cm (bottom) measured from the column top). The penetration resistance (N) and penetration depth (mm) were measured simultaneously (JSCE, 1991).

Table 1. Test program

Test Case	Temp. °C	Method	Bacteria or enzyme solutions		Solidification solution $CaCl_2$ mixed Urea (concentration = 1 M)		Solidification period
			Amount	Interval	Amount	Interval Injection	
Case 1	25	MICP	20 ml	1 time/ 7 days	20 ml	24 h	14 days
Case 2	25	EICP			20 ml	24 h	
Case 3	15	EICP			20 ml	24 h	
Case 4	25	EICP			10 ml	12 h	
Case 5	15	EICP			10 ml	12 h	
Control	15	-	-	-	20 ml	24 h	

2.5 Determination of $CaCO_3$ Content

The precipitated $CaCO_3$ content was measured by acid reaction method (Fukue et al., 1999). The specimens were first oven-dried at 60°C for 48 hours. Then, a representative sample of known weight was placed into a closed chamber maintained at a constant volume and temperature and reacted with concentrated HCl. When the $CaCO_3$ got into contact with HCl, dissolution occurred, which resulted in the formation of CO_2 gas. The internal pressure variations during the reaction were monitored using the digital manometer fabricated within the chamber. From the pressure reading obtained during the reaction, mass of the existing $CaCO_3$ could be determined using the developed calibration curve, hence the content of $CaCO_3$ was estimated using Equation 3.

$$\text{CaCO}_3 \text{ content (\%)} = \frac{\text{Mass of CaCO}_3}{\text{Mass of the oven dried specimen} - \text{Mass of CaCO}_3} \quad (3)$$

3 RESULTS AND DISCUSSIONS

3.1 Activity and Temperature Dependencies of the Urease Extract

The enzymes extracted are either filtered at the particle-to-particle contacts or adsorbed at the soil surface, enabling their possible distribution throughout the column depth. It should be stated that the adsorption capacity of the bacteria cells or urease rely on their biological nature such as the charge of the substance, shape and surface roughness (Zita and Hermansson, 1994).The previous study by Gowthaman et al. (2022) revealed an increase of around 18% compared to that of original bacteria culture ($p = 0.2251$) the effectiveness between ordinary MICP with extraction enzyme of this research. The above increase in activity could possibly be attributed to the reduced transport constraints of urea molecules (substrate) through cell membrane. During the sonication, the rupture of the bacteria cell membrane occurs, resulting in the release of free urease in the solution. This surges the exposure of substrate to urease enzymes that are suspended freely in solution, rather than those localized inside the cell membrane. Hoang et al. (2019) earlier pointed out that the sonication

(cyclic run-cool process) typically causes around 20% evaporation of the solution, resulting in a considerable increase in activity. The observation indicates that there is a general decrease in the activity of urease extract with storage time. This is attributed to enzyme denaturation, as urease is composed of protein subunits and could naturally be broken down into amino acids (Mobley et al., 1995). The finding here is that with the decrease in storage temperature, the rate of reduction in activity also decreases. For instance, a sharp drop in activity can be seen in the first 24 hours for 35 and 30°C, while the drop in activity is inconspicuous for 15°C. The initial activity measured at 35°C was 3.5 U/ml, and at the end of the storage time of 7 days, the extract almost lost all of its activity (>95%). On the other hand, the loss in the activity was only around 35% for the extract stored at 15°C, indicating high stability of the urease at low temperature. Assuming urea was always present, we can predict the total amount of urease activity by integrating the curves in Figure 2. Figure 3 shows the integrated value of urease activity after a given time.

