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**Urease active bio-slurry: a novel soil improvement approach
based on microbially induced carbonate precipitation**

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14 **Urease active bio-slurry: a novel soil improvement approach based on microbially**
15 **induced carbonate precipitation**

16 Liang Cheng and Mohamed A. Shahin

17 **Abstract:**

18 This paper presents a novel approach for soil stabilization by microbially induced carbonate
19 precipitation (MICP) using a new urease active catalyzer named herein as “bio-slurry”. The
20 bio-slurry, which was produced from reaction between bacterial culture and 400 mM of
21 CaCl_2 and Urea, is pre-formed urease active crystals consisting of CaCO_3 plus imbedded
22 urease active bacterial cells. By mixing the bio-slurry with sand, more than 95% of the bio-
23 slurry was retained in the soil matrix as a result of the mechanical trapping mechanism,
24 leading to high resistance to flushing of low salinity solution. The retained urease activity of
25 bio-slurry was uniformly distributed along the sand matrix, resulting in a rather uniform
26 CaCO_3 precipitation. Through repeated treatments with cementation solution, the unconfined
27 compressive strength of bio-slurry treated sand was significantly improved due to the
28 effective CaCO_3 precipitation at the contact points of soil grains. Scanning electron
29 microscopy analysis carried out on the bio-slurry treated sand revealed that the induced large
30 rhombohedral CaCO_3 crystals were localized around the bio-slurry spherical fine crystals.
31 The overall outcome of this work is that soil biocementation using the new bio-slurry
32 approach is controllable, reproducible and homogeneous.

33 **Keywords:** Bio-slurry; microbially induced carbonate precipitation (MICP); bio-
34 cementation; ground improvement; soil stabilization.

35 Introduction

36 Over the years, the growth of human population has rendered the construction on weak soils
37 inescapable (Ng et al. 2012). Current remedial methods for ground improvement include soil
38 replacement, chemical stabilization by grouts, mechanical stabilization by compaction,
39 consolidation by drainage, reinforcement with steel or geosynthetics, and use of electro-
40 osmosis. Among these methods, chemical stabilization by grouts is widely employed, where
41 the mineralogical structure of soil is altered by chemical additives (e.g. cement or lime) to
42 improve the physical and mechanical engineering properties of soil. However, chemical
43 grouts can cause serious environmental problems and contribute to ecosystem disturbance
44 and thus are considered to be hazardous (Soon et al. 2014). In fact, initiatives in several
45 countries, such as the USA, have proposed to ban most synthetic grouting materials (DeJong
46 et al. 2010) both because of their toxicity and due to a desire to reduce cement use as this is a
47 major contributor to global anthropogenic carbon dioxide emissions. As there are more than
48 40,000 projects/year performed worldwide employing chemically stabilized soils at a total
49 cost exceeding US\$6 billion, there is an immense need for alternative environmentally-
50 friendly and sustainable technologies that can fulfil the increasing demand for ground
51 improvement (DeJong et al. 2010; Ng et al. 2012; Achal and Mukherjee 2015).

52 An emerging soil stabilization method, known as microbially induced carbonate precipitation
53 (MICP) utilizing the concept of bio-mineralization, has been recently proposed as an
54 alternate, environmentally safe ground improvement technology. This technique is relatively
55 new in geotechnical engineering but has been potentially promising in terms of its capability
56 of improving the engineering properties of soil while preserving environmental sustainability
57 (Whiffin et al. 2007). MICP uses the metabolic pathways of ureolytic bacteria to form
58 calcium carbonate precipitation throughout the soil matrix, thereby, strengthening the soil

59 strength and stiffness while being able to retain sufficient permeability which is favored for
60 many geotechnical engineering applications.

