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Effect of chemical concentrations on strength and crystal size of biocemented sand

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Abstract. Biocementation due to the microbially induced calcium carbonate precipitation (MICP) process is a potential technique that can be used for soil improvement. However, the effect of biocementation may be affected by many factors, including nutrient concentration, bacterial strains, injection strategy, temperature, pH, and soil type. This study investigates mainly the effect of chemical concentration on the formation of calcium carbonate (e.g., quantity, size, and crystalline structure) and unconfined compressive strength (UCS) using different treatment time and chemical concentration in the biotreatment. Two chemical concentrations (0.5 and 1.0 M) and three different treatment times (2, 4, and 8 cycles) were studied. The effect of chemical concentrations on the treatment was also examined by making the total amount of chemicals injected to be the same, but using different times of treatment and chemical concentrations (8 cycles for 0.50 M and 4 cycles for 1.00 M). The UCS and CCC were measured and scanning electron microscopy (SEM) analysis was carried out. The SEM images revealed that the sizes of calcium carbonate crystals increased with an increase in chemical concentrations. The UCS values resulting from the treatments using low concentration were slightly greater than those from the treatments using high concentration, given the CCC to be more or less the same. This trend can be attributed to the size of the precipitated crystals, in which the cementation efficiency increases as the crystal size decreases, for a given CCC. Furthermore, in the high concentration treatment, two mineral types of calcium carbonate were precipitated, namely, calcite and amorphous calcium carbonate (ACC). As the crystal shape and morphology of ACC differ from those of calcite, the bonding provided by ACC can be weaker than that provided by calcite. As a result, the conditions of calcium carbonate were affected by test key factors and eventually, contributed to the UCS values.

Keywords: microbially induced calcium carbonate precipitation (MICP); unconfined compressive strength (UCS); calcium carbonate content (CCC); chemical concentration; Biocementation

1. Introduction

Cement is one of the most commonly used construction materials. However, cement production is an energy-intensive and environmentally unfriendly process. To produce one ton of Portland cement, approximately 0.81 tons of carbon dioxide (CO_2) are generated. The cement industry as a whole accounts for 5% of the global CO_2 emissions (Worrell *et al.* 2011).

New cementation methods utilizing microbiological techniques have been widely studied in the last decade. One method is the so-called microbially induced calcium carbonate precipitation (MICP), which precipitates calcium carbonate between soil particles (Mitchell and Santamaria 2005, Dejong *et al.* 2006, Van Paassen *et al.* 2010, Burbank *et al.* 2011, Chu *et al.* 2012, Feng and Montoya 2015, Choi *et al.* 2017c) or enhanced and repaired mortar/concrete (De Muynck *et al.* 2008, Pei *et al.* 2013, Ghosh *et al.* 2015, Choi *et al.* 2017a).

Another method uses biopolymers, extracted from bacteria, to develop binders in soil thereby improving soil stabilization (Chang and Cho 2014, Chang *et al.* 2015, Lee *et al.* 2017, Yasodian *et al.* 2012, Noh *et al.* 2016, Im *et al.* 2017, Qureshi *et al.* 2017, Demir *et al.* 2017, Ham *et al.* 2018).

One more soil cementation method that uses plant enzymes rather than bacteria is the so-called enzyme induced calcium carbonate precipitation (EICP) method. It has been studied for various purposes (Bang *et al.* 2009, Dakhane *et al.* 2018, Hamdan and Kavazanjian Jr. 2016, Park *et al.* 2014, Dilrukshi *et al.* 2016).

Microorganisms, such as *Sporosarcina pasteurii* and *Bacillus sphaericus* contain urease which can hydrolyze urea and release carbonate. The precipitation of calcium carbonate in the presence of free calcium ions is defined by Equation (1). The calcite crystals can bind soil particles through the process of biocementation.

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} + \text{Bacteria} \rightarrow CaCO_3 + 2NH_4^+ \quad (1)$$

The level of biocementation is associated with the calcium carbonate precipitated within soil particles. Many researchers have studied various combinations and optimizations of key factors in relation to calcium carbonate content (CCC) and strength.