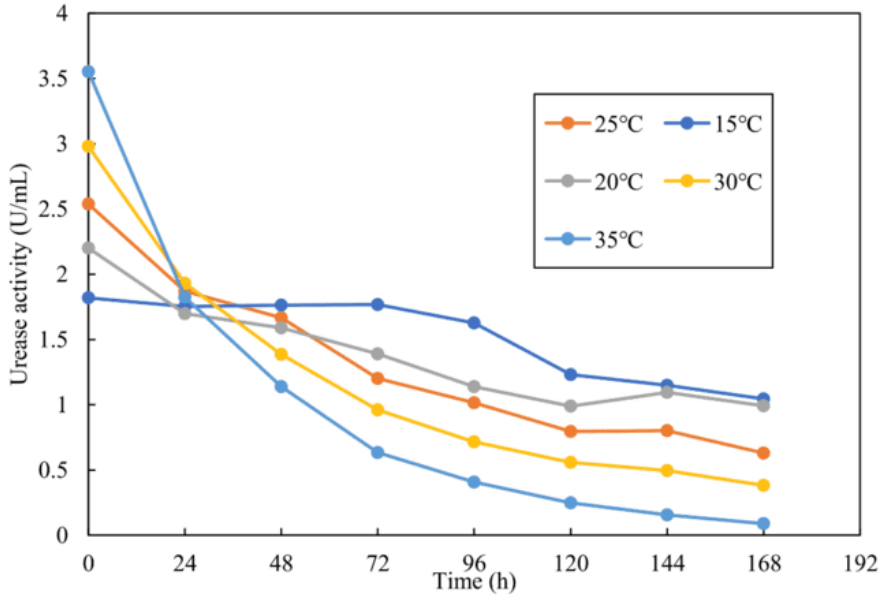

Figure 2. Urease enzyme extract activity versus time under different storage temperatures

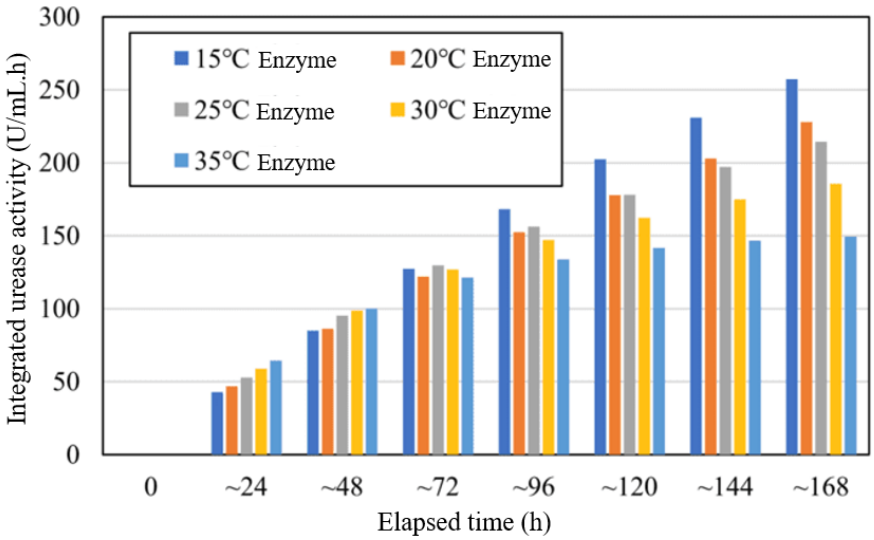

Figure 3. Elapsed time and integral value of urease activity

3.2 Solidification Test

Figure 4 shows the appearance of the specimens obtained from the syringe compaction test. The general characteristics of Cases 1 to 5, except for the control, were that the specimens became hard,

almost like concrete, from the top to a depth of about 0.7 cm and did not deform at all when squeezed with a finger. However, below 4 cm depth, the specimens became uniformly brittle, and all specimens near the middle of the bottom (5 cm depth) were easily squeezed with a finger. The control specimens were completely brittle, as no ureolytic bacteria nor urease was added, hence no solidification occurred. The control specimens and each bottom specimen immediately disintegrated, making it impossible to measure their UCS. Therefore, the UCS estimations using a needle penetrometer were only conducted at the top and middle of specimens for Cases 1 to 5. The results are shown in Figure 5. To correlate the UCS with calcium carbonate precipitated, the calcium carbonate concentration (%) at the top, middle, and bottom specimens for Cases 1 to 5 is shown in Figure 6. The results are discussed in the next section.

