Bacteria induced cementation for soil stabilization

Etude de la cimentation induite par les bactéries pour la stabilisation des sols

Liang Cheng, Mohamed A. Shahin
Department of Civil Engineering, Curtin University, Perth WA, Australia, M.Shahin@curtin.edu.au

ABSTRACT: In recent years, the use of microbiological processes to improve the mechanical properties of soil has gained some attention. This paper explores an emerging and promising biological soil stabilization technique, known as bio-cementation, using microbially induced calcite precipitation (MICP). In this work, uniform silica sand was treated using bio-cementation as well as ordinary Portland cement (OPC), and the results were compared in terms of the unconfined compressive strength (UCS) and permeability. The results indicate that bio-cementation is an effective soil stabilization technique in improving soil strength, with higher achieved UCS values and retained permeability than those of OPC-treated soil, demonstrating a major advantage for bio-cementation. The effectiveness of bio-cementation in harsh environment of extremely low and high temperatures was also examined, investigating the potential use of this technique in broader conditions in cold and arid regions. Furthermore, the performance of bio-cementation in marine environment was evaluated, showing the possibility of utilizing seawater as a natural calcium source to replace commercially available calcium chloride and demonstrating the feasibility of this technique in marine applications.

RÉSUMÉ : Ces dernières années, l'utilisation de procédés microbiologiques pour améliorer les propriétés mécaniques du sol a retenu l'attention des chercheurs. Cet article explore une technique biologique de stabilisation du sol qui est à la fois émergente et prometteuse. Elle est connue sous le nom de bio-cimentation (MICP) et elle est basée sur la précipitation de la calcite par les microbes. Cette technique utilise des bactéries uréolytiques à base de terre pour hydrolyser l'urée afin de donner des ions carbonates qui réagissent avec le chlorure de calcium pour produire du carbonate de calcium (calcite) qui lie les particules du sol, conduisant à une augmentation de la résistance du sol et de sa rigidité. Dans ce travail, un sable de silice uniforme a été traité à l'aide de bio-cimentation et de ciment Portland (OPC), et les résultats ont été présentés en termes de résistance à la compression non confinée (UCS) et de perméabilité. Les résultats indiquent que la bio-cimentation est une technique efficace de stabilisation du sol car elle donne des valeurs supérieures en termes de résistance UCS et de perméabilité que l'OPC montrant ainsi le grand avantage de la bio-cimentation. L'efficacité de bio-cimentation dans un climat rude correspondant à des températures extrêmement basses ou élevées a également été examinée pour investiguer sur l'utilisation potentielle de cette technique dans des conditions plus larges telles que les régions froides et arides. En outre, la performance de la bio-cimentation dans un environnement marin a été évaluée montrant la possibilité d'utiliser l'eau de mer comme source de calcium naturel pour remplacer le chlorure de calcium acheté dans le commerce et démontrant la faisabilité de cette technique dans des applications maritimes.

KEYWORDS: Biocementation, Microbially induced Calcite Precipitation (MICP), Ground Improvement, UCS, Permeability.

1 INTRODUCTION

The land demand for construction and expansion has increased significantly in the past few decades, owing to the growth of human population and scarcity of adequate land of suitable ground conditions, leading to an immense need for soil improvement. In the past, several methods have been utilized to improve the low bearing capacity and high compressibility of unstable (loose, soft and erodible) soils. However, the majority of current soil improvement techniques require a substantial energy for material production and/or installation (DeJong et al. 2010). For example, chemical treatment, one of the most commonly used soil improvement methods, can substantially enhance soil strength properties by binding soil particles together via injected synthetic chemical grouts (e.g. epoxy, acrylamide, silicates and polyurethane). However, grouts raise the issues of cost, health and safety, and many have been banned in several countries (Xanthakos et al. 1994; Karol 2003; DeJong et al. 2010).

Recently, research has been aimed at finding alternative methods for soil improvement that can achieve an optimum performance in terms of environmental sustainability and economic viability. Microbially induced carbonate precipitation (MICP) is an emerging attractive alternative that has undergone rapid developments in the last few years. MICP employs ureolytic (non-pathogenic) bacteria such as *Bacillus pasteurii* to hydrolyze urea in the presence of calcium ions to form calcium carbonate crystals, which act as a cementing agent that binds the soil grains together, resulting in increase in soil strength and stiffness. The chemical reactions of MICP can be described as follows (Stocks-Fischer et al. 1999):

- Urea is hydrolyzed by microbial urease to form ammonium and carbonate:

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

- In the presence of calcium source (in most cases calcium chloride), the produced carbonate ions react with the calcium ions to produce calcium carbonate (CaCO3) precipitates:

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

The produced $CaCO_3$ cements the adjacent soil particles together, leading to increased soil strength and forming cemented sand that is very similar to that of calcareous rocks. The MICP process simulates natural digenesis from sand to sandstone, only within a short time instead of million years.