In the aspect of chemical concentration, Al Qabany and Soga (2013) observed that low concentrations of chemical

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solutions (0.1, 0.25, 0.50, and 1.0 M) produced smaller size samples of calcium carbonate with higher unconfined compressive strength (UCS) in comparison with high concentrations of chemical solutions at a similar CCC (2.6 to 9.6%). But Soon *et al.* (2014) discovered that the highest shear strength resulted from the 0.50 M chemical concentration among the three different chemical concentrations (0.25, 0.50, and 1.00 M), while Sharma and Ramkrishnan (2016) tested the chemical effect (using 0.25, 0.50, 0.75, and 1.00 M) of the MICP method and claimed that 0.50 M of chemical solution is the most effective concentration for biocementation.

In addition to chemical concentration, there are other factors affecting the effect of biocement such as urease activity (Zhao *et al.* 2014), degrees of saturation of soil (Cheng *et al.* 2016), input ratio (Al Qabany *et al.* 2012), and calcium source (Choi *et al.* 2016a).

It is generally observed that UCS increases with the increase in CCC (Whiffin *et al.* 2007, Park *et al.* 2014, Choi *et al.* 2016a). The types of calcium carbonate will affect the effect of biocement, given the CCC the same. The types of calcium carbonate formed (i.e., quantity, size, and shape) are affected by a combination of factors. Al Qabany and Soga (2013) observed that higher UC strength is obtained from smaller sizes of calcium carbonate produced at a lower chemical concentrations.

With respect to the shape of calcium carbonate, some studies have reported that MICP results in the production of different phases of calcium carbonate (Rodriguez-Navaro *et al.* 2012; Dhami *et al.* 2013). Calcium carbonate forms three anhydrous polymorphs (calcite, aragonite, and vaterite) and one amorphous phase (amorphous calcium carbonate (ACC)) (Dhami *et al.* 2013). The engineering properties of biocement are also related to the difference in phase of the calcium carbonate (Ševčík *et al.* 2015, Verba *et al.* 2016). However, studies of engineering behaviors on MICP are still rare and experiments need to continue.

In this study, the effect of chemical concentration and times of treatment on the relationship between the conditions of calcium carbonate and UCS was investigated using different treatment times and chemical concentrations. The first set of tests used two different chemical (urea and calcium chloride) concentrations (0.50 and 1.00 M) under different treatment times (2, 4 and 8 cycles) and the second kept the total amount of chemicals the same, but using different times of treatment and chemical concentrations (8 cycles for 0.50 M and 4 cycles for 1.00 M).

2. Materials and test methods

2.1 Sand

The sand used was ASTM-graded Ottawa sand (as described in ASTM C778). This sand had a grain size of 0.6 mm (sieve #30) to 0.85 mm (sieve #20) and the mean grain size (D50) was 0.72 mm. The specific gravity of the sand was 2.65. The maximum and minimum void ratios were 0.739 and 0.519, respectively (Cheng *et al.* 2013).

2.2 Ureolytic bacteria

The urea producing bacteria employed was Bacillus *Sporosarcina pasteurii* (ATCC 11859), which has been commonly used for MICP (Chu *et al.* 2012, Zhang *et al.* 2016, Mahawish 2016 *et al.* Choi *et al.* 2016b). To cultivate bacteria culture, the medium was made of yeast extract (20 g/L), ammonium sulfate ($(NH_4)_2SO_4$) (10 g) and 1 L of 0.13 M tris buffer (pH = 9.0) solution. The sterilization of the medium was carried out in an autoclave at 121°C for 15 min.

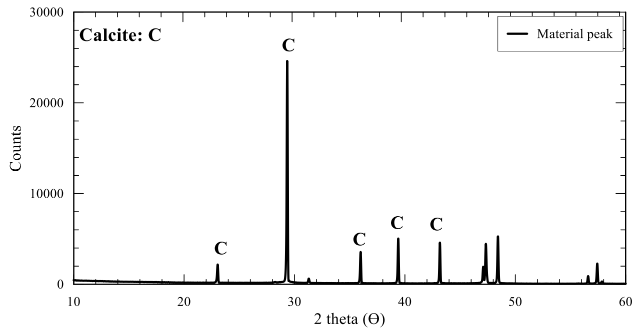


Fig. 1 Confirmation of calcite by MICP process

The seed of the bacteria was introduced into a sterilized medium and then cultivated in a temperature controlled (30 °C) shake-table incubator for 2 days. After 2 days, the urease activity was measured by an electric conductometer (Choi *et al.* 2017b). For the urease activity measurement, 2 mL bacterial culture was mixed with 10 mL 3 M urea and 8 mL deionized water. The urease from the bacteria hydrolyzes the urea and generates ammonium, which increases the conductivity of the solution.