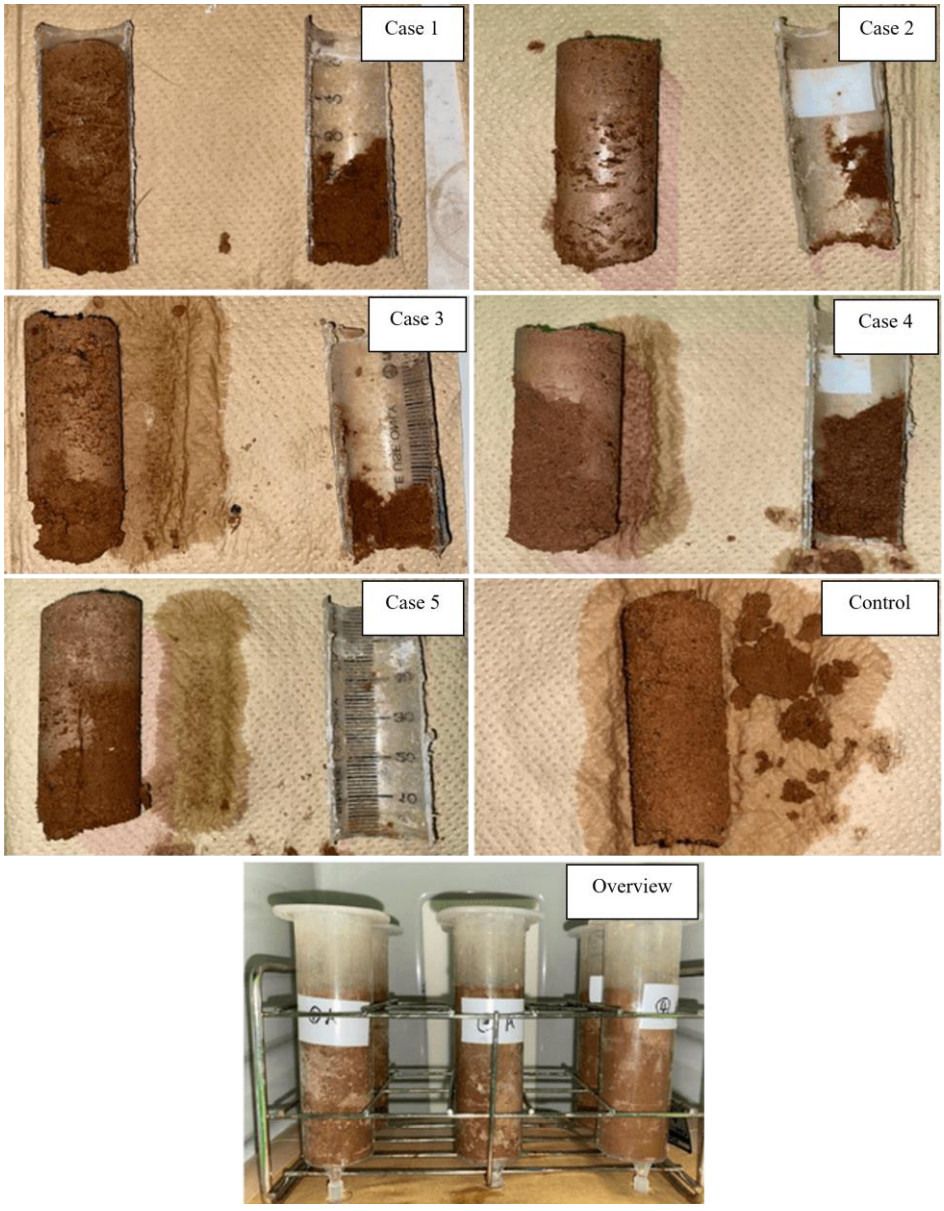

Figure 4. Physical look of the samples at the end of tests

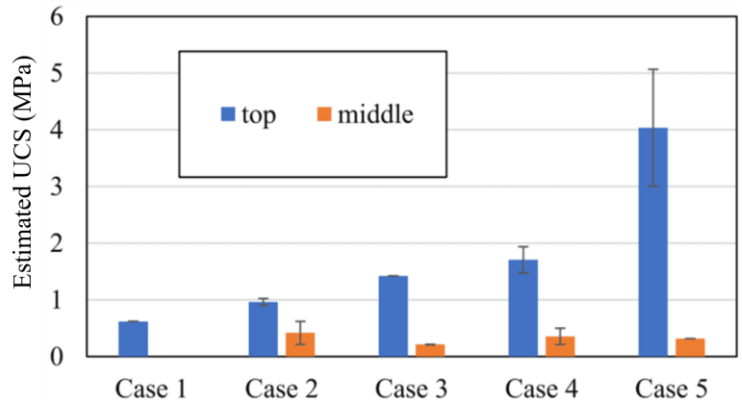

Figure 5. Estimated UCS of each sample

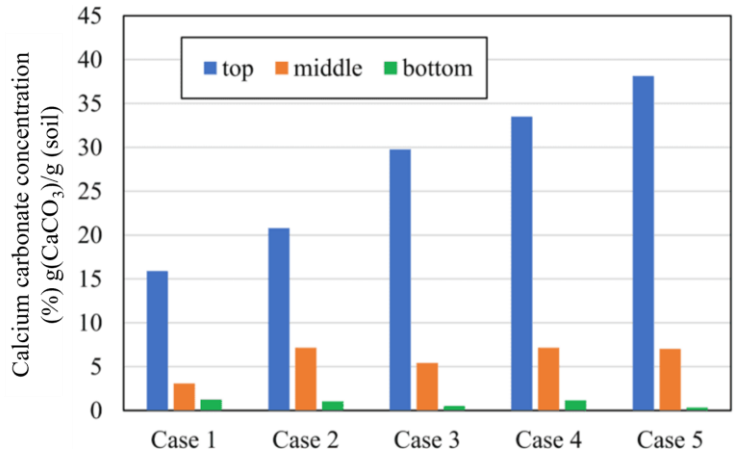

Figure 6. Calcium carbonate content of each sample

3.3 Discussion

From Figure 5, corresponding to the physical observation, the top part of the specimen has higher UCS than the middle part. For MICP case (case 1), UCS is only obtained for the top part, but not the middle part. For the EICP cases (case 2 to 5), the average estimated UCS ranges from 1 to 4 MPa, with the lowest UCS being in case 2. But the UCS obtained for case 2 is still double that of the MICP case. This results correspond to the calcium carbonate content concentration (Figure 6), with MICP calcium carbonate concentration being lower than that of EICP. The calcium carbonate content in the middle layer for case 1 is also lower than any of the EICP cases. This is probably due to the Wattsu fine sand grain size being too small for the bacterial cells. This result agrees with the initial hypothesis that MICP method is not optimum to be applied to base materials with fine grain size. EICP method does not have this weakness as the enzymes are smaller than bacterial cells and can pass through the pores easier.

Within the EICP cases themselves, it would be easier to present the results in a 3-dimensional graph as shown in Figure 7. That way, it is possible to compare the temperature as well as injection rate at the same time. It is evident that lower temperature results in higher UCS, and this corresponds well with the urease enzyme activity discussed in Figure 2 and 3. Looking at another angle, it can also be seen that adding the solidification solution at smaller interval (12 hours) is better than longer interval (24 hours), despite the total daily solidification solution added being the same (i.e., 2 