In the present study, the performance of MICP treated soil samples under different environmental conditions were investigated, including the degree of temperature and marine environment. In addition, the mechanical properties of MICP treated soil were compared with those obtained from soil treated

using ordinary Portland cement (OPC) in terms of unconfined compressive strength and permeability. The microstructure of precipitated crystals of MICP treated soil was also examined using scanning electron microscope.

2 MATERIALS AND METHODS

2 .1 *Soil used*

Natural silica sand obtained from Cook Industrial Minerals Pty. Ltd Western Australia was used in this study. The particle size distribution of the sand used is shown in Figure 1 and is classified as poorly graded (SP) in accordance with the unified soil classification system (USCS).

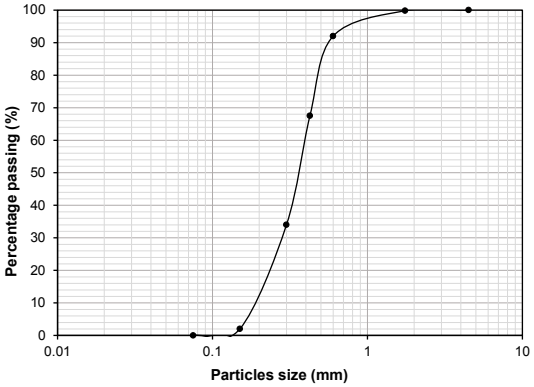

Figure 1. Particle size distribution of the sand used.

2 .2 *Bacterial culture and cementation solution*

The uratolytic bacteria used in this study were *Bacillus sp.* (DSM 23526) isolated from a previous work carried out by Al-Thawadi and Cord-Ruwisch (2012). The bacteria culture was cultivated aerobically in a sterilized growth medium similar to that used in a previous study by Cheng and Shahin (2016). After 24 hours of cultivation at 30ºC, the optical density at 600 nm (OD_{600}) of the bacterial culture was between 2.0−2.5 and the urease activity was about 5 U/ml (1 U = 1 µmol urea hydrolyzed per min). Urea-calcium chloride of 1 M was used as the cementation solution to facilitate the $CaCO_3$ production.

2 .3 *Preparation of sand columns for MICP and OPC*

The treatment process of MICP used in the current study was the same as that used in a previous study carried out by Cheng et al. (2016). PVC columns filled with sand were fully saturated with water at 100% degree of saturation prior to MICP treatment to ensure a relatively controlled flow field during the treatment injections. This procedure was carried out using an upward flow method that facilitates the removal of the air voids from inside the soil samples. Then MICP treatment was employed using a downward flow injection method, and the soil samples were kept fully saturated throughout the treatment by maintaining the water level in external U-type at the top level of the PVC columns.

A two-phase injection approach was employed for bacterial fixation by injecting a half void volume of bacterial culture followed by a half void volume of cementation solution. The sand was then cured for 24 h to allow for the fixation of bacteria on the surface of soil particles through the entire sand columns, which was attributed to the calcium ions that diffused into the bacterial culture phase (Cheng and Cord-Ruwisch 2012). Full void volume of cementation solution (1 M urea and 1 M $CaCl_2$) was then injected into each of the prepared sand column, which was left to cure for 24 h to allow for precipitation of calcite. For each individual sand column, treatment with the cementation solution was repeated several times to gain different degrees of cementation.

Additional sand specimens treated with OPC were also prepared by mixing different concentrations of Portland cement (7.0−14.0% cement/sand ratios were used because they are usually adopted in the literature) with a constant moisture content of 20%. The mixtures were then poured into PVC columns of the same dimensions as those used in bio-cementation, and a vibration was applied to avoid the air bubbles that might remain in the mixtures. The prepared mixtures were then cured at the room temperature (20±1 °C) for 7 days prior to UCS and permeability tests.