61 One of the main intrinsic obstacles of MICP technology as a practical ground improvement
62 methodology is the uniformity of CaCO_3 formation and corresponding mechanical strength
63 achieved, which are due to the non-uniform transport and attachment of bacteria to the
64 surface of soil particles. Several attempts have been found in the literature to overcome this
65 problem. For example, Stocks-Fischer et al. (1999) found that the injection of bacteria and
66 reagents together at lower rates can result in a full clogging of the system near the injection
67 point. Whiffin et al. (2007) developed a two-phase injection method, whereby one pore
68 volume of bacterial suspension was used followed by one pore volume of 50 mM CaCl_2
69 solution before injecting the cementation solution. Although the results showed that it was
70 possible to precipitate CaCO_3 at a distance of 5 m, the CaCO_3 content and consequent
71 mechanical behavior were not homogeneously distributed over the tested sand column. The
72 feasibility of such treatment method was further demonstrated in a large-scale trial (100 m^3)
73 by Van Paassen et al. (2010); however, the outcomes indicated varied strength of products
74 from loosely cemented sand to moderately strong rock with varied unconfined compressive
75 strength (UCS) between 0.7 and 12 MPa as a result of the non-uniform distribution of CaCO_3
76 precipitation. Feng and Montoya (2015) tested the behavior of sand treated with MICP under
77 confined pressure using the two-phase injection method and the results indicated a non-
78 uniform calcium carbonate distribution along the tested sand column (scale of cm), especially
79 after heavy cementation (i.e. $> 4\%$ calcium carbonate with variation more than 40%). Similar
80 heterogeneity of cementation distribution was also reported by Martinez et al. (2013). More
81 recently, Cheng and Cord-Ruwisch (2014) has developed a new treatment method that relies
82 on surface percolation to apply bacteria and cementation solution into unsaturated sandy soil,

83 and a development of heterogeneity of cementation along 2-m sand column (vertical direction)
84 was observed during treatment.

85 In fact, it is difficult to achieve MICP induced cementation that is highly reproducible and
86 homogeneous via the abovementioned commonly used bio-chemical injection methods. This
87 is because when the bacteria travel through the pore space of soils, they are likely to be
88 filtered through the soil grains with long-linear reduction of microbe concentration along the
89 injection path (Ginn et al. 2002). Furthermore, the attachment of bacteria to porous materials
90 is usually influenced by many physical, chemical and biological factors, and adsorbed
91 bacteria can be remobilized from the soil surface into the liquid phase by flushing of low
92 salinity solutions (Harkes et al. 2010). In the present work, a novel approach using pre-
93 formed urease active crystals, named herein as “bio-slurry”, is proposed as a source of urease
94 activity to induce a homogeneous biocementation for soil stabilization. In contrast to the
95 current usually adopted MICP treatment methods, the newly invented bio-slurry treatment
96 approach involves premixing bio-slurry with soil, similar to the traditional cement mixing
97 method, followed by flushing of cementation solution. Compared to the use of bacterial cells,
98 the advantages of using bio-slurry include more uniform and controllable activity
99 distribution, high urease activity retention, and firm attachment against water flushing. In the
100 current study, several parameters in relation to the use of bio-slurry approach for soil
101 stabilization are examined and discussed, including the urease activity retention and
102 distribution, long-term stability, microstructure and CaCO_3 content.

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104

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Materials and methods

Ureolytic bacteria and cementation solution

The urease active bacteria used in the current study were *Bacillus sp.* strain (DSM 23526) (Al-Thawadi et al. 2012), which were cultivated in a sterile aerobic batch growth medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate and 0.1 mM NiCl_2 , at a pH value of 9.25. The cultivated bacterial culture was collected at the stationary phase of culture growth after 24 hours of cultivation at 28°C. The optical density (OD_{600}) of collected bacterial culture varied between 2–2.5, and the urease activity was approximately 10 U/mL (1 U = 1 μmol urea hydrolyzed per minute). The culture was stored at 4°C refrigerator no longer than two days prior to use. The cementation solution used consisted of 1 M calcium chloride (111 g/L) and 1 M urea (60 g/L), and the pH was found to be between 6.6–6.7.

Bio-slurry preparation

The bio-slurry was prepared by adding specific amounts of urea and calcium chloride (equal moles) into the collected bacterial culture (100 mL) to reach target concentrations of 50, 100, 200, 400, 600, and 800 mM, followed by stirring at a speed of 600 rpm for about 12 hours. Because of the presence of urea and Ca^{2+} , the CaCO_3 crystals were formed due to the urea hydrolysis by the ureolytic bacteria (Fig. 1). The formed crystals were eventually precipitated out of the aqueous phase together with the bacterial cells. When the added urea and CaCl_2 were completely consumed, which was determined by measuring the amount of produced ammonia/ammonium in the solution, the mixture was allowed to settle for 6 hours. After settlement, a clean supernatant was disposed and settled crystals (bio-slurry) were then harvested. The produced bio-slurry possessed a solid content of about $15 \pm 1\%$ (w/w). The bio-

128 slurry was produced through 400 mM urea and CaCl_2 , and was used for long-term stability,
129 microstructure analysis and soil stabilization experiments.