A conductivity probe was used to detect the conductivity change as a measurement of urease activity, obtaining a value of 0.18 mS/min per unit volume of bacterial culture. Based on the conversion curve established by Whiffin *et al.* (2007), who adopted the same method for urease activity measurement, 0.05 to 0.07 mS/min of urease activity can hydrolyze 0.56 to 0.78 mM/min of urea. The urease activity of the bacteria used in this study was high enough to hydrolyze all the urea in the cementation solution of a single treatment round within 24 hours.

2.3 Chemical solution

Urea and calcium chloride were used as chemical solutions in the ratio of 1:1 (Cheng *et al.* 2016, Choi *et al.* 2017c). High concentrations of urea and calcium chloride solutions were prepared separately to produce different concentrations of the cementation solution used in the MICP treatment in this study.

2.4 Confirmation of precipitated calcite

By ureolytic bacteria with chemical solutions (urea and calcium chloride), calcite was precipitated to confirm MICP process. The urease producing bacteria culture grown for 2 days was mixed with 1 M urea solution in the ratio of 1:1 (v/v). This mixture was stored in a beaker for 1 day and then 1 M calcium solution, which was prepared based on the procedures, was added to the mixture.

Precipitation was observed immediately after the addition of the calcium solution. This precipitated material was filtered out using filter paper and dried at 60°C for 1 day. The dried material was then analyzed using X-ray diffraction (XRD). The XRD pattern of the precipitated materials (Fig. 1) perfectly matched that of the pure reagent grade of calcite.

2.5 Sample preparation

A total of 20 samples were prepared. Ottawa sand was put in PVC tubes with a diameter of 5.5 cm and height of 11 cm using an air pluviation method. The relative density achieved was 50-55%. A layer of gravel and a piece of scrub sponge pad was placed at the bottom of the sand sample to act as a drainage. Another piece of scrub sponge pad was placed on top of the sample for easy application of chemicals for biocementation. More details on the sample preparation method can be found in Choi *et al.* (2016b).

2.6 Treatment method

A one-phase biocementation method was used to treat all the samples.

First, 2.0 M of hydrochloric acid was used to adjust the pH of the cultivated bacteria strain to a lightly acidic condition of pH 5.2. Different chemical solutions (0.50 and 1.0 M) were then mixed with the bacteria strain suspension in a 1:1 volume ratio.

The low pH of the bacteria strain suspension prevented flocculation, which generally occurs in a neutral or alkaline environment. Finally, the mixture solution with two different urea and calcium concentrations were injected into the sand column from the bottom till the whole sample was immersed. Fig. 2 shows the prepared sample and injected direction.

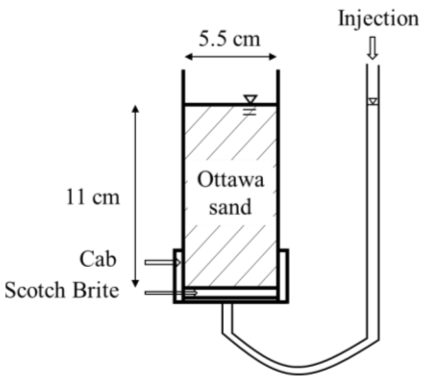


Fig. 2 Treatment method

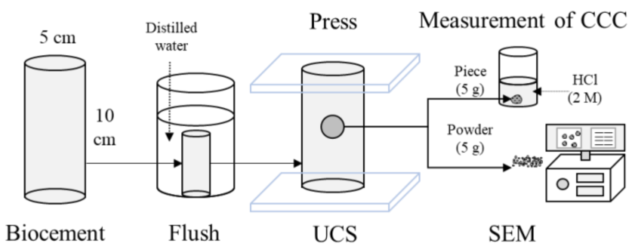


Fig. 3 Procedure of test

2.7 Testing procedure

After each biocementation treatment, the sample was washed using distilled water and left to air dry for 1 day at a temperature of 18°C. The samples were then used for unconfined compression tests based on ASTM D 4219. After the tests, pieces of samples, each weighing 5 g, were collected along the failure surface for CCC measurement using a washing method as detailed by Choi *et al.* (2017b). The same type of method was also used by other researchers (Zhao *et al.* 2014, Li *et al.* 2015). Finally, samples were also selected for (scanning electron microscopy) SEM tests. Fig. 3 illustrates the test procedure.