2 .4 *Unconfined compressive strength, permeability, $CaCO_3$ content and scanning electron microscopy*

The unconfined compression strength (UCS) tests were conducted according to the procedure reported in the ASTM D2166 (ASTM 2013). The diameter-to-height ratios of the soil samples used for the UCS tests were 1:1.5 or 1:2. The axial load was applied at a constant rate of 1.0 mm/min. The bio-cemented samples were flushed with 15 pore volumes of tap water prior to UCS measurements.

The permeability tests on untreated, bio-cemented, and OPC treated sand columns were conducted using the constant head permeability test in accordance with the Australian Standards AS 1289.6.7.1 (Australian Standards 2001).

$CaCO_3$ content tests were conducted on MICP samples by adding 2 mL of 2 M HCl solution into 0.5−2 g of dry samples. The volume of carbon dioxide gas produced was measured using U-tube manometer under standard conditions (25ºC, 1 atm). A calibration was made with the laboratory grade $CaCO_3$ powder in accordance with Cheng and Cord-Ruwisch (2012).

To characterize the shapes and locations of the precipitated $CaCO_3$ and investigate the crystal bonding between the grain hosts, scanning electron microscopy (SEM) analysis (Philips XL20, Eindhoven, The Netherlands) was also conducted on the MICP treated samples. The SEM analysis was conducted on crushed, cemented, sand samples after the UCS measurements.

2 .5 *MICP treatment at different temperatures*

The effect of temperature on the curing of OPC treated soils in terms of the strength gain has been extensively studied in the literature and will not be investigated in the current study. However, it is rather worthwhile to investigate the effect of temperature on MICP cementation as the urease activity of bacteria varies with the change in temperature, leading to different structures of microbially induced crystals. The temperature values used for bio-cementation were selected to simulate the subsurface soil temperature in cold regions (4°C), tropical regions (25°C) and arid regions (50°C). During the process of MICP treatment, the sand samples used were placed as follows: (1) inside 4°C refrigerator; (2) room temperature of 25°C; and (3) inside 50°C oven. The UCS tests and SEM analysis were performed for all bio-treated samples.

2 .6 *MICP treatment for marine environment*

The MICP bio-cementation was also investigated for marine environment using seawater as a source of calcium instead of commercially available calcium chloride, hence, reducing the cost of bio-cementation and bringing it closer to be commercially accepted. The seawater used in the current study was obtained from Coogee Beach, South Fremantle, Western Australia, and repeatedly flushed into the sand columns every 6 hours until a

desired strength was achieved. An amount of 60 mM of urea was added into the filtered seawater to produce CO_3^{2-} ions. The use of seawater as a relatively dilute calcium source requires many subsequent treatments; however, each treatment can be completed within a short period of time (6 hours in the current study) compared to 24 hours needed for a single treatment using the highly concentrated calcium source of 1 M calcium chloride.

3 RESULTS AND DISCUSSION

3 .1 *MICP vs OPC*

Figure 2 shows the results of the UCS tests carried out on sand samples treated with both MICP and OPC. Although only limited number of experiments were carried out and presented for the OPC treated soil, the general trend of the relationship between the UCS and cement content was relatively reflected, and the difference in strength improvement between the OPC and MICP treated soils was reasonable captured. It can be seen that MICP treated samples have higher strength in the range of the cement agents content tested compared to the OPC treated samples after 7 days of curing. The correlation between the UCS and content of cementing agents suggests that the $CaCO_3$ crystals are more effective in improving the soil strength compared to hydrated OPC. However, the obtained results may differ depending on the curing time of OPC treated samples.

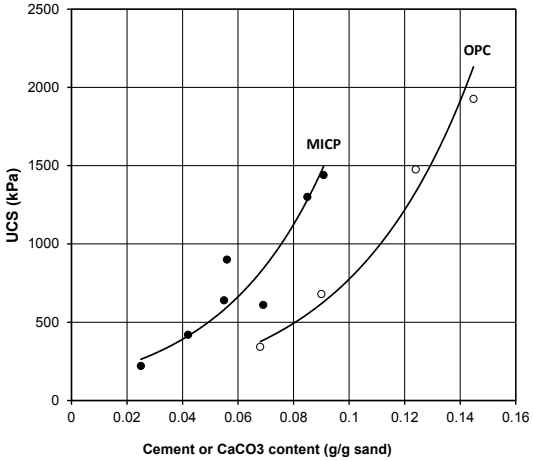

Figure 2. UCS of sand samples treated with MICP and OPC.

