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Abstract

Microbial-Induced Calcium Carbonate Precipitation (MICP) is evolving as a new method of improving the mechanical properties of soil. This environmentally friendly technique is a bio-geo-chemical process where microbes play a key role in increasing soil strength through precipitating calcium carbonate. Past studies at Boise State University have indicated that MICP via bio-stimulation could be a viable alternative for expansive clayey soil treatments. However, these studies raised a new question about the relationship between soil composition, urease activity, and calcite precipitation. To answer this question, batch studies were conducted using autoclaved-sterilized sand mixed with different percentages of non-sterile natural clay and tested for urease activity. Moreover, to investigate the difference in urease activity between sand and clay bacterial communities, experiments were repeated on samples with different amounts of non-sterile sand and autoclaved-sterilized clay. MICP-treated clay/sand mixes were then evaluated for calcite precipitation. Our results showed that soil mixes with higher clay content have more urease activity and higher levels of calcite precipitation for both sand-autoclaved and clay-autoclaved soil mixes. Test results indicate that urease activity could potentially be used as an indicator of MICP performance in different soil compositions.

Introduction

MICP is becoming a considerable soil improvement method for various civil engineering and environmental applications. In MICP, bacteria hydrolyze urea to raise the pH of the system, and carbonate is produced, which in turn results in the precipitation of calcium carbonate ($CaCO_3$) in the presence of a calcium source(Qabany & Soga, 2013). The precipitated calcium carbonate can coat soil particles, cement the soil matrix, and fill the void space between the particles, thereby increasing soil strength, stiffness, and dilatancy of soil (Lin et al., 2016).

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

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

MICP in soils is performed either by augmenting the soil with ureolytic bacteria (bio-augmentation) or by stimulating indigenous ureolytic bacteria (bio-stimulation). Both of these methods lead to changes in the indigenous bacterial population composition and the accumulation of large quantities of ammonium (Gat et al., 2016). As a part of the biostimulated MICP (Gat et al., 2016) studied the effect of urea and nutrient enrichment on indigenous bacterial populations in poor nutrient sand. Their result showed a significant change in the bacterial population following the

stimulation of ureolysis. These treatments also exhibited an increase in the relative abundance of Bacilli, suggesting that urea hydrolyzing bacteria belong to this bacterial class. Biogeochemical differences between bio-cementation mediated by native ureolytic microorganisms and augmented *S. pasteurii* was investigated by Gomez et al. (2019). Although their results confirmed that calcite and engineering properties were similar between approaches, they suggested that there is a significant difference in ureolysis rates and related precipitation rates, which may influence the temporal progression and spatial distribution of bio-cementation. Burbank et al. (2011) showed that the number of ureolytic bacteria and the precipitate calcite could be increased as a result of bio-stimulation in a variety of soils. In another research, Burbank et al. (2012) measured urease activity of 10 distinct strains of cultivable bacteria isolated from different sands and showed that there was at least one type of bacteria in each soil sample that was capable of urea hydrolysis even in the presence of a high concentration of ammonia. Many researchers also demonstrated that the prevalence of ureolytic communities had been widely seen in different soils irrespective of the type, mineralogy, and environmental conditions (Burbank et al., 2012; Fujita et al., 2008; Gat et al., 2016; Lloyd & Sheaffe, 1973; Zhu & Dittrich, 2016).

The soil's void ratio is one of the most important factors that can affect nutrient access and growth of the bacteria living in soil pores (Cardoso et al., 2018; Cheng et al., 2013; Cheng & Cord-Ruwisch, 2014; Terzis et al., 2016). Due to the large voids size and easy application, MICP is mainly used for the improvement of sandy soils. However, there are only very few studies about the effect of clay's chemical and physical interaction on MICP and calcite precipitation (Cardoso et al., 2018). Moreover, there are no studies on the impact of soil composition (clay content) on urease activity. Therefore, in this work, we investigate the changes in urease activity and efficiency of calcite precipitation in different soil compositions (clay/sand mixes). Also, the role of ureolytic bacterial communities in sand and clay are investigated.