3. Test results

In total, 20 tests were carried out on samples treated using different schemes as shown in Table 1. Two different test conditions were analyzed. First, 4 groups of cylinders were treated 2 times for different treatment cycles. Second, 2 different groups of samples were treated 8 times for 0.50 M and 4 times for 1.00 M. Table 1 shows the test conditions and results for these two cases.

3.1 Effect of treatment time

20 samples were treated with different treatment times (2, 4, and 8 cycles) using two chemical concentrations (0.50 and 1.00 M). To determine the relationship between CCC and UCS, different treatment times were tested. The test results in terms of CCC and UCS are presented in Table 1. Fig. 4(a) shows individual data of UCS and CCC with different treatment cycles using 0.5 M chemical concentration. Strength on samples treated two times were measured at 63-93 kPa on 0.94-1.28% of CCC. Strength on samples treated 8 times were measured at 402–594 kPa on 2.9-3.6% of CC. The UCS increased with increasing treatment times because

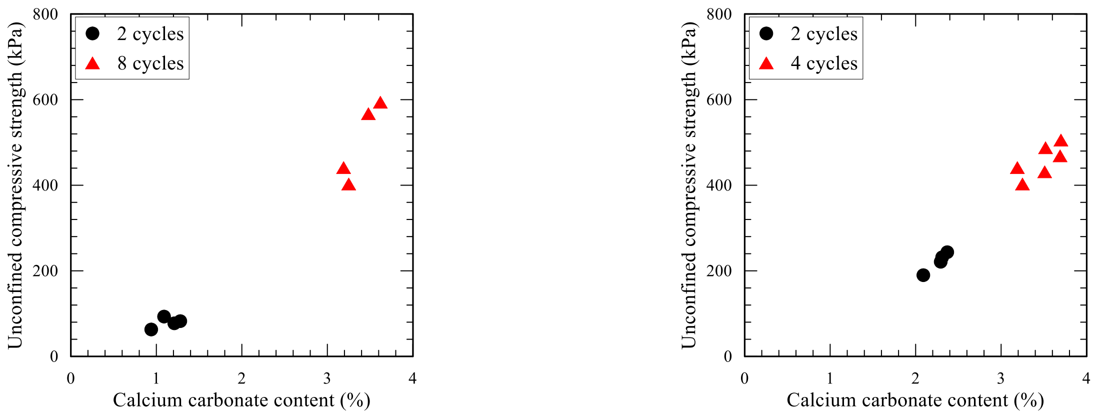
The increased number of treatment solutions induced more precipitated calcium carbonate, which bound and combined each calcium carbonate and sand particle (DeJong *et al.* 2010). Other researchers also found a relationship between CCC and UCS (Whiffin *et al.* 2007, van Paassen *et al.* 2010, Cheng *et al.* 2016).

Fig. 4(b) shows the individual data of UCS and CCC with different treatment times using 1.0 M of chemical concentration. Samples treated with 2 cycles were measured at 190-244 kPa on 2.1-2.4% of CCC and samples treated 4 times were measured at 403-488 kPa on 3.2-3.7% of CCC. The relationship between CCC and UCS indicated a similar trend for 0.5 M chemical concentration. Therefore, the amount of calcium carbonate is a main factor for determining UCS and engineering properties. These results also showed the relationship bewteen CCC and UCS in Table 1. The average and error of CCC and UCS increased with increasing treatment cycles, regardless of the chemical concentration. These results assume that the biocement using MICP method still needs to improve for high technology.

To compare the effect of chemical concentration, two

Table 1 Test conditions and results

Test ID	Type	Treatment cycle (Time)	Chemical concentration (Mol)	Calcium carbonate content (%)	Unconfined compressive strength (kPa)
C0501	Treatment time controlled	2	0.50	0.94	62.9
C0502				1.21	77.3
C0503				1.28	82.4
C0504				1.09	93
Ave.				1.13	78.9
C1001		2	1.00	2.37	243.5
C1002				2.31	231.6
C1003				2.09	189.8
C1004				2.29	221.1
Ave				2.27	221.5
T0501	Total chemical quantity controlled	8	0.50	2.9	445.6
T0502				3.1	402.1
T0503				3.37	449.5
T0504				3.51	584.1
T0505				3.48	567.2
T0506				3.62	594
Ave.				3.33	507.1
T1001		4	1.00	3.19	441.3
T1002				3.25	402.9
T1003				3.7	455.6
T1004				3.52	487.6
T1005				3.51	431.4
T1006				3.69	468.7
Ave.				3.48	447.9



