

Improvement in Plasticity Behavior of Residual Clay Soil via Bio-cementation Technique

Muttaqa Uba Zango^{1,2*}, Khairul Anuar Kassim¹, Abubakar Sadiq Muhammed^{1,3}, Kamarudin Ahmad¹, Murtala Umar⁴ and Jodin Makinda¹

¹School of Civil Engineering, Universiti Teknologi Malaysia, Johor, Malaysia

²Department of Civil Engineering, Kano University of Science and Technology, Kano, Nigeria

³Department of Civil and Water Resources Engineering, University of Maiduguri, Borno, Nigeria

⁴Department of Civil Engineering, Bayero University Kano, Kano, Nigeria

Received: 10 October 2020, Revised: 21 January 2021, Accepted: 4 February 2021

Abstract

Enzyme-induced calcium carbonate precipitation (EICP) is a bio-inspired technique that uses urease to activate the urea-hydrolysis reaction to produce CaCO_3 precipitation. This study was conducted to assess the effect of cementation solution concentrations on the plasticity and swell behavior of residual clay soil. The findings showed that the plasticity behaviour of the residual soil was improved. The liquid limit of the residual clay soil decreased from 79% to 58.8%, plastic limit increased from 30% to 47.8%, plasticity index decreased from 49% to 11% and linear shrinkage limit decreased from 16 to 4.3%, and these results reflected an increase in calcium carbonate precipitation from 0% in the untreated soil to 4.09% in the EICP soil sample treated at 1.00 M concentration of cementation solution. The SEM and EDX results indicated the presence of CaCO_3 crystals in the treated residual soil, while XRD analysis confirmed the formation of calcite crystals in the treated soil.

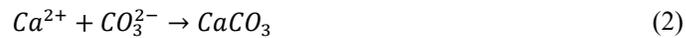
Keywords: biocementation; plasticity behaviour; residual clay soil; enzyme-induced calcium carbonate precipitation (EICP)

DOI.....

1. Introduction

Recently, research on soil improvement has shifted towards the use of green, environmentally friendly, and sustainable techniques [1-3]. Biocementation through either microbially-induced calcite precipitation (MICP) or enzymatically-induced calcium carbonate precipitation (EICP) is a green, environmentally friendly, and sustainable technique that utilizes ureolytic bacteria or free urease enzyme respectively, to synthesize calcium carbonate through the hydrolysis of urea and calcium-rich compounds [4-6]. The mechanism of MICP and EICP can be summarised in two equations (1 and 2), as shown below:

*Corresponding authour: Email: muttaqaubaz@yhoo.com



The majority of reported studies on biogeotechnology for soil improvement are centered around improvements in the strength, stiffness and permeability of granular soil via MICP [3, 7]. Despite several studies on MICP documented in various literature, only a few number of researches [8-12] were conducted on MICP in fine-grained soils. A limited number of studies on MICP in fine-grained soils is attributed to the inability of bacteria to pass through the pore throats of the soil, which are smaller than 0.4 μm [13].

In order to overcome treatment difficulties associated with MICP in fine-grained soils, enzymatically-induced calcium carbonate precipitation (EICP) has been adopted, which uses a free urease enzyme instead of urease produced by ureolytic bacteria [14, 15]. EICP has a similar mechanism to MICP, in which calcium carbonate is precipitated via ureolysis, as described in equations 1 and 2 above.

Previous studies conducted on EICP mostly focused mostly on improving strength the of sandy soils. For instance, Rohy *et al.* [16] adopted a one-phase injection method of various concentrations of EICP solution (urea, CaCl_2 , and urease solution) at low pH to improve a uniformly graded silica sand. Almajed *et al.* [17] improved the strength of silica sand using an EICP solution that was modified with an organic stabilizer (non-fat milk). Simatupang and Okamura [18] used the EICP technique to improve the liquefaction resistance of a sandy soil prepared at various degrees of saturation. Other research works [19-22] utilized the EICP solution to increase the shear strength of sandy soils.

Although a small number of studies such as Chandra and Karangat [23] and Cuccurullo *et al.* [24] were conducted on the use of the EICP technique to improve the engineering and physical properties of fine-grained soils, the use of the EICP technique to improve the plasticity behavior of fine-grained soils has not been investigated. There is a need to investigate the effect of calcite formation via the EICP technique on the plasticity behavior of fine-grained soils, especially for the application in the design of compacted clay liner.

This work presents the effect of a biocement produced via the EICP technique on the plasticity and swelling characteristics of residual clay soil. The effect of varying the concentration of cementation solution (urea and CaCl_2) on the Atterberg limits of the residual clay soil was also investigated.