It can be seen from Figure 3 that the permeability values of MICP treated samples is significantly higher than those of OPC treated samples. As an example, a mixture with 7% (0.07 g/g sand) Portland cement has much lower permeability compared to MICP treated sand with 7% (0.07 g/g sand) $CaCO_3$ content. Samples treated with OPC content of higher than 9.6% (0.096 g/g sand) produced a poor drainage material with permeability values less than 1×10^{-6} m/s. The significant reduction of permeability in OPC treated samples is due to the occupation of the pore space by water-insoluble hydrates formed from the cement hydration reaction with the pore water. In contrast, the reduced permeability in MICP treated samples is caused due to the pore spaces, which are occupied by the $CaCO_3$ crystals resulting in smaller volume changes compared to OPC hydrates, as indicated by the SEM image shown in Figure 4.

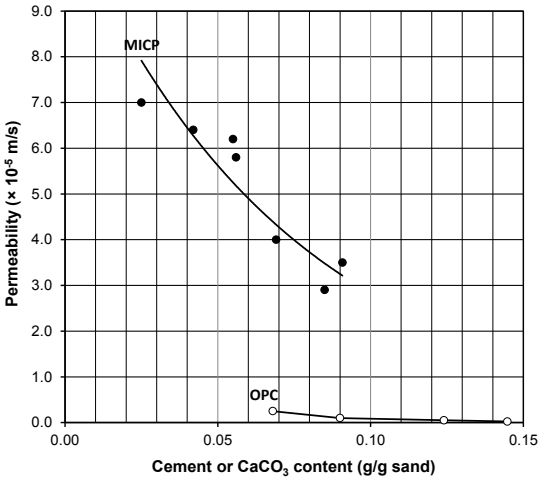

Figure 3. Permeability of sand samples treated with MICP and OPC.

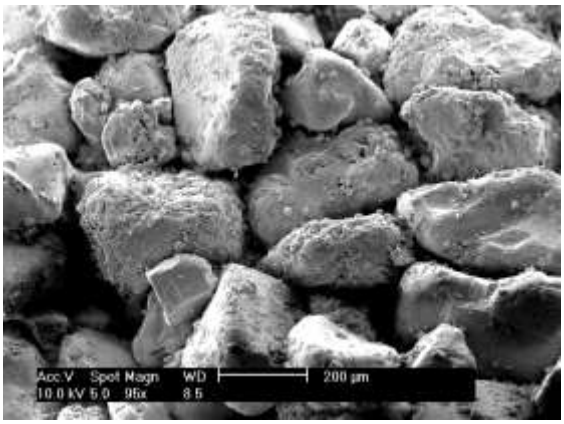

Figure 4. SEM image of MICP treated sample.

3 .2 *Effect of temperature on MICP treatment*

Figure 5 shows that with similar produced amount of $CaCO_3$ (0.028−0.034 g/g sand), the strength improvement is higher at 25°C compared to that at either lower temperature of 4°C or higher temperature of 50°C. The calcite crystals formed at 50°C were the least efficient to gain strength improvement. The microstructure examination indicates that MICP treatment at 50ºC results in typical individual small crystals of 2−5 µm, covering the entire sand grain surface (see Figure 6c-d) as a coating layer. However, the sand grains are not effectively connected, resulting in low strength improvement. The formation of such small crystals is probably due to the high temperature at which the rate of calcite nucleation becomes much faster, leading to a production of abundant small crystals. For sample treated at 25ºC, it was found that the average crystal size increased by 10 times (individual crystals size between 20 and 50 µm) compared to those formed at 50°C. These large calcite crystals were found to precipitate on the grain surface and also cover the contact areas of the sand grains (see Figure 6a-b). At low temperature of 4ºC, small individual calcite crystals of 5−10 µm were observed (data not shown), similar to those observed at 50ºC, which is due to the slow crystal growth rate because of the slow urea hydrolysis process.

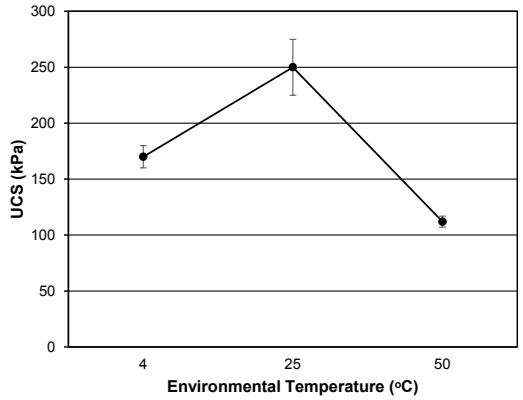

Figure 5. Effect of temperature on the UCS of MICP treated samples.