times 10 ml & 1 time 20 ml). Previous studies have reported that when a small amount of solution is added to a specimen, capillary forces cause the solution to remain at the contact points between sand particles as a meniscus, resulting in calcium carbonate crystals precipitating at locations that bridge the sand

particles and increase strength (Cheng and Cord-Ruwisch, 2012). On the other hand, when a larger amount of solutions is added, calcium carbonate precipitates uniformly in areas other than those that increase strength. In other words, when concentration the same cumulative amount of solidification solution, adding it gradually over a long period of time, as shown on the left side of Figure 8, will solidify the area involved in the bridge more efficiently than adding a large amount in a short time, as shown on the right side. Therefore, it is to be expected that UCS will be greater when the concentration is given at smaller time interval (10 mL/12 hours).

Many experimental studies report that unconfined compressive strength (UCS) roughly follows a power-law or exponential-type relationship with $CaCO_3$ content rather than a linear one. The correlations between UCS strength and content of calcium carbonate percentage concentration level are shown in figure 7. Compressive strength correlates positively with $CaCO_3$ content up to a threshold level, beyond which strength gain depends primarily on precipitation morphology, spatial distribution, and bonding efficiency rather than total carbonate content. To have a better overview of the best method in producing calcium carbonate and obtaining higher UCS, the results are plotted with UCS against calcium carbonate concentration for all cases in Figure 7. It can be seen that case 5 yields the best results. Therefore, it is recommended that solidification should be added at smaller time intervals, while maintaining lower temperature.