130 **Urease activity measurement**

131 The urease activity of bio-slurry was determined by measuring the urea hydrolysis rate of
132 tested bio-slurry. This was made by recording the change in the ammonium concentration of
133 a mixture consisted of 15 ml 3 M urea solution, 5 g bio-slurry (about 15% solid content) and
134 10 ml DI water, over 30 mins. The ammonium concentration was determined using the
135 Nessler method (Greenburg et al. 1992). The specific urease activity of bio-slurry was
136 presented as urease activity per g of completely dried bio-slurry (100% solid content). For
137 measuring the urease activity along the sand column, about 10 g of sand was sampled from
138 different locations and added to 30 mL of urea solution (1.5 M), followed by recording the
139 change in the ammonium concentration over 30 mins, as described above. After the urease
140 activity was measured, the sand sample was rinsed with deionized (DI) water and dried at
141 105°C for 12 hours. The weight of dry sand sample was recorded and the corresponding
142 urease activity was normalized per g of dry sand.

143 **Soil and cementation method**

144 Pure silica sand (Cook Industrial, Minerals Pty Ltd Western Australia) was selected for the
145 current study, with the following contents: > 0.425 mm (0.53%); 0.3–0.425 mm (50.78%);
146 0.15–0.3 mm (45.96%); and < 0.15 mm (2.73%). Before the bio-cementation treatment, the
147 sand was premixed with the bio-slurry at a weight ratio of 10:1 (w/w). A standard Proctor
148 compaction test was carried out on the mixture in accordance with the Australian Standards
149 AS 1289.5.4.2-1993 (2000), and a maximum dry density of 16.54 kN/m³ was obtained for the
150 mixture. The sand and bio-slurry were compacted into polyvinyl chloride (PVC) columns of

an inner diameter of 45 mm until the maximum dry density was reached. PVC columns of 300 mm in height were used to investigate the distribution of urease activity and CaCO_3 precipitation, whereas PVC columns of 120 mm in height were used to evaluate the strength improvement during the treatment process. The cementation solution was injected into the sand columns multiple times to reach different cementation levels. Each injection included 1.1 pore void volume of cementation solution introduced to the sand column from the bottom at a constant flow rate of 30 mL/min to generate a constant fully-saturated condition. For field applications, however, flushing can be carried out horizontally between the injection and extraction pipes, where the soil is fully saturated with the fluid, as proposed by van Paassen et al. (2010). Downward vertical flushing using the surface percolation method can also be used, as explained by Cheng and Cord-Ruwisch (2014). After injection was finished, the sand columns were kept at the room temperature ($25 \pm 1^\circ\text{C}$) for 48 hours to allow for a complete cementation reaction.

Unconfined compressive strength and CaCO_3 content determination

The bio-slurry treated sand specimens were flushed with at least five-void volume of tap water to wash away any excess soluble salts prior to the UCS measurements. Before carrying out the UCS tests, the sand samples were dried at 105°C for at least 24 hours. According to the procedure reported in the ASTM D2166 (ASTM 2013), the UCS tests were conducted on bio-cemented specimens of selected diameter-to-height ratio of between 1:1.5 and 1:2. The axial load was applied at a constant rate of 1.0 mm/min.

The calcium carbonate content of bio-treated soil samples was determined by adding 20 mL of 2 M hydrochloric acid (HCl) solution into 15-20 g of dry crushed cemented sample, and the volume of CO_2 gas was measured using a U-tube manometer under standard conditions of

25°C and 1 atm (Whiffin et al. 2007). The actual amount of CaCO_3 was calculated using an established relationship between the volume of CO_2 gas and amount of pure analytical grade CaCO_3 powder. It should be noted that, for each sample, measurements of CaCO_3 were carried out at least 3 times so as to obtain an average level of CaCO_3 precipitation.