Materials and Methods

To study the effect of variation of the bacterial source on MICP and to see if there is any correlation between calcite precipitation, microbial community, and urease activity in different soils, nine artificial soil samples each weighing 1600g were prepared. Soil samples were prepared by mixing different amounts (wt.%) of non-sterile natural clay (liquid limit=75, Plasticity Index=36.3), from Marsing, Idaho with an autoclaved and sterilized medium to fine sand (D_{60} = 0.68, D_{10} = 0.24, & Cu = 2.83). Another set of 9 samples were prepared by autoclaving the clays and mix with different amounts of natural sand (non-autoclaved).

Treatment Solutions

Two types of treatment solutions were used in this research to achieve bio-mineralization: *enrichment solution* and *cementation solution*. Enrichment solutions contained both carbon and nitrogen sources along with other necessary nutrients to facilitate bacterial growth. As recommended by (Burbank et al., 2011), the enrichment solutions consisted of 100 mM of sodium acetate, 333 mM of urea, and 0.5 g/L of corn steep liquor (CSL). Corn steep liquor consisted of amino acids, vitamins, and minerals and was provided in both enrichment solution and cementation solution (Burbank et al., 2011; Weaver et al., 2011), and is necessary for microorganism survival. The cementation solution differed from the enrichment solution only by the presence of calcium, which fuels calcium carbonate precipitation during MICP. Consequently, the cementation solution consisted of 100 mM of sodium acetate, 333 mM of urea, 0.5 g/L of corn steep liquor (CSL) along with 250 mM of calcium chloride.

Treatment Protocols

Calcite precipitation was achieved by placing soil samples inside sterilized plastic jars (about 3.5 L in volume) and mixing with about 600 mL of enrichment solution (Figure.1a). The samples were left for 48 hrs on the countertop, and then their pH was measured. All samples showed an increase in the pH (>9), indicating that 48 hours was sufficient time to trigger bio-stimulation. After 48 hours, each soil sample was mixed with 600 mL of cementation solution and was left for the bio-mineralization process to start. The pH of samples was measured every 48 hours for seven rounds of bio-cementation treatment. After pH measurement, each soil mix was drained with Whatman #42 (2.5 μm) filter paper, and 600 mL of fresh cementation solution was added. The total length of the bio-cementation rounds was 14 days, and the soil solution pH fluctuated between 9 and 9.5 during the treatment. The treatment stopped when the pH started to decrease. After treatment, soil samples were dried in an oven for 48 hours at 110°C, and their calcite content was measured.

Test Protocol for Urease Activity

Urease activity was determined by measuring ammonia concentration in the samples using the colorimetric method. First, plastic vials were used to mix 10 g of each soil sample with 8 ml of 4288 ppm urea solution in water. The vials were incubated for 5 hours at 37°C, as suggested by Douglas and Bremner (1971) (Figure1b). The contents of each vial were mixed with a 40ml solution of 2.5 M potassium chloride (KCl) - silver sulfate (Ag_2SO_4) (100 parts/10^6)(Tabatabai and Bremner,1971). The resulting mixture was filtered Whatman #42(2.5 µm), and 200 µl of the extract was pipetted into a reaction kit (Ammonia TNT 832, Hach) for colorimetric measurements of ammonia concentration. This was achieved by measuring the optical density (OD) of the extracts using a spectrometer (Figure1c). A higher OD corresponds to a larger concentration of ammonia, as explained by Tabatabai & Bremner (1972) and Verdouw et al. (1978) (Figure1d).

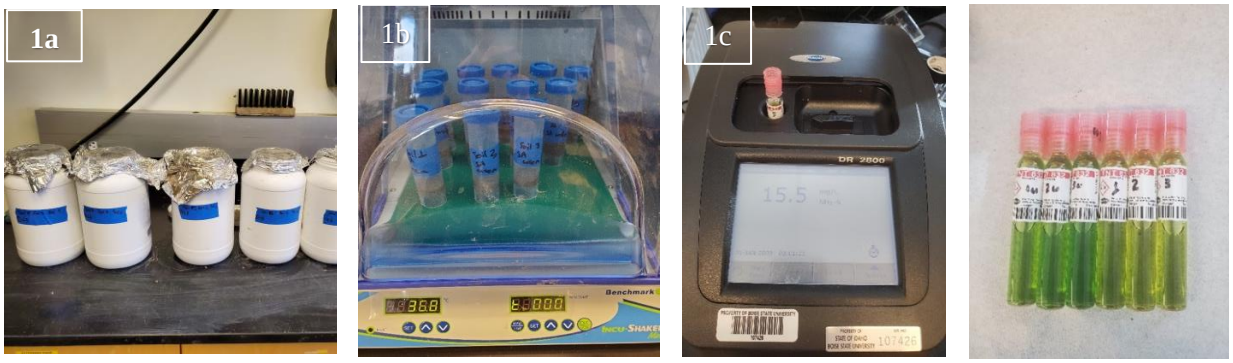

Figure (1a). Plastic jars containing soil mixes. Figure (1b). Incubation of soil samples for 5 hours at 37°C. Figure (1c). Portable spectrophotometer. Figure (1d).Sample vials showing the different concentrations of ammonia.