2. Materials and Method

2.1 Materials

The materials used to investigate the effect of enzymatically-induced calcite precipitation on the plasticity behavior of residuals are residual soil and EICP solution.

2.1.1 Tropical residual soil

The soil used for this work was retrieved through the disturbed sampling method from 1.5 m below the ground borrow pit at Universiti Teknologi Malaysia, Skudai Campus (1°33'35" N, 103°38'38" E). The climate of the sampling area is a tropical rainforest and has a basement complex geologic formation.

2.1.2 EICP recipe

The EICP solution prepared for treating the residual soil consisted of free urease enzyme and the cementation solution. The free urease enzyme was extracted from the jack bean. The free urease enzyme was procured from Fischer Scientific Sdn Bhd, Malaysia. The urease activity of the enzyme was reported to be 3,500 U/g. The cementation solution was a mixture of urea ($CO(NH_2)_2$) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$). The concentration and composition of the EICP solution used in this study are presented in Table 1 below.

Table 1. Composition of EICP solution

Concentration (M)	Urease (g/l)	Urea (g/l)	CaCl ₂ ·H ₂ O (g/l)
0.25	3	15	36.6
0.50	3	30	73.2
0.75	3	45	109.8
1.00	3	60	146.4

The EICP solutions were prepared by first dissolving the amounts of urea ($CO(NH_2)_2$) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) calculated as shown in Table 1 in a distilled and deionized water in Scott bottles. The dissolved urea and calcium chloride is herewith referred to as cementation solution. Then, equivalent free urease enzyme in powdered form was added to the cementation solution and the mixture was vigorously mixed until all the powdered urease enzyme were dissolved. The mixture of urea, calcium chloride and urease enzyme are known as EICP solution.

2.2 Methods

The work involved in this study involved mainly laboratory tests that have a connection with the plasticity behavior of a fine-grained soil. The tests were performed on both untreated natural soil and EICP treated soil.

2.2.1 EICP treatment

The soil to be treated with the EICP solution was initially prepared by oven drying and then sieving through a 425 μ m sieve. About 500 g of the sieved soil was then mixed homogeneously with the EICP solution at different concentrations, as prepared in section 2.1.2 and presented in Table 1. The volume of the EICP solution taken for each mixture was 79% of the mass of dry soil, which corresponded to the liquid limit of the untreated soil. The procedure for the treatment was adopted from Osinubi *et al.* [25]. The soil-EICP solution mixtures (in paste form) were then cured for three days in a humidity chamber that was operating at $25 \pm 2^\circ\text{C}$ and 100% relative humidity. The soil-EICP pastes were then subjected to Atterberg limits tests, as explained in section 2.2.2.

2.2.2 Determination of index properties of the natural soil

The natural soil was characterized by conducting a particle size analysis, and Atterberg limits and specific gravity tests. The particle size analysis was conducted on the natural soil by combining wet and dry sieving, as outlined in British Standard Methods of test for soils for civil engineering purposes [26]. The retrieved natural soil from the borrow pit was initially air-dried. About 1 kg of the air-dried soil was then soaked in a solution of sodium metahexaphosphate for about 24 h. The wet soil was then washed through a 2 mm BS sieve. The soil retained on the 2 mm sieve was dried

in an oven and then sieved through a series of BS sieves from 28 mm down to 2 mm. The particle size analysis of wet soil passing through 2 mm and down to nanoscale was performed using a laser light scattering, automated particle size analyzer.

The Atterberg limits tests, including liquid limit, plastic limit and linear shrinkage tests on the natural and EICP treated soil were performed following the procedure enshrined in British Standard Methods of test for soils for civil engineering purposes [26]. The specific gravity of the natural untreated soil was determined by conducting a pyknometer test using small bottles as prescribed in British Standard Methods of test for soils for civil engineering purposes [26].

2.2.3 Determination of calcium carbonate content (CCC)

The dried samples after the Atterberg limits tests were used for the determination of calcium carbonate content. A gravimetric acid wash method, as reported in Choi *et al.* [27], was adapted for the determination of calcium carbonate content. The CCC was determined by allowing about 20 - 25 g each of the natural and treated soils to dissolve in a 4 M HCl solution for 24 h. The wet soils were rinsed and washed thoroughly with water for about 10 min and then filtered. The soils retained on the paper were oven-dried. The weight of the dried sample before acid digestion and the dried sample after acid digestion was determined. Equation (3) below was used to calculate the calcium carbonate content in the soil.

$$CCC = 100 - \left(\frac{B}{A}\right) \times 100 \quad (3)$$

CCC = Calcium carbonate content (%)

B = Mass of oven-dried soil post washing

A = Mass of oven-dried treated soil before washing

2.2.4 Microstructural analysis

In order to determine the morphology and molecular nature of the precipitation formed, microstructural analyses such as SEM, EDX and XRD were conducted on the natural and EICP treated soils at 0.50 M cementation solution. The XRD analysis was performed using a Rigaku SmartLab 9kW XRD machine, while a Hitachi SU8020 SEM machine was the instrument employed to conduct SEM-EDX analyses.