Microstructure analysis

To characterize the crystals shape and size of bio-slurry and induced CaCO_3 precipitation, and to investigate the bonding behavior between the grain hosts and bio-cemented agent, a microscopy analysis was conducted on dried bio-slurry and cemented soil samples. By knowing exactly how and where the crystals were formed in the grain matrix of bio-cemented soil, it is possible to relate the effectiveness of precipitated CaCO_3 crystals to the corresponding obtained UCS values. Before conducting the microscopy investigation, all bio-cemented soil samples were flushed with tap water and dried at 105°C for 24 hours. The microscopy investigation was carried out using the scanning electron microscopy (SEM) (Tescan Mira3 XMU).

Presentation of results

Effect of concentration of cementation solution on specific urease activity of bio-slurry

In order to find the optimum conditions for bio-slurry production, various concentrations of urea and CaCl_2 were used. It can be seen in Fig. 2 that a linear relationship between the total amount of bio-slurry and concentration of urea and CaCl_2 was observed. For example, for the 800 mM concentration of urea and CaCl_2 about 8 g of bio-slurry (dry weight) was produced from 100 mL bacterial culture, which was 16 times higher than that produced using 50 mM concentration. It can also be seen that the bio-slurry produced using different concentrations

of urea and CaCl_2 indicated a remarkable difference in terms of the specific urease activity, which is defined as the urease activity per dry weight of bio-slurry. The specific urease activity decreased with the increase in concentration of urea and CaCl_2 . The maximum specific urease activity (about 1050 U/g) was observed when 50 mM concentration was used, which was about 15 times higher than that of the bio-slurry produced using 800 mM. This is attributed to the fact that higher concentration of urea and CaCl_2 caused larger quantity of crystal precipitation, with possibly thicker layer crystals around the bacterial cells. This thick layer of crystals would reduce the diffusion rate of urea to bacteria. As suggested by Cuthbert et al. (2012), the growth of crystals on the surface of bacterial cells encapsulates the cells leading to a reduction in the ureolysis rate. The loss of urease activity due to encapsulation of crystals was commonly found during MICP process and van Paassen (2009) suggested that the decrease in the bacterial urease activity might be represented by an exponential decay function.

It should be noted that although the highest specific urease activity in the present study was obtained using 50 mM of urea and CaCl_2 , the maximum total urease activity of bio-slurry was obtained using 400 mM concentration. This is due to both the relatively high bio-slurry yield and specific urease activity compared to the bio-slurry produced using other concentrations. It is also worthwhile noting that the optimum concentration of urea and CaCl_2 obtained in the current study may not be applicable to other bacterial culture of different urease activity and biomass concentration.

Achal et al. (2009) found that the calcite production by the ureolytic bacteria varies depending on the urease activity. Accordingly, a bio-slurry with the same total urease activity might not be able to induce the same amount of CaCO_3 precipitation. In order to investigate the efficiency of the CaCO_3 production using bio-slurry produced from different

concentrations of urea and CaCl_2 , various amounts of bio-slurry of different urease activities were added to an excess amount of 1 M CaCl_2 and urea solution (250 mL). After 48 hours and when no urea hydrolysis reaction was detected (i.e. urease activity decreased to zero), the solution was filtered through 0.45 μm filter paper and the amount of crystal was recorded. The correlation between the added amount of urease activity (U) and amount of induced CaCO_3 crystals is presented in Fig. 3. It can be seen from Fig. 3a that the yield efficiency of CaCO_3 precipitation was decreased using the bio-slurry produced from higher concentration of urea and CaCl_2 . The bio-slurry produced using 50 mM urea and CaCl_2 was recorded to be the most effective to induce CaCO_3 precipitation, which was about 0.0312 g CaCO_3 per 1 U urease activity consumed, and was about 3 times more efficient than that produced using 800 mM concentration. Considering the total urease activity and efficiency of CaCO_3 production, the optimum bio-slurry was obtained using 400 mM concentration of urea and CaCl_2 , which can produce a maximum amount of CaCO_3 crystals of about 21.9 g (see Fig. 3b).

Long-term stability of bio-slurry

The long-term stability of bio-slurry was tested over one month period. During testing, the bio-slurry was stored in a sealed glass bottle at 4°C. The bio-slurry was then placed at the room temperature for 2 hours prior to measurement of the urease activity. Fig. 4 shows that the decrease in the specific urease activity of bio-slurry is less than 15% after 27 days of refrigerator storage at 4°C. This indicates a high stability of bio-slurry at the given specific time.