Test Protocol for Calcite Content Determination

Precipitated calcium carbonate was detected using Rapid Carbonate Analyzer (D4373-96). In this method, calcium carbonate reacts with HCL, as shown in the following reaction (Equation (3)):

$$CaCO_3 + 2H^{+1} \rightarrow Ca^{+2} + H_2O + CO_2 \qquad (3)$$

About 20g pulverized dry soil specimen from the top and bottom was sieved (#10 sieve) and placed into a reactor chamber (Figure. 2). A plastic beaker containing 20±2 mL of HCL solution (1N) was inserted into the reactor. The chamber was then sealed by closing the lid and pressure relief valve. The chamber is swirled such that the acid was mixed and reacted with the soil sample. After 10 minutes of reaction time, the pressure was measured, and the amount of calcite was determined by reading from a calibration chart.

Figure 2. Placing soil mixes into the reactor.

Results and Discussion

Urease Test Results

The clay/sand mixes and their corresponding urease activity are shown in Table 1 and Figure 3. Urease activity exhibits a linear dependence on the sample's composition (R^2>0.97), where samples with higher clay content showed higher urease activity. Clay content, therefore, enhanced the activity of the urease enzyme in the soil samples. This might be because of the hydrophobic bond between enzyme and organic carbon on clays and chemical interaction between the feeding solution and the clay minerals (Boyd & Mortland, 1985; Cardoso et al., 2018; Theng, 2012). Moreover, we observed that for a given composition, urease activity is higher in sand-autoclaved samples compared to clay-autoclaved samples, indicating that the bacterial community in clay might be more active/efficient in catalyzing urea to ammonia.

Table 1. Different soil mixes and urease activity in sand autoclaved and clay autoclaved.

Soil mixes (%)			***sand autoclaved***	***clay autoclaved***
Soil Name	**Sand**	**Clay**	**Urease activity (mg/l)/g of soil**	**Urease activity (mg/l)/g of soil**
Soil1	95	5	0.938	0.898
Soil2	90	10	1.152	1.232
Soil3	85	15	1.539	1.289
Soil4	80	20	2.188	1.558
Soil5	70	30	3.357	1.907
Soil6	60	40	3.715	2.525
Soil7	50	50	4.06	2.43
Soil8	20	80	5.84	3.82
Soil9	0	100	6.62	4.3

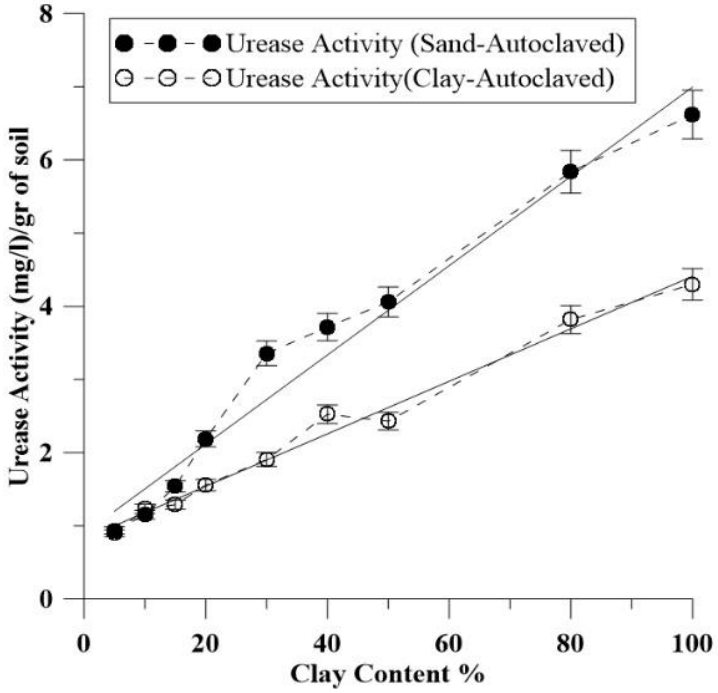

Figure 3. Lower urease activity in clay autoclaved mixes compared to sand autoclaved mixes. Error bars indicate a 10% error.