3. Results and Discussion

3.1 Index properties of the natural soil

The physical properties of the natural soil are summarised in Table 2. The soil is classified as gravelly clay of very high plasticity in the British Standard Classification System (BSCS). The dominant mineral in the soil is kaolinite as revealed by the XRD analysis conducted on the natural soil. The liquid limit and plastic limit of the natural soil are found to be 79% and 49%, respectively. It can be seen that the PI of the natural soil is higher than the recommended upper limit of 30% as suggested by Widomski *et al.* [28] and in Solid Waste Disposal Facility Criteria Technical Manual [29] for a compacted clay liner. As stated in EPA [29], clay soils with plasticity indices of higher than 30% tend to be challenging to work with when wet, and when dry, such soils could form hard clods that could provide a path for leachate to infiltrate. Therefore the soil plasticity index needs to be improved. One such method of improvement could be via enzymatically-induced calcium carbonate precipitation.

Table 2. Physical properties of the natural soil

Property	Quantity
Natural Moisture Content (%)	32.72
Percentage Passing 63 μm Sieve (%)	57
Gravel Fraction (%)	24.16
Sand Fraction	17.16
Liquid Limit (%)	79
Plastic Limit (%)	30
Plasticity Index (%)	49
Linear Shrinkage (%)	16
Specific Gravity	2.63
Loss on Ignition (LOI) (%)	12.28
BSCS Classification	CVG (Gravelly Clay of Very High Plasticity)
Colour	Reddish Brown
Clay Minerals	Kaolinite

3.2 Plasticity behaviour of EICP treated soil

Figure 1 shows the effect of concentration of cementation solution and calcium carbonate content on the Atterberg limits of treated soils. It can be seen from the graph that as the concentration of cementation solution increases, the liquid limit (LL), plasticity index (PI) and linear shrinkage limit (LS) decrease, while the plastic limit increases. The increase in the concentration of cementation reagents goes with the rise in calcium carbonate content formed in the treated soil. For instance, the natural soil has LL, PI, and SL of 79%, 49% and 16%, respectively. Upon treatment with EICP solution at 0.25 M concentration urea- CaCl_2 , the values of LL, PI and LS drop to 64.5%, 22.59%, 10%. Further increments in the concentration of urea- CaCl_2 lead to continuous decrease in the LL, PI and LS until the minimum values of 58.8%, 11.01% and 4.29%, respectively at 1.00 M urea- CaCl_2 concentration are reached. Similar pattern of results were reported by Choobbasti *et al.* [30] and Yazarloo *et al.* [31]. Moravej *et al.* [32] also reported that the reduction of LL and PI from 42 to 34% and 19 to 10%, respectively was due to the bio-treatment of dispersive soil via MICP. Kannan *et al.* [33] also determined that LL and PI of treated marine clay decreased upon treatment via both biostimulation and bioaugmentation with MICP. Furthermore, an increase in plastic limits was also reported by Choobbasti *et al.* [30]. The reduction in the LL, PI and LS in EICP treated soils can be attributed to the reduction in the thickness of the diffused double layer due to the replacement of hydrogen ions by the calcium from the calcium carbonate precipitation formed [30, 34]. The decrement in liquid limit is desirable in improving the plastic behavior of natural soils for compacted clay liner applications.

