



# Honors Lecture: Biological Cementation of Unstable Soils and Grounds for Civil Infrastructure Developments

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**Abstract.** Problematic soils (e.g., loose, erodible, and collapsible) are unstable and pose significant challenges to the geotechnical engineering communities due to their low bearing capacity and high compressibility. Such unstable soils are widespread in the world and greatly hinder civil infrastructure developments such as building foundations, roads, retaining walls, etc. Engineered ground improvement is often necessary to increase soil load-carrying capacity and prevent excessive post-construction deformations. However, existing techniques of ground improvement can be highly expensive (e.g., pile foundations) and some have significant environmental, sometimes toxic effects (e.g., chemical stabilization by Portland cement). In this presentation, an innovative technology for ground improvement, through biologically induced calcite cementation (bio-cementation), will be presented and discussed. This technology should replace most conventional soil stabilization methods, for superior urban and coastal infrastructure developments.

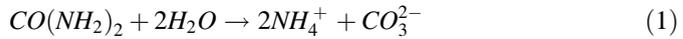
## 1 Introduction

Unstable soils encountered in civil and resource constructions possess poor geotechnical engineering properties, and thus pose significant construction, maintenance and safety issues. Among the conventional methods of ground improvement, chemical stabilization by grouts (e.g., lime or cement) is widely employed, where the chemical additives alter the mineralogical structure of soil to improve its physical and mechanical properties. However, most chemical grouts increase the pH of groundwater to highly alkaline levels and reduce soil permeability, thereby causing serious environmental problems and contributing to disturbance of local ecosystems. For instance, acrylamide grout was associated with five cases of water poisoning in Japan resulting in the ban of nearly all chemical grouts (Karol 2003). Recently, American agencies have proposed to ban most synthetic grouting materials (DeJong et al. 2010), both because of their toxicity and contribution to global carbon dioxide emissions (Li et al. 2013). As there are more than 40,000 worldwide projects/year employing chemically stabilized soils at a total cost exceeding US\$6 billion (DeJong et al. 2010), there is an immense

need for alternative environmentally-friendly and sustainable technologies to fulfil the increasing demand on ground improvement.

Bio-cementation via microbial-induced calcite precipitation (MICP) consists of injecting or mixing soil with cultivated ureolytic earth bacteria, typically *Bacillus pasteurii* (also known as *Bacillus sphaericus*) or *Bacillus megaterium*, in urea and calcium-rich solution. The microbial hydrolysis of urea generates carbonate and precipitates calcite (calcium carbonate) on the surfaces of the constituent soil grains. This process forms cementing bridges that bind soil particles together, leading to increased soil strength and stiffness. The chemical reactions of bio-cementation involves two main steps (Cheng et al. 2013):

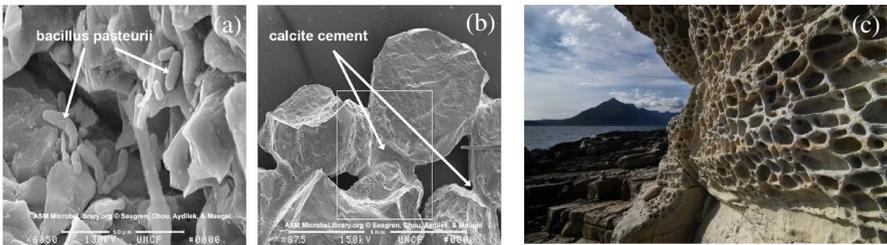
First, microbial urease hydrolyzes the urea to form ammonium and carbonate ions:



Second, in the presence of a calcium source (e.g.,  $\text{CaCl}_2$ ) the produced carbonate ions react with calcium ions and precipitate as calcium carbonate crystals:



Because calcite occurs on the soil grains, bio-cemented soils remain relatively permeable, which is desirable in most geotechnical applications. Advanced imagery revealed that bio-cemented soils have unique fabric and structure that differ from other artificially cemented soils, because the mineralogy and size of the calcium carbonate crystals attached to the native soil particles are dependent on the bio-chemical conditions (Al Qabany and Soga 2013; Cheng et al. 2017; Mujah et al. 2017). It has also been observed that calcite is as effective as other cements in increasing strength and stiffness, and that bio-cementation has proved its sustainability and capability to alter and improve most soil engineering properties (Cheng et al. 2013). This technology is eco-friendly and sustainable as it uses a bacteria-driven process that results in calcite precipitation, which mimics the natural diagenesis from sand to sandstone or lime to limestone (see Fig. 1), only within a short time instead of million years. This paper presents some features of the research undertaken at Curtin University (Australia) on bio-cementation technology.



