Effects of cementation reagent on the precipitation of calcium carbonate induced by Bacillus Megaterium

A S Muhammed1-2* M U Zango3 K A Kassim1 and K Ahmad1 M Umar1

1) Department of Civil Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
2) Department of Civil and Water Resources Engineering, Unimaid. P. M. B. 1069, Maiduguri. Borno State, Nigeria
3) Department of Civil Engineering, KUST Wudil P. M. B. 3244, Kano State, Nigeria

Abstract. A laboratory experiment was carried out to determine the concentration of cementation reagent that will produce the maximal amount of calcium carbonate induced by Bacillus Megaterium. The optimum condition for calcium carbonate precipitation was evaluated for its application in improving the geotechnical properties of soil. The process was studied using the test-tube experiment and evaluating the amount of calcium carbonate precipitated and subsequently verified using X-ray diffraction test. Five different concentrations of cementation reagent (0.25, 0.5, 0.75, 1.0 and 1.5M) were used in the study. Results showed calcium carbonate was higher with increase in concentration of cementation reagent irrespective of the curing period. Furthermore, the XRD scan confirmed the precipitate formed was calcium carbonate. Calcite formed acts ad bio-cement which is responsible for improving the geotechnical properties of various soil.

1. Introduction

Microbial induced calcium carbonate precipitation (MICP) is one of the most important bio-mineralization process that is receiving much attention in recent years because of their applications in the field of biotechnology, geotechnology and civil engineering [1]. More ever MICP was shown to be effective in increasing shear strength and stiffness of soil through bio-cement formation [2, 3, 4]; decreasing the permeability of soil through formation of bio-grouts [5, 6, 7]. Formation of microbial calcite precipitate (CaCO3) can occur through various biochemical pathways. So far, the identified biochemical processes capable of inducing calcium carbonates are ureolysis, denitriﬁcation, sulphate reduction and iron reduction [8]. MICP via ureolysis is the most efficient and easily controlled mechanism that can produce 3.8 - 7.4% precipitate

CaCO3 within 24 – 48 hours [9], thus it has become the most studied and utilized process of inducing calcium carbonate precipitates. In a simple form, MICP via ureolysis is accomplished in two stages. The first stage involves hydrolysis of urea by the action of urease enzyme secreted by urease producing bacteria. Equation 1 – 2 describe how urea is reduced to ammonium (NH4+) and carbonate ion (CO32-) [10].
\[ \text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{CO}_2 \] (1)

\[ \text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{CO}_2^{2-} + \text{OH}^- \quad \text{pH} \uparrow \] (2)

The second stage is the formation of calcium carbonate which is accomplished by the association of calcium ions and carbonates ion and the reaction is reversible but it is shifted to the right to form calcium carbonate as shown in equation 3 when the environmental condition is favourably alkaline – with pH between 7.5 – 9.5 [6] [11]. The source of calcium ions during the ureolysis reaction is normally calcium chloride otherwise known as cementation reagent when it is combined with urea.

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3 \] (3)

MICP controlled ureolysis as catalytic reaction is influenced by many environmental factors such as temperature, pH of the medium, bacterial concentration and cementation reagent. Many studies had been conducted to determine the optimum parameters that will give rise to maximum calcium carbonate precipitates via ureolysis [12, 13, 14]. Among those mentioned factors influencing MICP, initial concentration of cementation reagent remains the unverified result. For instance, [6] had conducted laboratory studied on the variation of cementation reagent to produce calcite precipitates in residual soil using Klebsiella pneumoniae UM123 urease producing bacteria strain. From the studies it was determine that the optimum cementation reagent was 0.5 M. [15] had studied the effect of cementation reagent on the pH, ammonium content, shear strength and the amount of calcite formed. It was determined that high amount calcite precipitates and maximum strength were determine at 0.5 M. But, [16] determined that optimum concentration of cementation reagent when Bacillus sp. strain was used to induce calcium carbonate precipitates was 0.25 M. In another experimental study [13] found that the amount of calcite formed when sporosarcina pasteurii was used for MICP increased by 100% when the initial concentration of Ca\(^{2+}\) increases by 10-fold from 0.025 M to 0.25 M. They also determined that the optimum concentration of cementation reagent solution for best MICP performance were at 0.666 M urea and 0.25 M initial Ca\(^{2+}\) concentrations. [17] argues that efficient ureolysis and calcite formation can be obtained when equal concentration of calcium chloride and urea are used in the formation of cementation reagent. Similarly, authors like [18] reported that the amount of calcium carbonate formed is proportional concentration of cementation reagent, while other authors like [19],[14] reported efficient urea hydrolysis and calcite formation at lower of cementation reagent. Thus, with thus contrasting results on the optimum concentration of cementation reagent there is need to conduct laboratory investigation to ascertain the actual concentration of cementation that will produce maximum amount of calcite precipitates