3.3 Relationship between Atterberg Limits and calcium carbonate content

Figure 2 shows the relationship between Atterberg limits and calcium carbonate precipitation formed in the EICP treated soils. The Figure also compared the results obtained in this study with those reported by Choobbasti *et al.* [30]. The reason for choosing Choobbasti *et al.* [30] for comparison with the results obtained in this research is to demonstrate that EICP treatment is equally viable in enhancing plasticity behaviour of soil as was the nano calcium carbonate from different

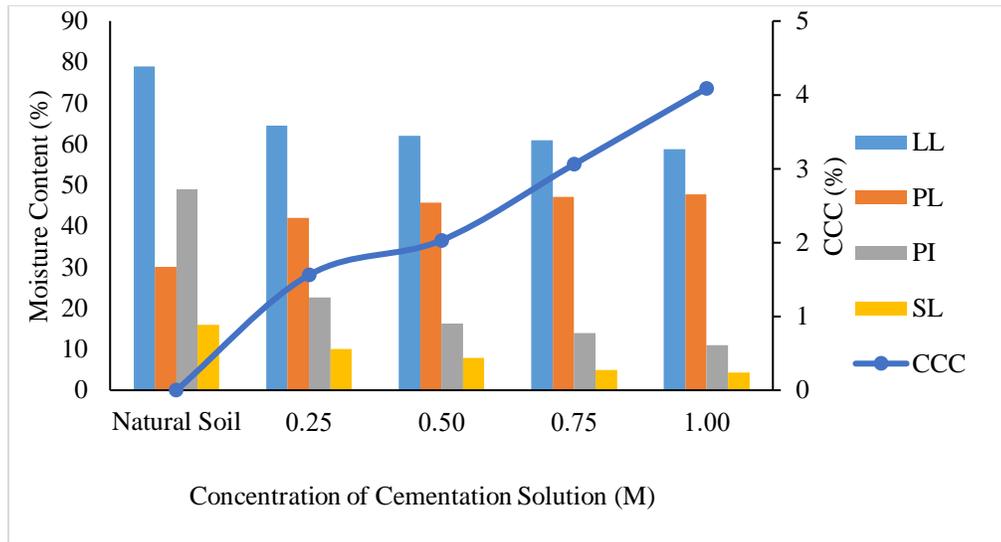


Figure 1. Variation of Atterberg limits with cementation solution and calcium carbonate content

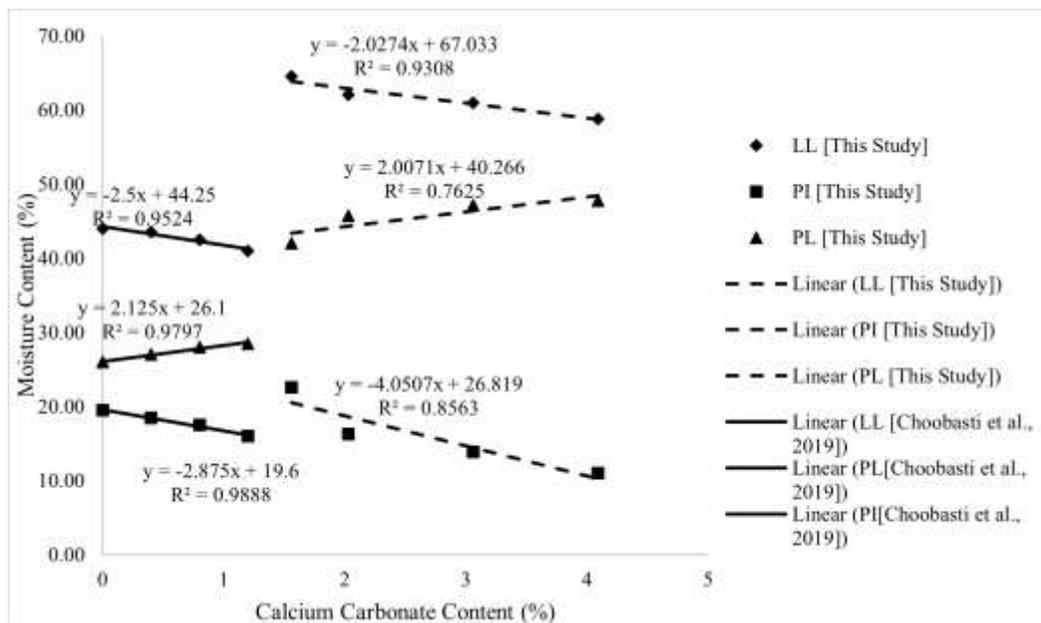


Figure 2. Relationship between Atterberg Limits with calcium carbonate content

sources of Choobasti *et al.* [30]. As can be seen in the Figure, there is a linear inverse relationship between liquid limit and calcium carbonate precipitations formed. The variation between LL and CCC obtained is consistent with that reported by Choobasti *et al.* [30]. The coefficient of regression for the variation of LL with CCC was found to be 0.9308 for this studies while that of Choobasti *et al.* [30] was 0.952. The Figure also depicts that plastic limits increase with the increment in the