**Fig. 1.** Natural bacteria induced bio-cementation: (a–b) [3dp.bio/research-labs/mica](https://3dp.bio/research-labs/mica); and (c) rock formation ([protocolarchitecture.wordpress.com](http://protocolarchitecture.wordpress.com))

## 2 Experimental Program

This section briefly presents and discusses some experimental results obtained from the work done by the authors, including the practicality of this technique towards its usage for ground improvement. The effects of some salient factors affecting bio-cementation are examined and presented, including the initial soil pH, degree of saturation of treated soil, degree of temperature at which bio-cementation occurs, presence of fines in the treated soil matrix, and attainment of treatment uniformity. In addition, the possibility of using the seawater as a calcium source for bio-cementation is also investigated, which can significantly reduce the cost of bio-cementation treatment in marine environment. Moreover, the paper presents the use of bio-cementation to produce sand bricks “bio-bricks” as a construction material.

### 2.1 Preparation of Materials

Bio-cementation via MICP is usually more successful for sand soils, which have pore sizes within the range of injected bacterial solutions (Mitchell and Santamarina 2005; Mujah et al. 2017). However, the feasibility of treating sand with fines and the corresponding maximum cementation strength have also been assessed by the authors (see Cheng and Shahin 2015). The authors also investigated the use of bio-cementation for treating oil-contaminated soils, utilizing a modified approach of MICP technique (Cheng and Shahin 2017), and enhancing fiber/matrix bonding in polypropylene fiber reinforced cementitious composites (Hao et al. 2018).

In this paper, sand specimens of 50 mm in diameter and 150 mm in height were prepared and used for the experimental program. Before treatment, highly ureolytic bacteria were cultivated aerobically in the laboratory as follows. The urease active strain used was *Bacillus sphaericus*. The liquid medium was prepared using 20 gm per liter of yeast extract added to deionized water. The following substances were added to the media: 0.17 M of Ammonium Sulphate and 0.1 mM of Nickel Chloride. Before inoculation, the growth medium was sterilized by supplying an inoculum of approximately 2–5% of pure bacteria culture into the medium to initiate microbial growth, then the medium was incubated for an extended period of time. After 24 h of cultivation at 28 °C, the bacteria culture was collected and stored at 4 °C prior to use. The optical density (OD600) of the culture varied between 0.6–1.0, and the urease activity was approximately 5 U/mL (1 U = 1  $\mu$ mol urea hydrolyzed per min). Reagent solutions containing a mixture of 1 M calcium chloride (111 g/L) and 1 M urea (60 g/L) were flushed through the sand specimens at an injection flow rate of 1 L/h. Throughout the flushing applications, fully saturated condition (i.e., 100% degree of saturation) was maintained (except for studying the impact of degree of saturation) using a pressurized vacuum to remove the previously supplied solution, leaving the next solution application as residual. For the seawater experiments, the cementation solution was prepared by adding 10 mM urea (0.6 g/L) into seawater. Figure 2 shows a typical example of the prepared sand specimens treated with MICP bio-cementation and the corresponding SEM image displaying the formation of calcite crystals at the soil contact points.



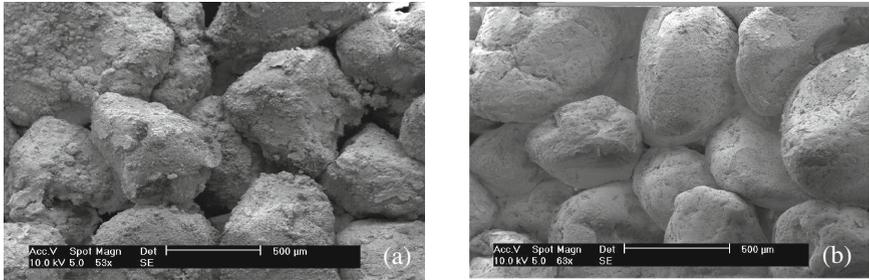
**Fig. 2.** Typical example of bio-treated sand (left) and its SEM image (right)

### 2.1.1 Initial pH of Soil

The results of the effect of initial pH of soil indicated that the acidity and alkalinity conditions of soil have negative impact on the bio-treated soil, resulting in decreased soil strength even in the presence of high content of calcite crystals. As mentioned by many researchers (e.g., Harkes et al. 2010), the soil pH value can influence the bacteria transport and adhesion, which is an essential factor for achieving homogeneously improved strength. The initial pH of soil can also affect the formation of crystals as the solubility of calcite varies according to the pH value.