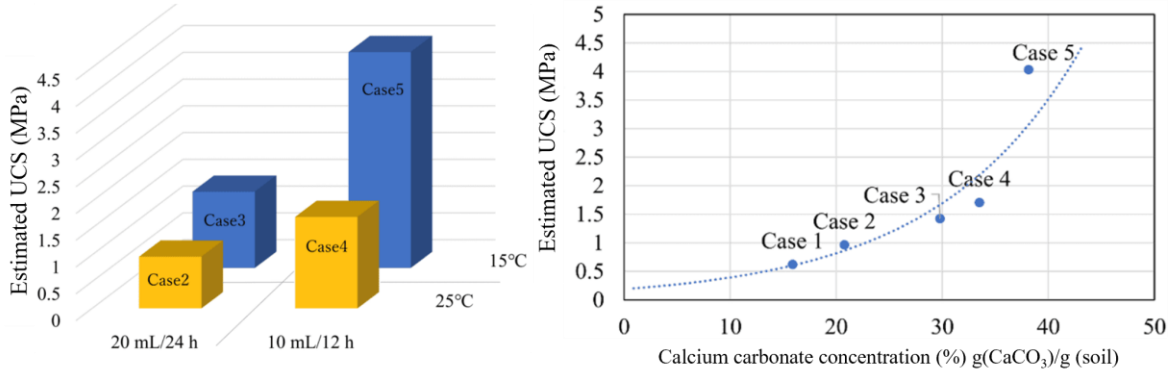

Figure 7. Upper solidification (left) and correlation diagram (right) between upper mass calcium carbonate content and estimated UCS for Cases 1-5

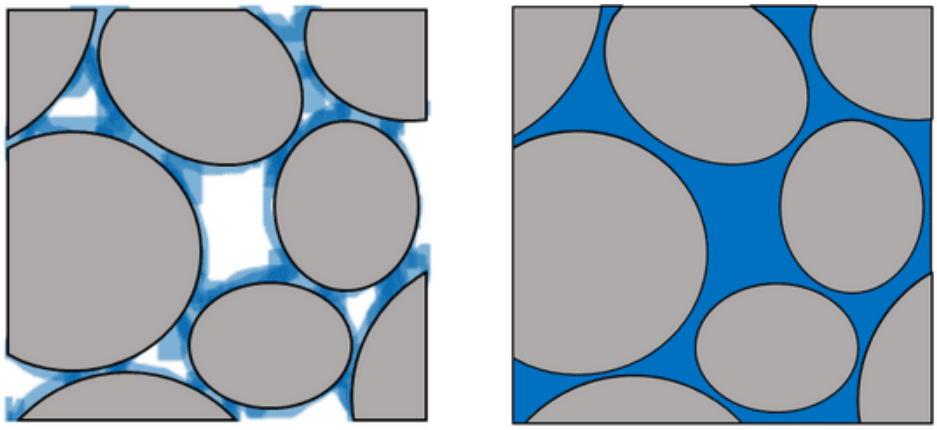

Figure 8. Illustration of the solidification solution by amount added

4 CONCLUSIONS

This experiments in this study provides the following findings:

1. Through the urease enzyme activity test, the urease enzyme was found to have higher initial activity at higher temperatures (30 and 35 ºC). In the long term, it is better to use lower temperature to maintain longer urease enzyme activity.

2. It was further confirmed through solidification tests that lower temperature of 15°C is more suitable for the EICP method than elevated temperature of 25°C. That way, higher calcium carbonate content and higher UCS can be obtained.

3. It was also found that in the EICP methods, higher calcium carbonate content and higher UCS can be obtained by increasing the frequency of dripping the solidification liquid.

4. It was confirmed that the EICP method is more suitable for solidification than the MICP method for soil with high fine sand content.

DISCLAIMER

The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

All data are available from the authors.

ACKNOWLEDGMENTS

The authors extend their heartfelt gratitude to all the individuals and entities involved in this collaborative research conducted between Hokkaido University and NITTOC Construction Ltd. While it is not feasible to acknowledge each contributor individually, their collective efforts and support have played a pivotal role in the successful completion of this study.