2. Materials and methods

2.1 Bacillus Megaterium

The urease-producing microorganism used in this study was B. megaterium (strain ATCC 14581). B. megaterium is a Gram-positive bacterium that can be found in a broad range of habitat; however, it is mainly found in soil [20]. B. megaterium has been proven to have the ability to induce calcite precipitation in natural soils [21]. The bacteria cells required for the experiment were initially grown on a solid nutrient medium (tryptic soy agar) and a single colony was then transferred to liquid growth medium (nutrient broth). The liquid growth media containing the bacteria colony was incubated at 30 °C under agitation for a period of 24 hours to attain the stationary stage of the cell growth curve.

The concentration of cells suspended in the stock culture was estimated by the expression shown in equation 1, after [22].

\[ Y = 8.59 \times 10^7 \cdot Z^{1.3627} \] (4)

Where Z is reading at OD\(_{600}\), and Y is the concentration of cells mL\(^{-1}\).
2.2 Cementation Reagents
Cementation reagents serve as important ingredients for promoting calcite precipitation. The cementation reagents used consists of urea, calcium chloride at different concentrations in addition to other supplements such as nutrient broth, ammonium chloride and sodium bicarbonate. The concentrations were varied to evaluate their influence on ureolytic-driven calcium carbonate precipitations. Table 1 shows the components of the cementation reagents and their masses.

Table 1: Chemical composition of cementation reagents.

| Concentration (M) | 0.25 | 0.5  | 0.75 | 1.0  | 1.5  |
|-------------------|------|------|------|------|------|
| Urea (g/l)        | 15   | 30   | 45   | 60   | 90   |
| CaCl₂ (g/l)       | 27.8 | 55.5 | 83.3 | 111  | 165.5|
| Nutrient broth (g/l) | 3   | 3    | 3    | 3    | 3    |
| Ammonium Chloride (g/l) | 10  | 10   | 10   | 10   | 10   |
| Sodium Bicarbonate(g/l) | 2.12| 2.12 | 2.12 | 2.12 | 2.12 |

2.3 Test-tube experiments
In this work, the precipitation of carbonate was evaluated directly in polypropylene (PP) tubes. The experimental procedures developed by [23, 24] were adopted in this work. Predetermined masses of the cementation reagents were dissolved into distilled water based on the different equimolar requirements (Table 1), the solution was thoroughly mixed to ensure the chemical products were completely dissolved. Then, 15ml of bacterial solution and cementation reagents each were mixed in the test tubes, resulting in a total solution volume of 30 ml. The test tubes were then placed in a temperature-controlled room (30± 2°C) for a specified curing time of 3days. At the end of the curing period, the solution was filtered, the particles deposited on the filter paper and remaining in the tubes were dried, and their amounts are measured. The total amount of calcium carbonate was determined as the sum of the material deposited on the filter paper and on the bottom of the test tube. Each stage of the test was repeated two times to guarantee the reliability of the procedure.

X-ray diffraction test was carried out on the precipitate to verify the calcium carbonate formed during the process. The output data of the XRD test was analysed and compared using the standard calcite (PDF 01-080-9775) and Vaterite (PDF 01-080-4618) Phases collected from the Power Diffraction File Database of the International Centre for Diffraction Data (ICDD).