### 2.1.2 Degree of Saturation

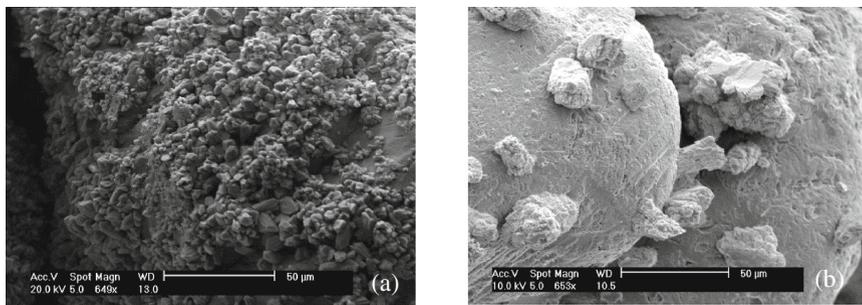
To provide the desired degree of saturation (i.e., 20%, 40%, 80%, and 100%) within the soil matrix, the reagent solution (i.e., calcium chloride and urea) was flushed through the sand specimens at various amounts of water. Throughout the flushing applications, a specified degree of saturation was maintained using a pressurized vacuum to remove the previously supplied solution, leaving the next solution application as residual. The results indicated that at any degree of saturation, there is an increase in the soil compressive strength with the increase in the produced calcite content, at any degree of saturation (Cheng et al. 2013). The results also proved that at the same calcite content, the soil treated at lower degree of saturation exhibited significantly higher values of shear strength. This can be attributed to the effectiveness of the calcite crystals formation within the soil matrix, which is clearly demonstrated by the images taken from the scanning electron microscopy (SEM) shown in Fig. 3, for soil treated at fully and partially saturated conditions. For fully saturated treatment condition (Fig. 3a), the produced calcite is not fully formed at the inter-particle contact points of the soil grains but rather floccules either on the grain surface or suspends in the pore space between the soil grains. These nucleation sites are ineffective; hence, the calcite formation provides no significant soil strength improvement. In the case of partially saturated treatment condition (Fig. 3b), the calcite formation effectively coats over the soil particles and predominantly occurs at the effective areas of the granular contact points. This calcite formation provides rationale to the significant reduction in the calcite content, obtaining similar strength to that witnessed for the saturated condition. The above results indicate that the bio-treated soils gain its mechanical strength mainly through the effectiveness of the calcite crystals formation rather than the total amount of produced calcite.



**Fig. 3.** SEM images of bio-cemented soil treated at: (a) fully saturated condition; and (b) partially saturated condition (Cheng et al. 2013)

### 2.1.3 Degree of Temperature

The values of degree of temperature used in the present study simulate the subsurface soil temperature in the cold (4 °C), tropical (25 °C), and arid (50 °C) regions. During the process of MICP treatment, the sand samples used were placed: (1) inside 4 °C refrigerator; (2) at the room temperature  $\pm 25$  °C; and (3) inside 50 °C oven. The results indicated that at any similar produced amount of calcite, the strength improvement was higher at 25 °C compared to that at either lower temperature of 4 °C or higher temperature of 50 °C (Cheng et al. 2017). The calcite crystals formed at 50 °C were the least efficient to gain strength improvement. The microstructure examination indicated that treatment at 50 °C leads to typical individual small crystals of 2–5  $\mu\text{m}$ , covering the entire sand grain surface as a coating layer (see Fig. 4a). Small calcite crystals cannot effectively connect the soil grains, 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 production of abundant small crystals. For soil treated at 25 °C, the average crystal size increased by 10 times (individual crystals size between 20–50  $\mu\text{m}$ ) compared to those formed at 50 °C (see Fig. 4b). These large calcite crystals precipitate on the grain surface, covering the contact areas of the sand grains. At low temperature of 4 °C, small individual calcite crystals of 5–10  $\mu\text{m}$  were observed, similar to those observed at 50 °C, and this is due to the slow crystal growth rate due to the slow urea hydrolysis process.