calcium carbonate precipitations as shown in this study and also PL rises with the increment in nano-calcium carbonate as reported in Choobbasti *et al.* [30]. It can also be seen in the Figure that plasticity index falls steadily with the increment in both calcium carbonate precipitations (as found in this study) and nano calcium carbonate as reported in Choobbasti *et al.* [30]. The variation between PI and CCC have R^2 values of 0.8563 and 0.9888 in this study and Choobbasti *et al.* [30], respectively. Similarly Musso *et al.* [35] and Howayek *et al.* [36] also reported decrease in liquid limit with increase in calcium carbonate. The decrease in LL and corresponding increase and decrease in PL and PI, respectively can also be explained in terms of cementation of soil particles through the action of calcium carbonate precipitates formed from biocementation technique [32]. Howayek *et al.* [36] also attributed increase in liquid limit and plasticity index of soil to the increase in surface area when cementation between, in this case carbonate, was removed from the soil aggregates. Thus, the decrease in LL and PL limits in this study can be associated with the formation of cementation between clay particles due to the formation of calcium carbonates via EICP.

3.4 Microstructure analysis

3.4.1 X-Diffraction (XRD) analysis

XRD analysis was conducted on both the untreated and treated EICP residual to determine the change in the mineral content due to EICP treatment. Figure 3 (a and b) depicts XRD patterns in both the untreated and EICP treated soil. Kaolinite was found to be the dominant mineral in the untreated soil, and the peaks showing the presence of calcite or calcium carbonate minerals detected as shown in Figure 3(a). XRD pattern depicted in Figure 3 (b) shows the presence of calcium carbonate and calcite minerals in addition to kaolinite mineral. Thus, EICP treatment resulted in the formation of calcite minerals in the soil. The presence of calcite minerals due EICP treatment was reported in sandy soils by Yasuhara *et al.* [19].

3.4.2 Scanning electron microscope (SEM) and electron dispersive X-ray (EDX)

The scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analyses were performed on the natural soil and treated soil in order to confirm the formation of calcium carbonate in the EICP treated soil. Figure 4 shows the SEM and EDX analyses of both natural and treated soils. Calcium carbonate crystals were deposited on the treated soil (see Figure 4 (b)). The presence of calcium carbonate was confirmed by EDX analysis and is shown on the SEM images. As shown in Figure 4 (b), in addition to oxygen, calcium and carbon were detected in the treated soil which indicates the production of calcium carbonate as result of the biocementation via EICP technique, where as there was no detection of calcium and carbon in the untreated soil as depicted in Figure 4 (a). This indicates that CaCO_3 was responsible for the improvement in the plasticity behaviour of the EICP treated soil. The finding in this study is supported by studies reported by Almajed *et al.* [17] and Kavazanjian and Hamdan [37]. For instance, Kavazanjian and Hamdan [37] observed formation of visible white precipitates on the SEM images of EICP treated soil. The precipitations were verified to be CaCO_3 minerals by EDX analysis.

It should be noted that the amount of calcium carbonate content (CCC) determined using gravimetric acid for 0.50 M EICP treated soil was 2.03%, which was higher than the percentage of calcium, 1.7%, as determined by the EDX method. The reason for this discrepancy as explained by Choi *et al.* [27], could be that CCC determined from gravimetric acid wash of the EICP treated soil may tend to be overestimated due to dissolution of non-calcium carbonate substance. Nevertheless, the results obtained from gravimetric acid wash, XRD, SEM-EDX have confirmed the production of calcium carbonate precipitation due to EICP treatment.