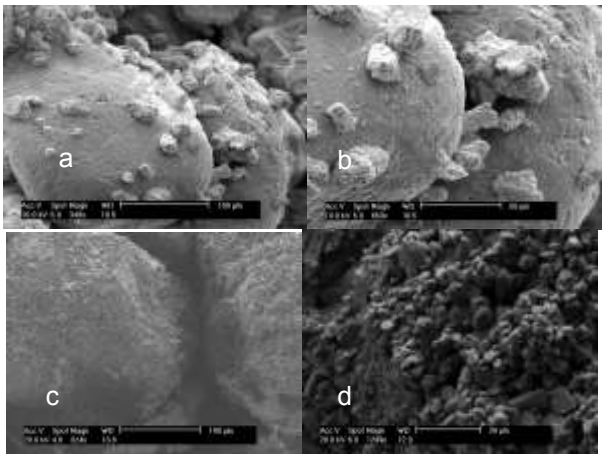

Figure 6. SEM images of MICP samples treated at different temperatures: (a) & (b) 25°C, UCS = 250 kPa, $CaCO_3$ = 0.028 g/g sand); (c) & (d) 50°C, UCS = 121 kPa, $CaCO_3$ = 0.034 g/g sand).

3 .3 Use of seawater for MICP treatment

The compressive strength of soil samples, which were treated with MICP using filtered real seawater as calcium source, increased significantly to about 300 kPa. The total amount of carbonate precipitates was about 0.022 g/g sand. In comparison with normal MICP using high concentration of $CaCl_2$ and urea (1 M), it was found that for similar amount of precipitate carbonate salts, the compressive strength of samples treated with seawater was higher than that of the control samples treated with high concentration of cementation solution. On the other hand, the use of filtered seawater supplemented with urea instead of concentrated cementation solution did not affect the permeability reduction (see Table 1). However, if considering a long-term effect of marine biofilm formation, there could be a further reduction in permeability of the sample treated with seawater, which is due to the effect of bio-clogging.

Table 1. Comparison between MICP using seawater and highly concentration $CaCl_2$ source.

Calcium source	UCS (kPa)	Permeability ($\times 10^{-5}$ m/s)	Carbonate precipitates (g/g sand)
Seawater	300 ± 15	7.2± 0.3	0.022 ± 0.001
$CaCl_2$ (1 M)	250± 10	7.0± 0.2	0.027± 0.002

The X-ray diffraction (XRD) testing of the obtained crystal samples indicated a typical XRD pattern of calcite and magnesium carbonate trihydrate ($MgCO_3 \cdot 3H_2O$) (data not shown). This is expected because the seawater magnesium ion concentration is about 50 mM, which is five times higher than the calcium ion concentration (10 mM). The MICP treatment with seawater showed abundant crystals precipitated on the sand grain surface and at the contact points between adjacent sand grains. The size of the precipitated crystals ranged from 2−5 μm (individual small crystal) to large clusters of about 50 μm.

The proof of concept of bio-cementation using filtered seawater as calcium sources has been established. Although the calcification rate (crystals formed per time) using seawater is much slower than that of the concentrated cementation solution, resulting in more treatment cycles required to gain a certain desired strength, it allows saving in cost of chemicals. This opens up a novel and cost-effective way of bio-cementation for marine and coastal applications, where continued gain of strength would be desirable, by naturally occurring calcium.

4 CONCLUSIONS

Based on the resutls obtained from the current study, the following concludions are drawn:

- In the presenc of similar amount of cement agents, MICP treated sand achieved higher UCS strength and retain suffiencint permeabilty compared to OPC treated samples.
- MICP treated sand achieved the highest stregnth at 25°C and this is attributed to the effective large crystals precipiated at the contact points of sand particles. MICP treatment conducted at extremely low temperature (4°C) and high temperature (50°C) indicated low efficiency in terms of strength improvement and this is due to the ineffecieve calcite precipiation.
- This study has demonsrated the feasibility of using MICP treatment for marine environment using the calcium ions directly from seawater as the sole calcium source instead of commercially available calcium chloride. The strength of sand columns in such a case can be significantly increased by repeated treatment of seawater. The application of using natural calcium source of seawater can possibly offer a cost-effective and sustainable way to strengthen the ground in marine and coastal environments.

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