Carbonate Precipitation Measurements

The difference in calcite precipitation between sand-autoclaved and clay-autoclaved mixes are shown in Figure 4. The amount of precipitation correlates well with urease activity, and the result confirmed that calcite precipitation is more in mixes with higher clay (samples 5 to 9) and less in mixes with higher sand content (samples 1 to 4) (Table 1). Moreover, in tested samples, unlike urease activity, which increased monotonically with clay content (Figure. 3), calcite precipitation saturated at ~40% clay (Figure. 4). This means that calcite precipitation in the samples is independent of urease activity beyond the 40% threshold, and having more clay content increased urease activity but did not result in the formation of more calcite precipitates. Also, we observed that the saturation level for calcite precipitation is higher in sand-autoclaves samples compared with clay-autoclaved samples. This is further evidence that clay bacterial communities might play a more dominant role in MICP compared to bacterial communities in the sand.

We measured calcite precipitation at the top and the bottom of our dried samples. The results are summarized in Figures 5 and 6. A significant gradient is observed in sand-rich mixtures (Figure. 7), which reduces as clay content increases. For samples with >50% clay, the gradient completely diminishes, indicating that uniform precipitation is obtained (Figure. 8). These observations confirm that bio-stimulation and bio-cementation using the mixing method produces a more homogeneous distribution of calcite in the treated clay-rich soils.

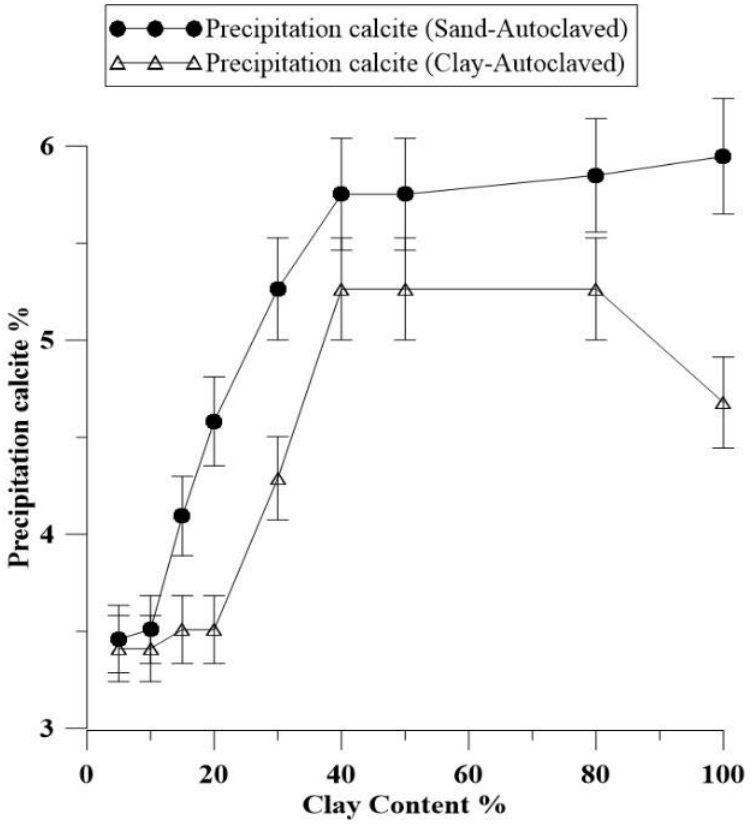

Figure 4. Less calcite precipitation in clay autoclaved mixes compared to sand autoclaved mixes.