(a) 0.5 M of chemical concentration (b) 1.0 M of chemical concentration

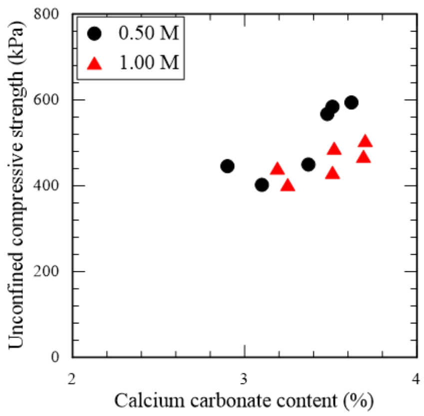
Fig. 4 Relationships of CCC and UCS by different treatment cycles

types of samples with chemical concentration of 0.50 and 1.00 M were tested. The results are presented in Table 1 and Fig. 5. To achieve the same CCC level, samples treated 4 times were used for the solution with 1.00 M chemical concentration and samples treated 8 times were used for the solution with 0.50 M chemical concentration. The CCC obtained was in the narrow range of 2.90-3.70% as shown in Table 1.

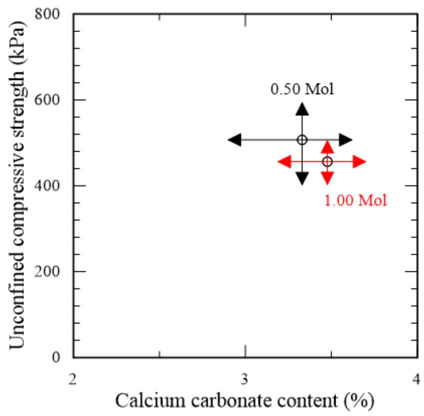
3.2 Effect of chemical concentration

UCS versus CCC relationships are plotted in Fig. 5(a) for tests with two different chemical concentrations of 0.5 M and 1.0 M. These data show that the UCS increases with increasing CCC regardless of the chemical concentration. However, the UCS for tests using 1.0 M is lower in general.

In terms of average, the UCS for samples treated using

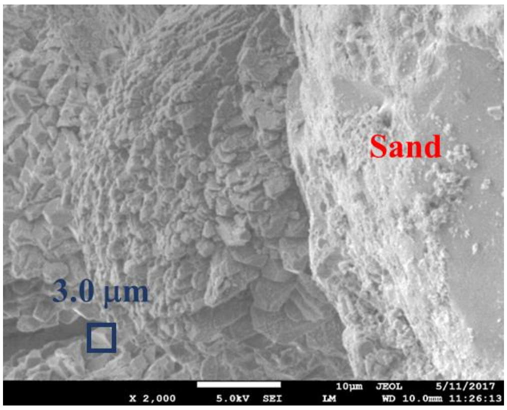


(a) Individual data

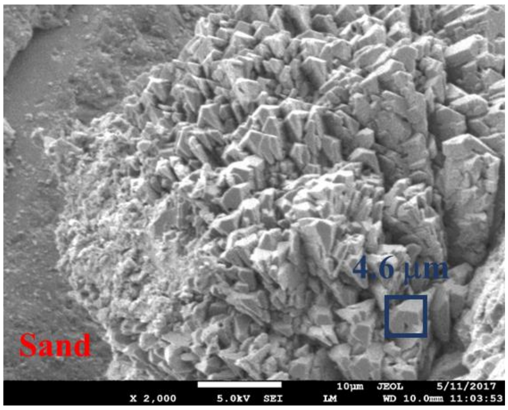


(b) Average and errors for CCC and UCS

Fig. 5 Relationships of CCC and UCS of same chemical concentrations with different treatment times