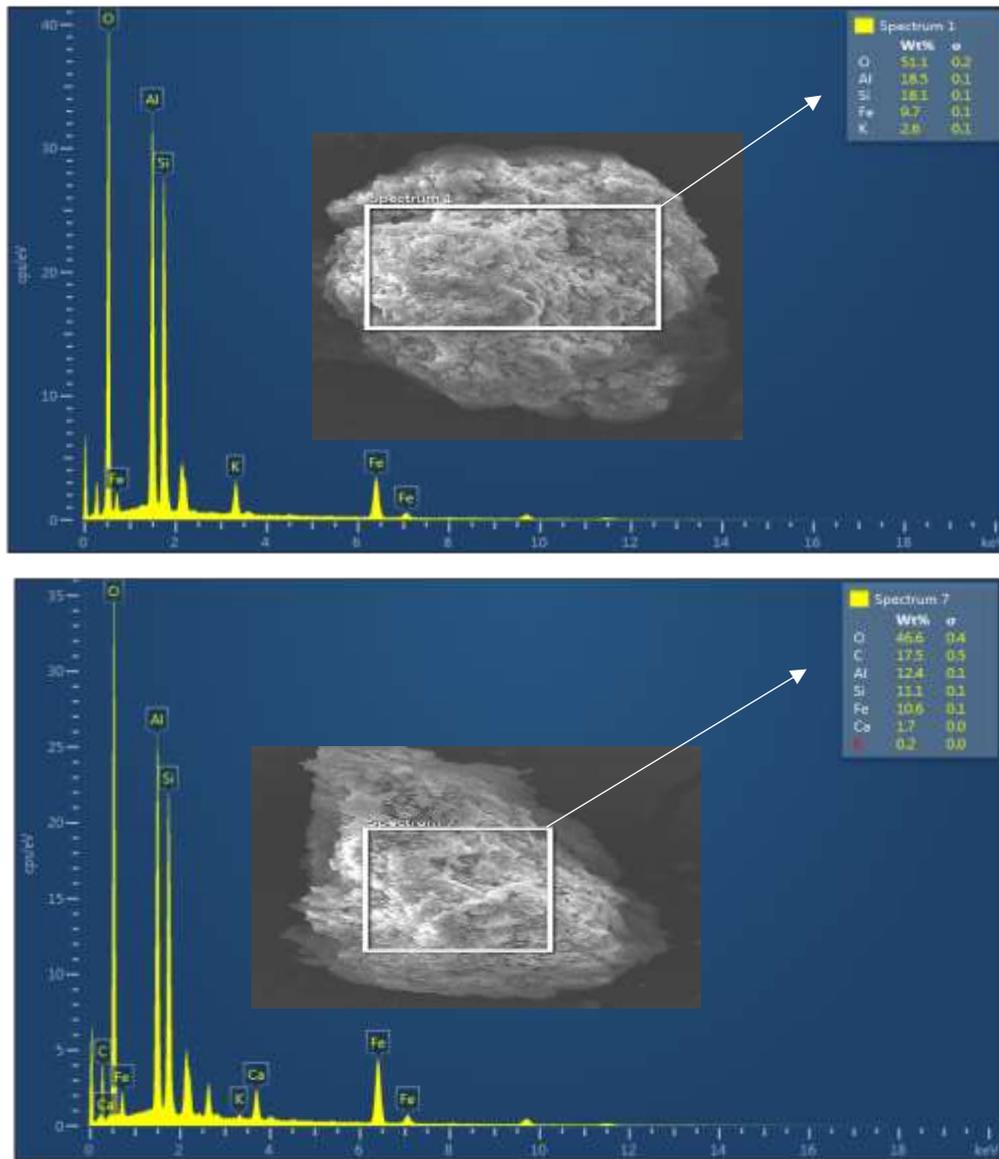


Figure 4. SEM and EDX analysis of (a) untreated residual, (b) EICP-treated soil at 0.50 M

4. Conclusions

In this study, an enzymatically-induced calcite precipitation technique for improving the plasticity and swelling behavior of residual clay soil was presented. The following conclusions are made:

- i. It was found that due to the EICP treatment on the residual soil, the plasticity and swelling characteristics of treated soil were improved.

- ii. The liquid limit, plasticity index and MFSI of the treated soil were found to decrease with an increase in the calcium carbonate content due to an increment in the concentration of cementation solution.
- iii. The formation of calcium carbonate precipitation was confirmed via SEM and EDX analyses, and the formation of calcite was established through XRD analysis.

5. Acknowledgements

The authors gratefully acknowledge the financial support from the Malaysian Ministry of Higher Education (MOHE) through the Fundamental Research Grant Scheme (FRGS): (R.J130000.7851.5F256), GUP from UTM: Q.J130000.2522.20H21 and Q.J130000.2451.04G57 from UTM. The first and last authors also acknowledge the funding coming from the Nigerian Government through TETFUND.