Microstructure of bio-slurry and induced CaCO_3

The micro-features of pure bio-slurry were examined using SEM (see Fig. 5, left column). It can be seen that the bio-slurry was dominant by spherical shape crystals with particle size

around 0.2-5 μm . There were imprints of the bacterial cells shape at the surface of the spherical crystals (Fig 5b, dashed circles). By simply mixing the pure bio-slurry with the sand without applying a subsequent treatment of cementation solution, no cementation was obtained (data not shown). This suggests that the bio-slurry itself could not provide a bonding force between the sand grains. It was also found that the bio-slurry had high and stable urease activity, and was able to induce crystals precipitation (Figs. 2-4). Therefore, it is fair to assume that, similar to the ureolytic bacteria induced crystal precipitation, the crystals induced by bio-slurry were able to adhere on the sand particle surface, connecting them together and enabling soil strength improvement. Therefore, before applying the bio-slurry approach for soil stabilization, it is worthwhile examining the micro-feature of bio-slurry induced CaCO_3 crystals.

The bio-slurry induced crystals was prepared by adding 5 g of bio-slurry produced from 400 mM urea and CaCl_2 into 100 mL of 1 M cementation solution followed by 24 hours reaction period at the room temperature without stirring. The precipitated crystals were filtered using a filtering paper (0.45 μm) and was rinsed with DI water, followed by drying at 105°C for 24 hours. The micro-feature analysis of the bio-slurry induced crystals was presented in Fig. 5 (right column). It was found that large rhombohedral crystals of size of around 20 μm were observed. These crystals were aggregated together with fine spherical-shape crystals, as indicated by the arrows in Fig. 5(d). This type of rhombohedral crystals was not observed in the pure bio-slurry sample (see Fig. 5, left column). Therefore, it can be concluded that the rhombohedral crystals were induced by the bio-slurry. The absence of the imprints of bacterial cells on the rhombohedral surface suggests that a pure chemical reaction might have occurred to precipitate this type of crystals. This in turn suggests a diffusion mechanism of CO_3^{2-} ions from the inside bacterial cells (where the intercellular urea hydrolysis occurs) to

the surrounding solution which drives the precipitation of such rhombohedral crystals once the critical supersaturation of Ca^{2+} and CO_3^{2-} was achieved, as discussed by Al-Thawadi and Cord-Ruwisch (2012). According to the literature, the rhombohedral crystals of cubic faces are a typical calcite (De Yoreo and Dove 2004).

Soil stabilization using bio-slurry

Having examined the characteristics of bio-slurry as a carrier of urease activity and inducer of CaCO_3 precipitation, it is then appropriate to test the soil stabilization using bio-slurry induced CaCO_3 precipitation for the purpose of exploring a new manner of ground improvement, as described below.

Improving urease activity retention

The retention of urease activity inside the soil is crucial for a successful biocementation. In the present study, the retention of urease activity using bio-slurry was tested. The amount of bio-slurry in the effluent was collected by filtering the effluent using 0.45 μm filter paper. After drying at 105°C, the weight of collected solids was compared with the solid weight of bio-slurry. It was found that, in all tested sand columns, more than 95% of bio-slurry was retained. It was also interesting to note that although the bio-slurry crystals were well retained within the compacted sand columns, they could be easily separated from the sand grains by re-suspending the mixture in bulk water. This suggests that there was no strong bonding force or notable chemical-physical interaction between the bio-slurry crystals and the sand particles. Therefore, the trapping of fine particles (bio-slurry) in a medium of porous materials (sand grain matrix) was considered to be a mechanical trapping, which was likely to occur at the pore “throat” (contact points) of the sand grains.

It can be seen in Fig. 6 that this type of urease activity retention as a form of bio-slurry cannot be washed out by flushing of the low salinity solution (tap water). The chemical conversion efficiency, which is defined as the percentage of injected urea and CaCl_2 that precipitate as CaCO_3 , was found to decrease from about 98% to 30% throughout the MICP process for the control sample (no tap water flushing). In comparison, the chemical conversion efficiency of the sand columns with regular flushing using tap water (i.e. 2-void volume tap water flushed before the 2nd and 4th cementation solution treatments) was only about 10% less than that achieved for the control sample.