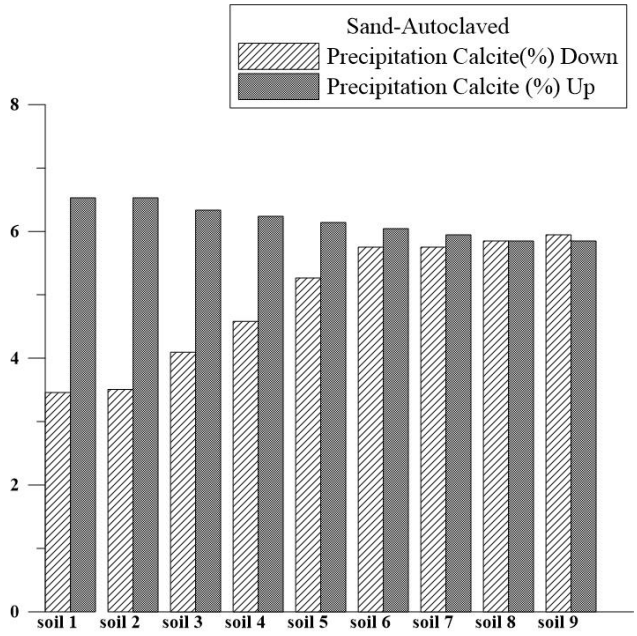

Figure 5. The difference in precipitation calcite in the top and bottom of soil mixes.

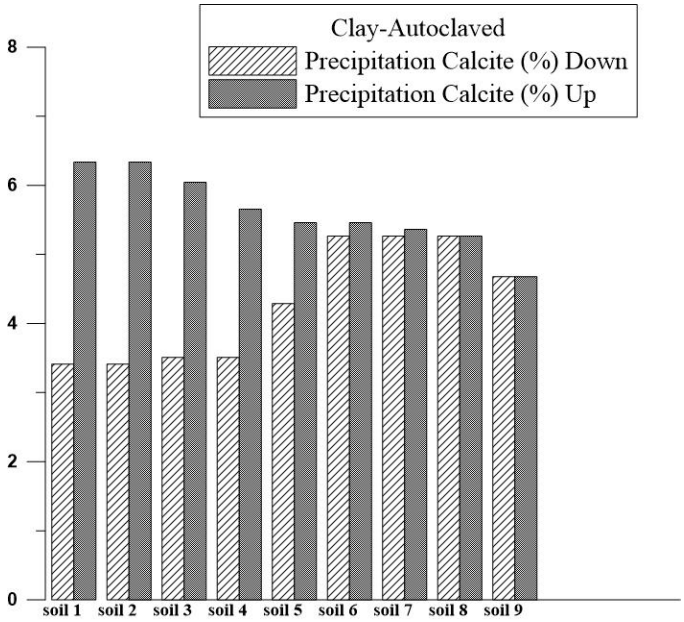

Figure 6. The difference in precipitation calcite in the top and bottom of soil mixes.

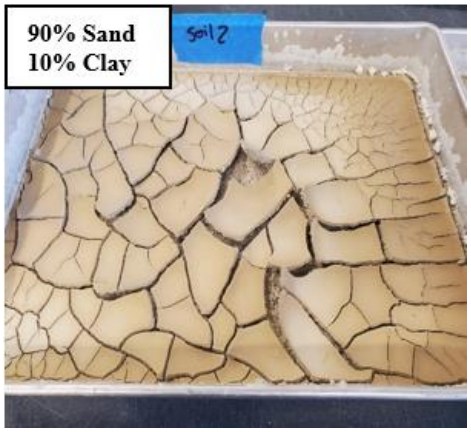

Figure 7. Non-uniform precipitation of calcite in mixes with higher sand content.

Figure 8. Uniform precipitation of calcite in mixes with higher clay content.

Summary and Findings

The effect of soil composition and urease activity on calcite precipitation was studied using different mixes of natural and autoclaved clay/sand. Urease activity exhibited a linear dependence on the sample's composition, where samples with higher clay content showed higher urease activity. Also, urease activity was larger in sand-autoclaved samples compared to clay-autoclaved samples, which confirmed the importance/abundance of microbial communities in clay. The amount of calcite precipitation correlated well with the urease activity in soils with lower than 40% clay content. For soils with higher than 40% clay, while the urease activity increased with clay content, the calcite precipitation did not. This can be related to a decrease in pore space with increasing clay content and filling all of the spaces by calcite. We observed a gradient in the amount of calcite precipitation in sand-rich mixtures, which diminished in soil-mixes with >50% clay. These observations showed mixing method could produce a more homogeneous distribution of calcite in clayey soils.