REFERENCES

Almajed, A., Abbas, H., Arab, M., Alsabhan, A., Hamid, W. and Al-Salloum, Y., 2020. Enzyme-Induced Carbonate Precipitation (EICP)-Based methods for ecofriendly stabilization of different types of natural sands. *Journal of Cleaner Production*, 274, p. 122627. https://doi.org/10.1016/j.jclepro.2020.122627

Almajed, A., Khodadadi Tirkolaei, H. and Kavazanjian Jr, E., 2018. Baseline investigation on enzyme-induced calcium carbonate precipitation. *Journal of Geotechnical and Geoenvironmental Engineering (ASCE)*, 144(11), p. 04018081. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001973

Almajed, A., Lateef, M. A., Moghal, A. A. B. and Lemboye, K., 2021. State-of-the-art review of the applicability and challenges of microbial-induced calcite precipitation (MICP) and enzyme-induced calcite precipitation (EICP) techniques for geotechnical and geoenvironmental applications. *Crystals*, 11(4), p. 370. https://doi.org/10.3390/cryst11040370

Amarakoon, G. G. N. N. and Kawasaki, S., 2018. Utilization of Microbially Induced Calcite Precipitation for Sand Solidification Using Pararhodobacter sp. In *Ecological Wisdom Inspired Restoration Engineering* (pp. 69-91). Singapore: Springer Singapore.

Bolleter, W. T., Bushman, C. J. and Tidwell, P. W., 1961. Spectrophotometric determination of ammonia as indophenol. *Analytical Chemistry*, 33(4), pp. 592-594.

Chen, M., Ichinohe, A., Nakashima, K., Takano, C., Gowthaman, S., Daryono, L. R. and Kawasaki, S., 2024. Effect of humic acid on soil solidification by enzyme induced carbonate precipitation. *Biogeotechnics*, p. 100133. https://doi.org/10.1016/j.bgtech.2024.100133

Cheng, L. and Cord-Ruwisch, R., 2012. In situ soil cementation with ureolytic bacteria by surface percolation. *Ecological Engineering*, 42, pp. 64-72. https://doi.org/10.1016/j.ecoleng.2012.01.013

Danjo, T. and Kawasaki, S., 2014. Formation mechanisms of beachrocks in Okinawa and Ishikawa, Japan, with a focus on cements. *Materials Transactions*, 55(3), pp. 493-500. https://doi.org/10.2320/matertrans.M-M2013844

Daryono, L. R., Nakashima, K., Kawasaki, S., Suzuki, K., Suyanto, I. and Rahmadi, A., 2020. Investigation of natural beachrock and physical–mechanical comparison with artificial beachrock induced by MICP as a protective measure against beach erosion at Yogyakarta, Indonesia. *Geosciences*, 10(4), p. 143. https://doi.org/10.3390/geosciences10040143

DeJong, J. T., Gomez, M. G., San Pablo, A. C., Graddy, C. M. R., Nelson, D. C., Lee, M., Ziotopoulou, K., El Kortbawi, M., Montoya, B. and Kwon, T.H., 2022. State of the Art: MICP soil improvement and its application to liquefaction hazard mitigation. *Proceedings of the 20th ICSMGE-State of the Art and Invited Lectures*.

Dilrukshi, R. A. N. and Kawasaki, S., 2018. Effect of plant-derived urease-induced carbonate formation on the strength enhancement of sandy soil. In *Ecological wisdom inspired restoration engineering* (pp. 93-108). Singapore: Springer Singapore.

Fukue, M., Nakamura, T. and Kato, Y., 1999. Cementation of soils due to calcium carbonate. *Soils and Foundations*, 39(6), pp. 55-64. https://doi.org/10.3208/sandf.39.6_55

Gowthaman, S., Chen, M., Nakashima, K. and Kawasaki, S., 2021. Effect of scallop powder addition on MICP treatment of amorphous peat. *Frontiers in Environmental Science*, 9, p. 690376. https://doi.org/10.3389/fenvs.2021.690376

Gowthaman, S., Iki, T., Nakashima, K., Ebina, K. and Kawasaki, S., 2019. Feasibility study for slope soil stabilization by microbial induced carbonate precipitation (MICP) using indigenous bacteria isolated from cold subarctic region. *SN applied sciences*, 1(11), p. 1480. https://doi.org/10.1007/s42452-019-1508-y

Gowthaman, S., Nakashima, K. and Kawasaki, S., 2022. Effect of wetting and drying cycles on the durability of bio-cemented soil of expressway slope. International Journal of Environmental Science and Technology, 19(4), pp.2309-2322.