For conventional MICP treatment, the fixation of urease active bacteria inside the soil is usually achieved by injecting the bacteria suspension followed by a fixation solution containing CaCl_2 . However, Harkes et al. (2010) found that flushing a low salinity solution may result in a large portion of fixed bacteria to be remobilized from the solid surface into the liquid phase. On the contrary, the results presented in the current study demonstrate that the use of bio-slurry results in a stable retention of urease activity against the flushing of low salinity water (i.e. tap water), enabling a successful biocementation. This has a potential implication in terms of further extending MICP application to continuous water flow regions, such as control of seepage-induced internal erosion of dams.

Improving distribution of urease activity and CaCO_3 precipitation

After mixing the bio-slurry with sand, a highly uniform distribution of urease activity (variation less than 15%) along the 300 mm sand columns was achieved (see Fig. 7a). This homogeneous distribution of urease activity was due to the uniform bio-slurry retention, which was evidenced from the homogeneous distribution of CaCO_3 content obtained before the cementation solution treatment (Fig. 7b). After treatment, the distribution of CaCO_3

content (bio-slurry plus bio-slurry induced CaCO_3) was also detected, as shown in Fig. 7b. It can be seen that the CaCO_3 content increased uniformly along the entire sand columns from 0.016 to 0.035 g/g sand for one injection and to 0.054 g/g sand for two injections, showing that the variation of CaCO_3 content along the sand columns was less than 10% (the difference between the highest and lowest levels). These results demonstrate that the bio-slurry approach can significantly improve the uniformity of urease activity and distribution of CaCO_3 . The uniform distribution of crystals after flushing the cementation solution also revealed a stable entrapping of bio-slurry, which assumes that the bio-slurry crystals were not transported and this was evident from the high retention of urease activity.

It should be noted that although the current study indicates a uniform CaCO_3 content distribution along the 30-cm sand columns, gaining highly uniform distribution at large scale (meters) is still challenging. A uniform distribution of urease activity using the bio-slurry approach is not seemed to be restricted to the distance; however, many other factors can affect the outcomes of CaCO_3 content distribution such as the concentration of cementation solution, flow rate, and preferential flow path (Cheng and Cord-Ruwisch 2014; Martinez et al. 2013).

Reproducibility of biocementation using bio-slurry

In order for the bio-slurry approach to be a reliable bio-cementation soil stabilization method, it should lead to reproducible and homogeneous bio-treated soils. Fig. 8 shows the stress-strain curves of two identical sand columns treated equally using the bio-slurry approach. It can be observed that the results of the two samples were quite comparable (both samples followed almost similar failure patterns), indicating a good reproducibility. The peak UCS

values of the two treated specimens were found to be equal to 683 kPa and 716 kPa, indicating less than 5% variation.

Microstructure of bio-slurry stabilized soils

Fig. 9 portrays the SEM results of sand particles cemented with CaCO_3 crystals induced by the bio-slurry mechanism. Details of the SEM indicate numerous rhombohedral crystals with various sizes ranging from 10 – 50 μm . This type of rhombohedral crystals is suggested to be due to the bio-slurry induced crystals (also according to the SEM results presented earlier in Fig. 5). It is interesting to find that the precipitated crystals were dominated at the gaps between the sand grains, whereas the sand grains surface possesses minimum crystals precipitation (see the dash circles in Fig. 9b). This is possibly due to the bio-slurry entrapping at the connecting points of sand grains. It has been suggested in many previous studies that this type of crystals precipitation pattern can effectively bridge the adjacent crystals and provide sufficient inter-particles bonding strength (Cheng et al. 2013; Al Qabany et al. 2012; Martinez et al. 2013). The reason that the precipitated crystals are dominating at specific locations (i.e. connecting points) in the bio-slurry treated soils can be explained as follows.