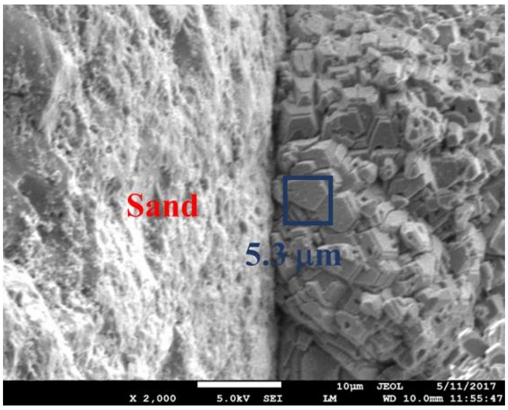


(a) Calcium carbonate sizes in area a

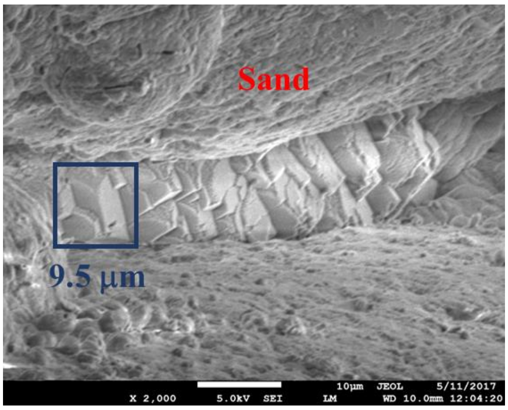


(b) Calcium carbonate sizes in area b

Fig. 6 Calcium carbonate sizes by 0.50 M of chemical concentration



(a) Calcium carbonate sizes in area a



(b) Calcium carbonate sizes in area b

Fig. 7 Calcium carbonate sizes by 1.00 M of chemical concentration

0.50 M chemicals was 11% higher than that for samples treated using 1.00 M, for similar CCC, as shown in Fig. 5(b).

The UCS values for two tests with different chemical concentrations of 0.50 and 1.00 M had different values even though the same amount of chemical concentrations was injected. Lower chemical concentrations (0.50 M) induced higher strength than higher chemical concentrations.

There are two explanations for this observation. The first explanation is that the sizes of calcium carbonate crystals formed are different. Under a lower chemical concentration (0.50 M), the precipitated calcium carbonate crystals tend to be smaller.

The SEM of samples from two tests treated using two different chemical concentrations (0.50 and 1.00 M) are shown in Figs. 6 and 7. The SEM shown in the figures have a fixed magnification of 2,000 times. The box in each image indicates the largest calcite size observed in that image. For the two images in Fig. 6 (for 0.5 M), the maximum calcite size is 3.0 and 4.6 µm, respectively. For the two images in