**Fig. 4.** SEM images of bio-cemented soil treated at different temperature: (a) hot weather 50 °C; and (b) room temperature  $\pm 25$  °C (Cheng et al. 2017)

#### 2.1.4 Percentage of Fines

Sand specimens containing clay fines of 5% and 10% were treated. The results of bio-treated sand containing 5% clay showed a slight increase in the UCS values compared to those obtained from bio-treated pure sand (Cheng and Shahin 2015). This is due to the increased cohesion of the sand with fines, or the increase in the larger contact surface area provided by the clay fine particles, which may facilitate the bridging formation between the sand particles via the calcite crystals. The specimens of sand containing 10% clay fines encountered clogging by the excessive cementation at the injection end and the minor calcite precipitation inside the treated sand specimens (Cheng and Shahin 2015). This observation occurred after three flushes of treatment, and further treatment became difficult to conduct due to the serious clogging. This is due to the high amount of hydrolysed urea and the calcite formed at the injection end. The sand samples containing 10% clay content, which have smaller pores, acted as a filter to the bacteria and resulted in accumulation of the bacterial cells around the injection end. The accumulated urease activity associated with the low infiltration rate of the cementation solution resulted in excessive bio-cementation occurred at the injection end rather than uniformly distributed along the sand columns. This indicates that bio-cementation using the injection method may not be applicable to treat soils that contain more than 5% fines due to the immediate bio-clogging at the injection end (the bacterial cells block the pores) and thus alternative treatment processes are necessary for bio-cementation treatment of coarse grained soils containing fines.

#### 2.1.5 Attainment of Treatment Uniformity

One of the main intrinsic obstacles of MICP bio-cementation technology as a practical ground improvement methodology is the uniformity of calcite formation and the corresponding mechanical strength achieved, which are due to the non-uniform transport and attachment of bacteria to the surface of soil particles. This is because when the bacteria travel through the pore space of soils, they are likely to be filtered through the soil grains with long-linear reduction of microbe concentration along the injection path (Ginn et al. 2002). Furthermore, the attachment of bacteria to porous materials is usually influenced by many physical, chemical and biological factors, and adsorbed bacteria can be remobilized from the soil surface into the liquid phase by flushing of low salinity solutions (Harkes et al. 2010). In this regard, the authors proposed a novel approach that can achieve a good MICP treatment uniformity (see Cheng and Shahin 2016). The approach uses pre-formed urease active crystals, named as “bio-slurry”, as a source of urease activity to induce a homogeneous bio-cementation for soil stabilization. In contrast to the other usually adopted MICP treatment methods, this treatment approach involves premixing of bio-slurry with soil, similar to the traditional cement mixing method, followed by flushing of the cementation solution. Compared to the use of bacterial cells, the advantages of using bio-slurry include more uniform and controllable activity distribution, high urease activity retention and firm attachment against water flushing. Figure 5 shows photos of the bio-slurry used in the bio-slurry treatment approach, taken during the reaction period and settling period.



**Fig. 5.** Photos of bio-slurry taken during the reaction period (left) and settling period (right) (Cheng and Shahin 2016)

### 2.1.6 Use of Seawater

The cost of MICP process including bacterial cultivation, chemical usage, equipment, and labour may prevent the progress of further commercial development of this emerging ground improvement technique. Consequently, the authors have made an attempt (see Cheng et al. 2014) to exploit the potential benefit of using the seawater as a calcium source in the cementation solution instead of the commercially available calcium chloride, for marine environment. This attempt reduces the cost of bio-cementation and brings it closer to be cost-effective and commercially acceptable ground improvement alternative. The results clearly demonstrated the feasibility of using the seawater as a calcium reagent for bio-cementation. However, the use of seawater as a relatively dilute calcium solution requires several number of subsequent treatments (probably 60 or 80 flushes) to get similar UCS values of those obtained from the traditional cementation solution of urea and calcium chloride.

### 2.1.7 Production of Bio-bricks

Bricks are usually used as building blocks for structures; however, the manufacturing of commonly used bricks is either energy intensive (e.g., fired clay bricks) or relies on unsustainable or un-environmentally-friendly materials that contribute to the global carbon dioxide emissions (e.g., cement bricks). The authors used MICP to form sustainable bricks called “bio-bricks” that do not utilize energy consumption. The work aimed to determine the viability of using bio-bricks as a construction material based on the associated strength, durability, and masonry properties, by means of comparison to relevant standards. The properties investigated include the uniformity of calcite precipitation, compressive strength, salt attack resistance, water absorption, and fire resistance. Figure 6 shows typical examples of the produced bio-bricks.