The presence of the bio-slurry crystals can act as nucleation sites for the new CaCO_3 crystals formation, by providing existing solid surface, which has lower surface energy than that of the new solute particles (Dirksen and Ring 1991). The bio-slurry crystals hydrolyze urea via the imbedded bacterial cells and release CO_3^{2-} ions into the surrounding solution. The supersaturation degree reaches the highest level on the surface of the bio-slurry crystals and decreases with the distance towards the bulk solution. The existing nucleation sites and high degree of supersaturation favor the precipitation of new CaCO_3 crystals on the surface of the bio-slurry crystals. Furthermore, the production of ammonia as a result of the enzymatic urea

hydrolysis creates an alkaline microenvironment around the bio-slurry crystals. The high pH values of these localized areas commence the growth of CaCO_3 crystals, similar to the crystal precipitation around the bacterial cells (Stock-Fisher et al. 1999). As discussed earlier, the bio-slurry is most likely to be entrapped at the pore throats (contact points). Therefore, the restricted distribution of bio-slurry enables an induced crystals formation at the particular position and contributes the most to strength development. It is interesting to note that the aggregated spherical shape crystals, which were found in both pure bio-slurry and bio-slurry induced crystal samples (see Fig. 5), were not observed clearly in the cemented soil (see Fig. 9). This is probably due attributed to the fact that the bio-slurry attached to the sand surface was gradually covered by the induced rhombohedral crystals during the cementation solution treatment.

Effect of flushes on UCS results

As indicated earlier, the development of micro-feature of crystals precipitation was observed during the treatment of cementation solution. It was believed that the regular-shape rhombohedral crystals which appeared during the treatment process would contribute to the strength improvement. In order to evaluate the strength improvement versus the number of cementation solution flushes, UCS tests were conducted for treated soil specimens for various number cementation solution flushes. It was found that the UCS values increased with the increase of the number of flushes (see Fig. 10a). However, it was also found that the rate of strength improvement decreased with the increase of the number of flushes. This can be explained by the gradually decreased chemical conversion efficiency in each flush (Fig. 10a). The decreased chemical conversion efficiency was probably due to the decreased urease activity as a result of the encapsulation of bacterial cells, resulting in a decrease in the amount of crystals precipitation and bridging bond formation. Fig. 10b shows the stress-strain curves

of the bio-slurry treated soils. It can be seen that the stress-strain curves of soil specimens treated with less number of flushes showed a noticeable plastic region, which was not observed for the samples treated with higher number of flushes. In comparison, these soil specimens failed immediately after the maximum stress was reached, indicating a typical mechanical behavior of brittle materials. It was also found that the stiffness of samples (stiffness was represented by Young's modulus obtained from the initial tangent of UCS stress-strain curve) was increased with the number of flushes, which was improved from 7 MPa (1 flush) to 190 MPa (8 flushes).

Discussion

It has been reported in the literature that the urease activity of ureolytic bacteria decreases during the MICP process (Van Paassen et al. 2010; Whiffin et al. 2007). It is also true that the ureolytic bacteria can be active for several flushes of cementation solution before the full loss of urease activity. This suggests that the ureolytic bacteria can survive from the preliminarily precipitated crystals until the bacterial cells are completely encapsulated or died. Based on this phenomenon, a bio-slurry having high urease activity was successfully produced in aqueous medium. In conventional MICP process, multiple injections of bacteria are needed to maintain the urease activity and achieve high level of cementation (Cheng and Cord-Ruwisch 2012; Feng and Montoya 2015). However, in the proposed bio-slurry approach, an introduction of different levels of urease activity can be simply achieved by controlling the level of urease activity per amount of soil during the mixing step via regulating the ratio between the bio-slurry and soil.

Predicable outcomes of MICP biocementation process are essential for field application. To achieve this, the first step is to know and predict where the bacteria are located in the soil

matrix as the bacteria catalyze the urea hydrolysis to produce cementing agents of CaCO_3 crystals. Van Wijngaarden et al. (2012) developed a mathematical model for the fixation of bacteria in MICP bio-cementation process. However, it is a big challenge to use such a model in the field since many important phenomena need to be included such as the advection, dispersion, adsorption, desorption, fixation decay, growth and systematic motion of bacteria (Fontes et al. 1991; Foppen and Schijven 2006). Because of complexity of bacteria distribution, Van Wijngaarden et al. (2010) developed a numerical model to predict behavior of MICP ground improvement based on an assumption of a homogenous distribution of bacteria, which was not likely reachable by the conventional two-phase injection method. Compared with the conventional MICP method, one of the major advantages of the proposed bio-slurry approach is the homogeneous distribution of the bacteria/urease activity distribution, which can be beneficial for establishment of a reliable numerical model that can predict the outcomes of ground improvement more precisely.