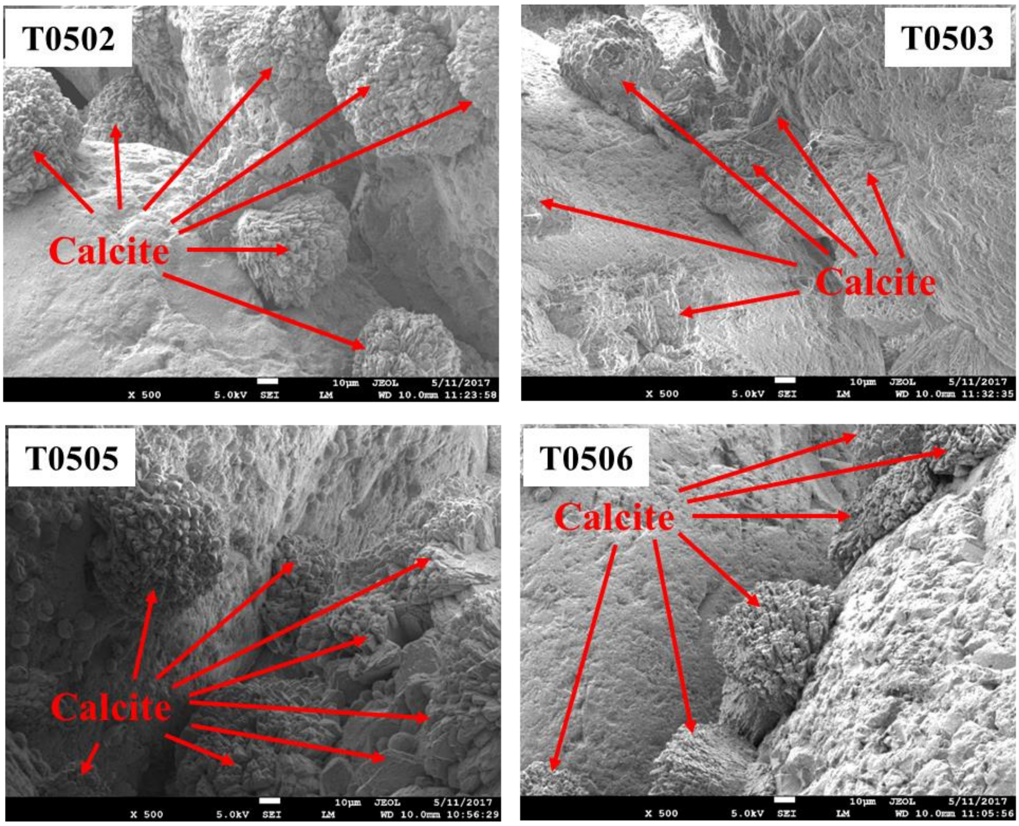


Fig. 8 Calcium carbonate sizes by 0.50 M of chemical concentration

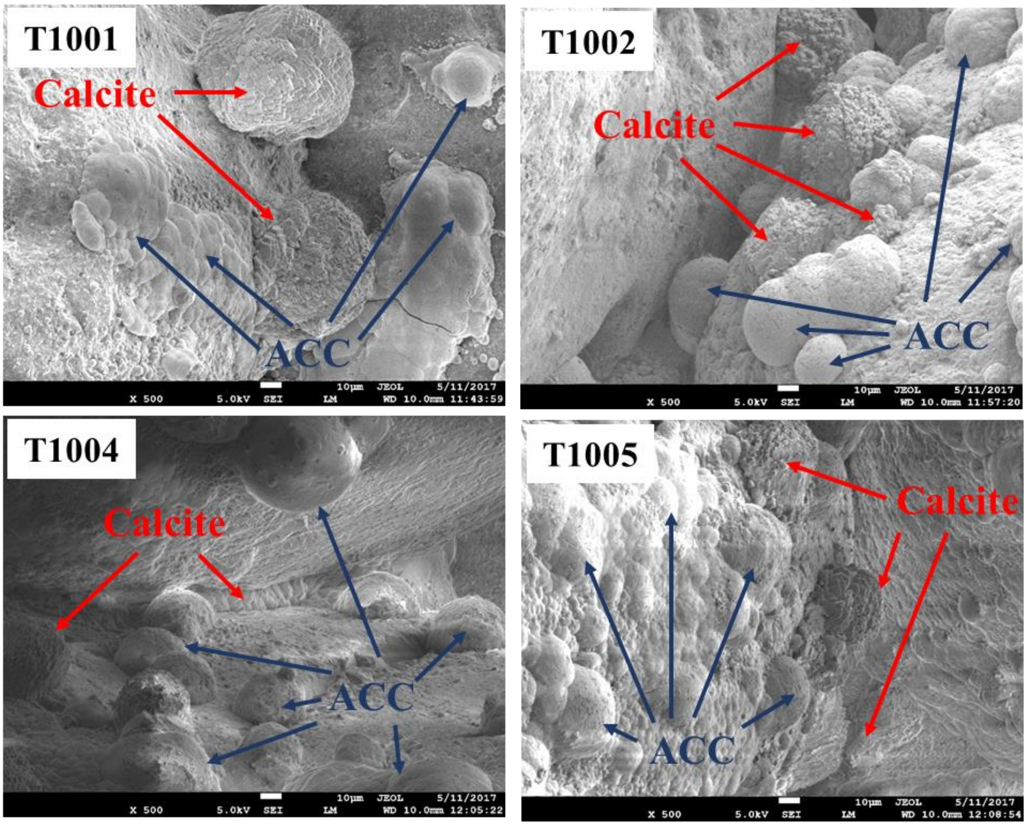


Fig. 9 Calcium carbonate sizes by 1.00 M of chemical concentration

Fig. 7 (for 1.0 M), the maximum calcite size is 5.3 and 9.5 μm, respectively. Similar observations have been made by Al Qabany and Soga (2013).

In general, the smaller the crystals, higher the specific

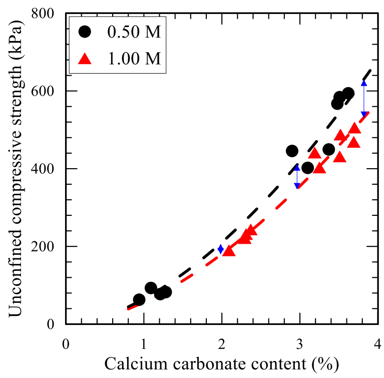


Fig. 10 Relationships of CCC and UC as different chemical concentration by total results

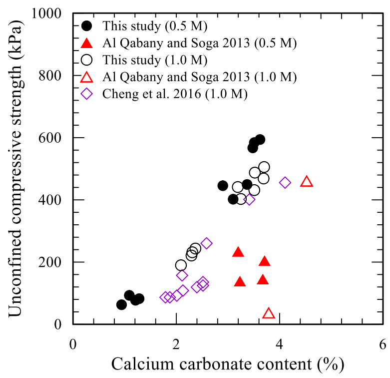


Fig. 11 UCS plotted against calcium carbonate precipitation with our study and references

surface area, and thus, greater the contact area between calcium carbonate crystals and the sand grains, which results in a greater shear strength. It has related with different strength.