3. Results and discussions
Figure 1 show the amounts of calcium carbonate precipitation by B. Megaterium over the range of cementation reagent concentration. Results showed a general increase in carbonate precipitates with increase in concentration of cementation reagent irrespective of the curing period. For the 3 days curing period, the calcite precipitate increased from 60 – 490mg for concentration of cementation reagent of 0.25 – 1.5M having a percentage increase of 87.8%.
Similarly, for the curing periods of 7 and 14 days, the percentage increase in the precipitate over the same range cementation reagent concentration are 72.5 % and 61% respectively. The increase in calcite precipitate with higher concentration of cementation reagent could be attributed to the fact that higher urea concentration gave rise to more localized rise in pH around the bacterial cells as more urea molecules are available, and thus making the precipitate higher [25].

Similar trend was observed by [26] when S. aquimarina and S. pasteurii where used to optimize the calcium carbonate precipitation at different combination, urea and calcium. [13] investigated various non-equimolar cementation solution (CS) and concluded that a higher amount of deposited calcium carbonate was associated with a higher CS concentration.

![Figure 1: Variations of calcium carbonate precipitate with different concentration of concentration reagent.](image1)

![Figure 2: XRD analysis of the material precipitated in the test-tube experiment.](image2)
The formation of calcium carbonate was confirmed by the results of XRD shown in figure 2. According to ICCD, the major peak for calcium carbonate is usually found at 29° while other lower peaks were found at 36°, 39°, and 48° which corresponds to the peaks on the diffractogram for the precipitate. Traces of Vaterite were also detected as part of the minerals contained in the precipitate formed.

4. Conclusions
From the results of the test-tube experiments carried out to study the efficacy of calcium carbonate precipitation for various concentration of cementation reagent (urea - calcium chloride), the following conclusion were drawn.

1. Bacillus Megaterium was able to produce urease enzyme that was required for the process of calcium carbonate precipitation through urea hydrolysis.
2. Higher calcium carbonate precipitation was observed with higher concentration of cementation reagent.
3. Precipitate formed from the experiment was able to be confirmed that calcium carbonate was formed.

References
[1] N. K. Dhami, M. S. Reddy, and A. Mukherjee, “Biomineralization of calcium carbonates and their engineered applications: a review,” Front. Microbiol., vol. 4, Oct. 2013.
[2] A. Zamani and B. M. Montoya, “Shearing and Hydraulic Behavior of MICP Treated Silty Sand,” in Geotechnical Frontiers 2017, 2017, pp. 290–299.
[3] J. T. Dejong, “Biologically induced improvement of loose sand,” no. January, 2006.
[4] G. G. N. N. Amarakoon and S. Kawasaki, “Factors Affecting the Improvement of Sand Properties Treated with Microbially-Induced Calcite Precipitation,” Geo-Chicago 2016, pp. 72–83, 2016.
[5] J. T. Dejong et al., “Biogeochemical processes and geotechnical applications: progress, opportunities and challenges,” Géotechnique, vol. 63, no. 4, pp. 287–301, 2013.
[6] Murtala Umar & Khairul Anuar Kassim, “Effect of cementation reagent concentrations on microbial calcite precipitation in residual soil,” Malaysian J. Civ. Eng. 29 Spec Issue, vol. 78, no. 1, pp. 79–90, 2017.
[7] N. W. Soon, L. M. Lee, T. C. Khun, and H. S. Ling, “Improvements in engineering properties of soils through microbial-induced calcite precipitation,” KSCE J. Civ. Eng., vol. 17, no. 4, pp. 718–728, Oct. 2013.
[8] T. Zhu and M. Dittrich, “Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review,” Front. Bioeng. Biotechnol., vol. 4, no. January, pp. 1–21, 2016.
[9] M. B. Burbank, T. J. Weaver, B. C. Williams, and R. L. Crawford, “Urease Activity of Ureolytic Bacteria Isolated from Six Soils in which Calcite was Precipitated by Indigenous Bacteria,” Geomicrobiol. J., vol. 29, no. 4, pp. 389–395, 2012.
[10] C.-W. Chou, E. A. Seagren, A. H. Aydilek, and M. Lai, “Biocalcification of Sand through Ureolysis,” J. Geotech. Geoenvironmental Eng., vol. 137, no. 12, pp. 1179–1189, 2011.
[11] S. Stocks-Fischer, J. K. Galinat, and S. S. Bang, “Microbiological precipitation of CaCO3,” Soil Biol. Biochem., vol. 31, no. 11, pp. 1563–1571, 1999.
[12] F. Hammes, W. Verstraete*, and W. Verstraete, “Key roles of pH and calcium metabolism in microbial carbonate precipitation,” Re/Views Environ. Sci. Bio/Technology, vol. 1, no. Morita 1980, pp. 3–7/2002.
[13] G. D. O. Okwadha and J. Li, “Optimum conditions for microbial carbonate precipitation,” Chemosphere, vol. 81, no. 9, pp. 1143–1148, 2010.
[14] W. De Muynck, N. De Belie, and W. Verstraete, “Microbial carbonate precipitation in construction materials: a review,” *Ecol. Eng.*, vol. 36, no. 2, pp. 118–136, Oct. 2010.