References

- [1] Achal, V., Mukherjee, A., Kumari, D. and Zhang, Q., 2015. Biomineralization for sustainable construction - A review of processes and applications. *Earth-Science Reviews*, 148, 1-17.
- [2] Anbu, P., Kang, C.-H., Shin, Y.-J. and So, J.-S., 2016. Formations of calcium carbonate minerals by bacteria and its multiple applications. *Springerplus*, 5, 250, <https://doi.org/10.1186/s40064-016-1869-2>.
- [3] Wang, Z., Zhang, N., Cai, G., Jin, Y., Ding, N. and Shen, D., 2017. Review of ground improvement using microbial induced carbonate precipitation (MICP). *Marine Georesources & Geotechnology*, 35(8), 1135-1146.
- [4] Omoregie, A.I., Ngu, L.H., Ong, D.E.L. and Nissom, P.M., 2019. Low-cost cultivation of *Sporosarcina pasteurii* strain in food-grade yeast extract medium for microbially induced carbonate precipitation (MICP) application. *Biocatalysis and Agricultural Biotechnology*, 17(11), 247-255.
- [5] Wen, K., Li, Y., Amini, F. and Li, L., 2020. Impact of bacteria and urease concentration on precipitation kinetics and crystal morphology of calcium carbonate. *Acta Geotechnica*, 15(1), 17-27.
- [6] Umar, M., Kassim, K.A. and Chiet, K.T. P., 2016. Biological process of soil improvement in civil engineering: A review. *Journal of Rock Mechanics and Geotechnical Engineering*, 8(5), 767-774.
- [7] Tian, Z.-F., Tang, X., Xiu, Z.-L. and Xue, Z.-J., 2020. Effect of different biological solutions on microbially induced carbonate precipitation and reinforcement of sand. *Marine Georesources & Geotechnology*, 38(4), 450-460.
- [8] 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(11), 105389, <https://doi.org/10.1016/j.enggeo.2019.105389>.
- [9] Morales, L., Garzón, E., Romero, E. and Sánchez-Soto, P.J., 2019. Microbiological induced carbonate (CaCO₃) precipitation using clay phyllites to replace chemical stabilizers (cement or lime). *Applied Clay Science*, 174(3), 15-28.
- [10] Vail, M., Zhu, C., Tang, C.S., Anderson, L., Moroski, M. and Montalbo-Lombay, M.T., 2019. Desiccation cracking behavior of MICP_treated bentonite. *Geosciences*, 9(9), 385, <https://doi.org/10.3390/geosciences9090385>

- [11] Li, M., Fang, C., Kawasaki, S. and Achal, V., 2018. Fly ash incorporated with biocement to improve strength of expansive soil. *Scientific Report*, 8, <https://doi.org/10.1038/s41598-018-20921-0>
- [12] Hataf, N. and Baharifard, A., 2020. Reducing soil permeability using microbial induced carbonate precipitation (MICP) method : A case study of Shiraz landfill soil. *Geomicrobiology Journal*, 37(2), 147-158.
- [13] Mitchell, J.K. and Santamarina, J.C., 2005. Biological considerations in geotechnical engineering. *Journal of Geotechnical and Geoenvironmental Engineering*, 131(10), [https://doi.org/10.1061/\(ASCE\)1090-0241\(2005\)131:10\(1222\)](https://doi.org/10.1061/(ASCE)1090-0241(2005)131:10(1222))
- [14] Hamdan, N., Kavazanjian, E. and O'Donnell, S., 2013. Carbonate cementation via plant derived urease. *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering*, Paris, France, 2013, 2489-2492.
- [15] Almajed, A., Tirkolaei, H.K. and Kavazanjian, E., 2018. Baseline investigation on enzyme-induced calcium carbonate precipitation. *Journal Geotechnical and Geoenvironmental Engineering*, 144 (11), [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0001973](https://doi.org/10.1061/(ASCE)GT.1943-5606.0001973)
- [16] Rohy, H., Arab, M., Zeiada, W., Omar, M., Almajed, A. and Tahmaz, A., 2019. One phase soil bio-cementation with eicp-soil mixing. *Proceedings of the 4th World Congress on Civil, Structural, and Environmental Engineering*, <https://doi.org/10.11159/icgre19.164>
- [17] Almajed, A., Tirkolaei, H.K., Kavazanjian, E. and Hamdan, N., 2019. Enzyme induced biocementated sand with high strength at low carbonate content. *Scientific Reports*, 9(1), 1135, <https://doi.org/10.1038/s41598-018-38361-1>
- [18] Simatupang, M and Okamura, M., 2017. Liquefaction resistance of sand remediated with carbonate precipitation at different degrees of saturation during curing. *Soils and Foundations*, 57(4), 619–631.
- [19] Yasuhara, H., Neupane, D., Hayashi, K and Okamura, M., 2012. Experiments and predictions of physical properties of sand cemented by enzymatically-induced carbonate precipitation. *Soils and Foundations*, 52(3), 539-549.
- [20] Neupane, D., Yasuhara, H., Kinoshita, N. and Unno, T., 2013. Applicability of enzymatic calcium carbonate precipitation as a soil-strengthening technique. *Journal of Geotechnical and Geoenvironmental Engineering*, 139(12), 2201-2211.
- [21] Zhao, Z., Hamdan, N., Shen, L., Nan, H., Almajed, A., Kavazanjina, E. and He, X., 2016. Biomimetic hydrogel composites for soil stabilization and contaminant mitigation. *Environmental Science and Technology*, 50(22), 12401-12410.
- [22] Oliveira, P.J.V., Freitas, L.D. and Carmona, J.P.S.F., 2017. Effect of soil type on the enzymatic calcium carbonate precipitation process used for soil improvement. *Journal of Materials and Civil Engineering*, 29(4), [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0001804](https://doi.org/10.1061/(ASCE)MT.1943-5533.0001804)
- [23] Chandra, A. and Karangat, R., 2019. Effect of magnesium incorporation in Enzyme Induced Carbonate Precipitation (EICP) to improve shear strength of soil. In A. Prashant, A. Sachan, and C.S. Desai, eds. *Advances in Computer Methods and Geomechanics*, https://doi.org/10.1007/978-981-15-0890-5_28
- [24] Cuccurullo, A., Gallipoli, D., Bruno, A.W., Augarde, C., Hughes, P. and Borderie, C.L., 2019. Advances in the enzymatic stabilisation of soils. *Proceedings of the XVII European Conference: Geotechnical Engineering Foundation of the Future*. September, 2019, <https://doi.org/10.32075/17ECSMGE-2019-0987>.
- [25] Osinubi, K.J., Eberemu, A.O., Gadzama, E.W. and Ijimdiya, T.S., 2019. Plasticity characteristics of lateritic soil treated with *Sporosarcina pasteurii* in microbial-induced calcite precipitation application. *SN Applied Sciences*, 1, 829, <https://doi.org/10.1007/s42452-019-0868-7>.
- [26] BS 1377-2, 1990. *Methods of Test for Soils for Civil Engineering Purposes-Part 2: Classification Tests*. London: British Standards Institute.