Another explanation for the results is the difference in structures of calcium carbonate. Figs. 8 and 9 show SEM results of several samples. Figs. 8 and 9 show samples treated with 0.50 M and 1.0 M of chemical concentration, respectively. In Fig. 9, two types of calcium carbonate precipitations were found in the SEM images: one was calcite and the other was ACC shapes.

The low chemical concentration tends to induce crystallization quickly, whereas the high chemical concentration may take a longer time to convert ACC to calcite. This is because calcite is known to be the most stable form of calcium carbonate (de Leeuw and Parker 1998), whereas the bonding provided by ACC may not be as strong.

Verba *et al.* (2016) also examined the precipitated calcium carbonate in tests with a high urea and low urea condition, respectively, in the same treatment cycle. They found that the initial amorphous calcium carbonate formed in the low urea concentration solution turned into crystalline calcium carbonate quicker than that in the high urea concentration solution.

All the test data are summarized in Fig. 10, in which two UCS versus CCC curves are obtained. The UCS values obtained from tests with 0.50 M chemical concentration are higher than that obtained from tests with 1.0 M for the same CCC, although the difference is not significant.

3.3 Comparisons and discussion

The UCS results of this study were compared with references (Al Qabany and Soga 2013, Cheng *et al.* 2016) for 0.5 and 1.0 M chemical concentrations. The comparisons were performed between UCS values for similar CCC (0-5%). As mentioned above, there are various key factors of engineering properties of biocement using MICP, such as urease activity, temperature, chemical concentration, flow rate, and pH. However, the combinations of key factors affected the conditions of calcium carbonate (quantity, size, and crystalline structure) and induced the precipitation of calcium carbonate.

Eventually, the UCS values may follow these precipitated calcium carbonate results. This observation can explain Fig. 11 results. The figure shows that UCS increased with increasing CCC, regardless of test cases. Additionally, the figure shows that the amount of calcium carbonate mainly contributed to the increment of UCS. Lower chemical concentrations (0.5 M for this study) commonly measured higher strength than high chemical concentrations (1.0 M for this study, Al Qabany and Soga 2013 and Cheng *et al.* 2016). This is because lower chemical concentration induced the precipitation of uniform and smaller calcium carbonate in comparison to higher chemical concentration. Each test case induced different calcium carbonate sizes. For example, smaller sizes of calcium carbonate (less than 10 μm for this study (0.5 and 1.0 M), Cheng *et al.* 2016 (1.0 M)) contributed higher UCS than larger sizes of calcium carbonate (about than 35 μm for Al Qabany and Soga (0.5 and 1.0 M)). The results also indicated that lower chemical concentrations, alone, did not induce smaller sizes of calcium carbonate, but relied on combinations of key factors. So, Al Qabnay and Soga 2013 using lower chemical concentration (0.5 M) results showed lower UCS values than Cheng *et al.* 2016 using higher chemical concentration (1.0 M).

Finally, all referenced observations showed the same calcium carbonate shape (calcite) except one result: this study for 1.0 M, which found two types of calcium carbonate shapes (ACC and calcite). The shapes of calcium carbonate also affected UCS values.

The results suggest that the engineering properties of biocement using MICP are related to calcium carbonate conditions (quantity, size, and crystalline structure). To analyse the relationship between CCC and the engineering properties, these conditions must be considered.

4. Conclusions

In this study, two different biocementation conditions (time of treatment and chemical concentration) were examined to investigate the effect of time of treatment and chemical concentration on the relationship between calcium carbonate and UCS of biocemented sand. The following conclusions can be drawn from the study:

• UCS increases with increasing CCC. The strength of biocemented sand increases with the increase in the times of treatment given the chemical concentration used is the same.

• For the same amount of total chemical usage, a lower chemical concentration leads to a higher shear strength. This is related to the observation that a lower chemical concentration induces smaller size calcium carbonate crystals. Furthermore, a lower chemical concentration leads to a quicker precipitation of crystals.

• The types of calcium carbonate (amount, size and crystalline structure) also affect the shear strength of the biotreated soil. The UCS for samples dominated by calcite crystals will be higher than those by ACC.

Acknowledgements

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