**Fig. 6.** Photo of produced bio-bricks

### 3 Conclusions

This paper demonstrated the use of bio-cementation via microbial-induced calcite precipitation (MICP) as a promising technique for ground improvement and production of bricks. However, the efficiency of bio-cementation in improving the soil strength varies significantly according to the physical and environmental treatment conditions. Findings presented in this paper confirmed that higher soil strength can be obtained at lower degree of saturation, challenging the widely belief that bio-cemented soils need to be treated under full saturated conditions. The results also indicated that bio-cementation is able to process in different environmental conditions, such as extreme high temperature. However, the compressive strength of bio-treated soils can vary significantly, depending on the treatment environmental condition, with lower strength gained at hot temperature of 50 °C compared to lower temperature of 4 °C or room temperature of 25 °C. The paper also indicated that bio-cementation of sand with fines (e.g., clayey sand) is not successful as it faces a tremendous challenge; thus, requires further investigation in the future. The paper also emphasised the potential use of the bio-slurry in providing a better treatment uniformity for MICP bio-treated soil compared to the usually used injection method of bacteria-cementation solution. The paper also confirmed that it is possible to use the seawater as a chemical reagent for bio-cementation to replace the calcium chloride, to provide a considerable strength improvement after repeated treatments. This finding is interesting as it extends the application of bio-cementation to broader areas, such as ground improvement in marine environment. Marine bio-cementation can potentially contribute to more sustainable human activities and significantly benefit society in areas of offshore and onshore infrastructure protection and maintenance, as well as coastline erosion prevention. Finally, the paper demonstrated that bio-cementation was successful in producing bio-bricks that possess good construction material attributes, complying with available codes and standards.

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## References

- Al Qabany, A., Soga, K.: Effect of chemical treatment used in MICP on engineering properties of cemented soils. *Geotechnique* **63**(4), 331–339 (2013)
- Cheng, L., Cord-Ruwisch, R., Shahin, M.A.: Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. *Can. Geotech. J.* **50**(1), 81–90 (2013)
- Cheng, L., Shahin, M.A. Assessment of different treatment methods of microbial-induced calcite precipitation for clayey soil improvement. In: 68th Canadian Geotechnical Conference (GeoQuebec), Quebec, Canada (2015)
- Cheng, L., Shahin, M.A.: Urease active bio-slurry: a novel soil improvement approach based on microbially induced calcite precipitation. *Can. Geotech. J.* **53**(9), 1376–1385 (2016)
- Cheng, L., Shahin, M.A.: Stabilisation of oil-contaminated soils using microbially induced calcite crystals by bacterial flocs. *Geotech. Lett.* **7**(2), 1–6 (2017)
- Cheng, L., Shahin, M.A., Cord-Ruwisch, R.: Bio-cementation of sandy soil using microbial-induced carbonate precipitation (MICP) for marine environments. *Geotech.* **64**(12), 1010–1013 (2014)
- Cheng, L., Shahin, M.A., Mujah, D.: Influence of key environmental conditions on microbially induced cementation for soil stabilization. *J. Geotech. Geoenviron. Eng.* **143**(1), Article Number 04016082 (2017)
- DeJong, J.T., Mortensen, B.M., Martinez, B.C., Nelson, D.C.: Bio-mediated soil improvement. *Ecol. Eng.* **36**(1), 197–210 (2010)
- Ginn, T.R., Wood, B.D., Nelson, K.E., Scheibe, T.D., Murphy, E.M., Clement, T.P.: Processes in microbial transport in the natural subsurface. *Adv. Water Resour.* **25**, 1017–1042 (2002)
- Hao, Y., Cheng, L., Hao, L.H., Shahin, M.A.: Enhancing fiber/matrix bonding in polypropylene fiber reinforced cementitious composites by microbially induced calcite precipitation pre-treatment. *Cement Concr. Compos.* **88**, 1–7 (2018)
- Harkes, M.P., van Passen, L.A., Booster, J.L., Whiffin, V.S., van Loosdrecht, M.C.: Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecol. Eng.* **36**(2), 112–117 (2010)
- Karol, R.H.: *Chemical Grouting and Soil Stabilisation*. Marcel Dekker, New York (2003)
- Li, J., Tharakan, P., Macdonald, D., Liang, X.: Technological, economic and financial prospects of carbon dioxide capture in the cement industry. *Energy Policy* **61**, 1377–1387 (2013)
- Mitchell, J.K., Santamarina, J.C.: Biological considerations in geotechnical engineering. *J. Geotech. Geoenviron. Eng.* **131**(19), 1222–1233 (2005)
- Mujah, D., Shahin, M.A., Cheng, L.: State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. *Geomicrobiol J.* **34**(6), 524–537 (2017)