[15] L. M. Lee, W. S. Ng, C. K. Tan, and S. L. Hii, “Bio-Mediated Soil Improvement under Various Concentrations of Cementation Reagent,” *Appl. Mech. Mater.*, vol. 204–208, pp. 326–329, 2012.

[16] K. T. P. Chiet, K. A. Kassim, K. B. Chen, U. Martula, C. S. Yah, and A. Arefnia, “Effect of Reagents Concentration on Bio cementation of Tropical Residual Soil,” in *IOP Conference Series: Materials Science and Engineering*, 2016, vol. 136, no. 1.

[17] M. Nemati, E. A. Greene, and G. Voordouw, “Permeability profile modification using bacterially formed calcium carbonate: Comparison with enzymic option,” *Process Biochem.*, vol. 40, no. 2, pp. 925–933, 2005.

[18] V. S. Whiffin, “Microbial CaCO3 Precipitation for the Production of Biocement,” 2004.

[19] A. Al Qabany et al., “Factors affecting efficiency of microbially induced calcite precipitation,” *J. Geotech. Geoenvironmental Eng.*, vol. 138, no. 8, pp. 992–1001, 2012.

[20] P. S. Vary, “Prime time for Bacillus Megaterium,” *Prime time Bacillus megaterium*, vol. 140, pp. 1001–1013, 1994.

[21] B. Lian, Q. Hu, J. Chen, J. Ji, and H. H. Teng, “Carbonate biomineralization induced by soil bacterium Bacillus megaterium,” *Carbonate Biominer. Induc. by soil Bact. Bacillus megaterium*, vol. 70, pp. 5522–5535, 2006.

[22] S. S. B. Santhosh K. Ramachandran, V. Ramakrishnan, “Microorganisms, Remediation of Concrete Using,” *Microorg. Remediat. Concr. Using*, vol. 98, no. 1, pp. 3–9, 2001.

[23] D. Neupane, A. M. Asce, H. Yasuhara, N. Kinoshita, and T. Unno, “Applicability of Enzymatic Calcium Carbonate Precipitation as a Soil-Strengthening Technique,” *J. Geotech. Geoenvironmental Eng.*, vol. 139, no. 12, pp. 2201–2212, 2013.

[24] H. Putra, H. Yasuhara, and N. Kinoshita, “Optimum Condition for the Application of Enzyme-Mediated Calcite Precipitation Technique as Soil Improvement Method Optimum Condition for the Application of Enzyme-Mediated Calcite Precipitation Technique as Soil Improvement Method,” *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 7, no. 6, pp. 2145–2151, 2017.

[25] D. Li, K. Tian, H. Zhang, Y. Wu, K. Nie, and S. Zhang, “Experimental investigation of solidifying desert aeolian sand using microbially induced calcite precipitation,” *Constr. Build. Mater.*, vol. 172, pp. 251–262, 2018.

[26] H. A. Keykha, A. Asadi, B. B. K. Huat, and S. Kawasaki, “Laboratory Conditions for Maximal Calcium Carbonate Precipitation Induced by Sporosarcina pasteurii and Sporosarcina aquimarina Bacteria,” *Environ. Geotech.*, no. January, pp. 1–20, 2018.

**Acknowledgement**

The authors wish to acknowledge with appreciation the financial support received from high impact research grants HIR 04G57, Malaysia Government and technical support by the Geotechnical Engineering laboratory staff, Universiti Teknologi Malaysia.