Gowthaman, S., Koizumi, H., Nakashima, K., & Kawasaki, S., 2023. Field experimentation of bio-cementation using low-cost cementation media for preservation of slope surface. *Case Studies in Construction Materials*, 18. https://doi.org/10.1016/j.cscm.2023.e02086

Hamdan, N. and Kavazanjian Jr, E., 2016. Enzyme-induced carbonate mineral precipitation for fugitive dust control. *Géotechnique*, 66(7), pp. 546-555. https://doi.org/10.1680/jgeot.15.P.168

Hoang, T., Alleman, J., Cetin, B., Ikuma, K. and Choi, S. G., 2019. Sand and silty-sand soil stabilization using bacterial enzyme–induced calcite precipitation (BEICP). *Canadian Geotechnical Journal*, 56(6), pp. 808-822. https://doi.org/10.1139/cgj-2018-0191

Imran, M. A., Nakashima, K. and Kawasaki, S., 2021. Bio-mediated soil improvement using plant derived enzyme in addition to magnesium ion. *Crystals*, 11(5), p.516. https://doi.org/10.3390/cryst11050516

Ivanov, V. and Chu, J., 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. *Reviews in Environmental Science and Bio/Technology*, 7(2), pp. 139-153. https://doi.org/10.1007/s11157-007-9126-3

Japan Society of Civil Engineers. 1991. *Soft Rock Penetrometer SH-70, Patent No. 2147438, Complies with the needle penetration test method of the "Guidelines for the Investigation and Testing of Soft Rock*"

Kubo, R., Kawasaki, S., Suzuki, K., Yamaguchi, S., & Hata, T. Beachrock mapping by geophysical exploration: Application to Yagaji Island in Okinawa, Japan. In *ISRM International Symposium - EUROCK 2013*.

Lin, H., Suleiman, M. T., Brown, D. G. and Kavazanjian Jr, E., 2016. Mechanical behavior of sands treated by microbially induced carbonate precipitation. *Journal of Geotechnical and Geoenvironmental Engineering (ASCE),* 142(2), p. 04015066. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001383

Liu, B., Zhu, C., Tang, C. S., Xie, Y. H., Yin, L. Y., Cheng, Q. and Shi, B., 2020. Bio-remediation of desiccation cracking in clayey soils through microbially induced calcite precipitation (MICP). Engineering geology, 264, p. 105389. https://doi.org/10.1016/j.enggeo.2019.105389

Mobley, P. W., Lee, H. F., Curtain, C. C., Kirkpatrick, A., Waring, A. J. and Gordon, L. M., 1995. The amino-terminal peptide of HIV-1 glycoprotein 41 fuses human erythrocytes. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1271(2-3), pp.304-314. https://doi.org/10.1016/0925-4439(95)00048-9

Montoya, B. M. and DeJong, J. T., 2015. Stress-strain behavior of sands cemented by microbially induced calcite precipitation. *Journal of Geotechnical and Geoenvironmental Engineering (ASCE)*, 141(6), p.04015019. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001302

Omoregie, A. I., Palombo, E. A., Ong, D. E. and Nissom, P. M., 2020. A feasible scale-up production of Sporosarcina pasteurii using custom-built stirred tank reactor for in-situ soil biocementation. *Biocatalysis and Agricultural Biotechnology*, 24, p.101544. https://doi.org/10.1016/j.bcab.2020.101544

Sharma, M., Satyam, N., Reddy, K. R. and Chrysochoou, M., 2022. Multiple heavy metal immobilization and strength improvement of contaminated soil using bio-mediated calcite precipitation technique. *Environmental Science and Pollution Research*, 29(34), pp. 51827-51846. https://doi.org/10.1007/s11356-022-19551-x

Sun, X., Miao, L., Xia, J. and Wang, H., 2021. Evaluation and prediction for the cementation effect of MICP based on electrical resistivity. *Journal of Materials in Civil Engineering*, 33(10), p.06021006. https://doi.org/10.1061/(ASCE)MT.1943-5533.0003896

Zita, A., and Hermansson, M., 1994. Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Applied and Environmental Microbiology*, 60(9), pp. 3041–3048. https://doi.org/10.1128/aem.60.9.3041-3048.1994

- This page is intentionally left blank -