- [27] Choi, S.-G., Park, S.-S., Wu, S. and Chu, J., 2017. Methods for calcium carbonate content measurement of biocemented soils. *Journal of Material and Civil Engineering*, 29(11), [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0002064](https://doi.org/10.1061/(ASCE)MT.1943-5533.0002064).
- [28] Widomski, M.K., Stepniewski, W. and Musz-Pomorska, A., 2018. Clays of different plasticity as materials for landfill liners in rural systems of sustainable waste management. *Sustainability*, 10(7), 2489, <https://doi.org/10.3390/su10072489>
- [29] EPA, 1993. *Solid Waste Disposal Facility Criteria. Technical Manual*. Washington D.C.: United States Environmental Protection Agency.
- [30] Choobasti, A.J., Samakoosh, M.A. and Kutanaei, S.S., 2019. Mechanical properties soil stabilized with nano calcium carbonate and reinforced with carpet waste fibers. *Constructions and Building Materials*, 211, 1094-1104
- [31] Yazarloo, R., Katooli, F.A., Golestani, M., Asadi, M. and Ebrahimi, S., 2017. Adding calcite and nanocalcite to improving the plastic properties of the lean clay. *Proceeding of the 3rd World Congress on New Technology*, Rome, Italy, June 6-8, 2017, <https://doi.org/10.11159/icnfa17.106>.
- [32] Moravej, S., Habibagahi, G., Nikooee, E. and Niazi, A., 2017. Stabilization of dispersive soils by means of biological calcite precipitation. *Geoderma*, 315, 130-137.
- [33] Kannan, K., Bindu, J. and Vinod, P., 2020. Engineering behaviour of MICP treated marine clays. *Marine Georesources and Geotechnology*, 38(7), 761-769.
- [34] Pei, X., Zhang, F., Wu, W. and Liang, S., 2015. Physicochemical and index properties of loess stabilized with lime and fly ash piles. *Applied Clay Science*, 114, 77-84.
- [35] Musso, G., Chighini, S. and Romero, E., 2008. Mechanical sensitivity to hydrochemical processes of Monastero Bormida clay. *Water Resources Research*, 44(5), <https://doi.org/10.1029/2007wr006533>
- [36] Howayek, A.E., Bobet, A. and Santagata, M., 2019. Microstructure and cementation of two carbonatic fine-grained soils. *Canadian Geotechnical Journal*, 56(3), 320-334.
- [37] Kavazanjian, E. and Hamdan, N., 2015. Enzyme induced carbonate precipitation (EICP) columns for Ground Improvement. *Proceedings of the International Foundations Congress and Equipment Expo*, Texas, USA, <https://doi.org/10.1061/9780784479087>