References

Boyd, S. A., & Mortland, M. M. (1985). Urease Activity on a Clay-Organic Complex. *Soil Science Society of America Journal*, *49*(3), 619–622. https://doi.org/10.2136/sssaj1985.03615995004900030018x

Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B. C., & Crawford, R. L. (2011). Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils. *Geomicrobiology Journal*, *28*(4), 301–312. https://doi.org/10.1080/01490451.2010.499929

Burbank, M. B., Weaver, T. J., Williams, B. C., & Crawford, R. L. (2012). Urease Activity of Ureolytic Bacteria Isolated from Six Soils in which Calcite was Precipitated by Indigenous Bacteria. *Geomicrobiology Journal*, *29*(4), 389–395. https://doi.org/10.1080/01490451.2011.575913

Cardoso, R., Pires, I., Duarte, S. O. D., & Monteiro, G. A. (2018). Effects of clay's chemical interactions on biocementation. *Applied Clay Science*, *156*, 96–103. https://doi.org/10.1016/j.clay.2018.01.035

Cheng, L., & Cord-Ruwisch, R. (2014). Upscaling Effects of Soil Improvement by Microbially Induced Calcite Precipitation by Surface Percolation. *Geomicrobiology Journal*, *31*(5), 396–406. https://doi.org/10.1080/01490451.2013.836579

Cheng, L., Cord-Ruwisch, R., & Shahin, M. A. (2013). Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. *Canadian Geotechnical Journal*, *50*(1), 81–90. https://doi.org/10.1139/cgj-2012-0023

Fujita, Y., Taylor, J. L., Gresham, T. L. T., Delwiche, M. E., Colwell, F. S., McLing, T. L., Petzke, L. M., & Smith, R. W. (2008). Stimulation of Microbial Urea Hydrolysis In Groundwater To Enhance Calcite Precipitation. *Environmental Science & Technology*, *42*(8), 3025–3032. https://doi.org/10.1021/es702643g

Gat, D., Ronen, Z., & Tsesarsky, M. (2016). Soil Bacteria Population Dynamics Following Stimulation for Ureolytic Microbial-Induced $CaCO_3$ Precipitation. *Environmental Science & Technology*, *50*(2), 616–624. https://doi.org/10.1021/acs.est.5b04033

Gomez, M. G., Graddy, C. M. R., DeJong, J. T., & Nelson, D. C. (2019). Biogeochemical Changes During Bio-cementation Mediated by Stimulated and Augmented Ureolytic Microorganisms. *Scientific Reports*, *9*(1), 11517. https://doi.org/10.1038/s41598-019-47973-0

Lin, H., Suleiman, M. T., Brown, D. G., & Kavazanjian, E. (2016). Mechanical Behavior of Sands Treated by Microbially Induced Carbonate Precipitation. *Journal of Geotechnical and Geoenvironmental Engineering*, *142*(2), 04015066. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001383

Lloyd, A. B., & Sheaffe, M. J. (1973). Urease activity in soils. *Plant and Soil*, *39*(1), 71–80. https://doi.org/10.1007/BF00018046

Qabany, A. A., & Soga, K. (2013). Effect of chemical treatment used in MICP on engineering properties of cemented soils. *Géotechnique*, *63*(4), 331–339. https://doi.org/10.1680/geot.SIP13.P.022

Tabatabai, M. A., & Bremner, J. M. (1972). Assay of urease activity in soils. *Soil Biology and Biochemistry*, *4*(4), 479–487. https://doi.org/10.1016/0038-0717(72)90064-8

Terzis, D., Bernier-Latmani, R., & Laloui, L. (2016). Fabric characteristics and mechanical response of bio-improved sand to various treatment conditions. *Géotechnique Letters*, *6*(1), 50–57. https://doi.org/10.1680/jgele.15.00134

Theng, B. K. G. (2012). *Formation and Properties of Clay-Polymer Complexes*. Elsevier.

Verdouw, H., Van Echteld, C. J. A., & Dekkers, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Research*, *12*(6), 399–402. https://doi.org/10.1016/0043-1354(78)90107-0

Weaver, T. J., Burbank, M., Lewis, A., Lewis, R., Crawford, R., & Williams, B. (2011). Bio-Induced Calcite, Iron, and Manganese Precipitation for Geotechnical Engineering Applications. *Geo-Frontiers 2011*, 3975–3983. https://doi.org/10.1061/41165(397)406

Zhu, T., & Dittrich, M. (2016). Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. *Frontiers in Bioengineering and Biotechnology*, *4*. https://doi.org/10.3389/fbioe.2016.00004