Controlling the location of crystals precipitation is also crucial to gain an effective strength improvement because only crystals that precipitate at the connecting points can provide enough bonding force among the adjacent soil grains (Cheng et al. 2013; Al Qabany et al. 2012; Martinez et al. 2013). Cheng et al. (2013) has achieved a highly effective crystal formation by restricting the crystals precipitation at the particle-particle contact points via controlling the degree of saturation of the cementation solution inside of the soil matrix. However, this method is only applicable to unsaturated soils. In the current study, the SEM images of soils treated by bio-slurry under fully saturated conditions revealed that the induced crystals were dominated at the connecting points, suggesting that the use of bio-slurry favors an effective crystals formation even in water logging areas.

Future research on this topic may include a thorough investigation on the behavior of bio-slurry using different concentrations of urease activity, uniformity at large scale (meters), and incorporation of bio-slurry method into the real world of deep mixing technology. Another investigation could be the possibility of mixing the bio-slurry and other treatment ingredients with the soil to avoid the repeated injections of cementation solution, which in turn can reduce the field operation cost. However, to achieve a good soil strength using a full mixing methodology, high content of CaCO_3 crystals precipitation will be necessary and this will require the highly concentrated cementation solution to provide enough urea and Ca^{2+} source to form sufficient amount of CaCO_3 precipitates. Such highly concentrated CaCl_2 and urea solution may dramatically reduce the urease activity, leading to very low efficiency in CaCO_3 production and thus poor cementation. Furthermore, the correlation between the soil mechanical response and amount of bio-slurry used is worthwhile an investigation.

Conclusion

This paper presents a novel approach for soil stabilization based on microbially induced carbonate precipitation (MICP) using urease active bio-slurry as catalyzer. By mixing the bio-slurry with sand, more than 95% of the bio-slurry content was retained and evenly distributed along the soil matrix, leading to a rather uniform CaCO_3 precipitation. SEM analysis carried out on bio-slurry treated sand samples revealed that the induced large rhombohedral CaCO_3 crystals were localized around the bio-slurry crystals and were also dominated at the connecting points of the sand grains. Through repeated treatments with the cementation solution, the UCS value of bio-slurry treated sand was significantly improved to 1 MPa.

The current study emphasizes the potential use of the newly proposed bio-slurry approach for soil stabilization. As an efficient alternative to the injection method of bacteria-cementation solution, the bio-slurry approach was proved to significantly improve the uniformity of the urease activity and CaCO_3 precipitation distribution. The bio-cemented sand columns using the bio-slurry approach resulted in controllable and reproducible outcomes, with an increasing strength by increasing the number of cementation solution flushes.

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551 **Figure Captions**

552 **Fig. 1.** Photos of bio-slurry taken during: (a) reaction period; and (b) settling period.

553 **Fig. 2.** Specific urease activity, total amount of bio-slurry and total amount of urease activity
554 of the bio-slurry produced from 100 mL of raw bacteiral culture using varied concentrations
555 of urea and CaCl_2 .

556 **Fig. 3.** Relationship between: (a) amount of precipitated CaCO_3 crystals and amount of
557 applied urease activity; and (b) total amount of CaCO_3 precipitation and different bio-slurries
558 produced from 100 mL of raw bacteiral culture using varied concentration of urea and CaCl_2 .

559 **Fig. 4.** Long-term stability of bio-slurry produced from 400 mM urea and CaCl_2 .

560 **Fig. 5.** SEM images of pure bio-slurry produced from 400 mM urea and CaCl_2 (left column)
561 and the same bio-slurry with induced CaCO_3 precipitation (right column).

562 **Fig. 6.** Effect of low salinity water flushing (tap water) on chemical conversion efficiency
563 during biocementation process using bio-slurry.

564 **Fig. 7.** Distribution of: (a) urease activity; and (b) CaCO_3 along 300 mm sand column.

565 **Fig. 8.** Reproducibility of bio-slurry treated sand with 3 flushes of cementation solution.

566 **Fig. 9.** SEM images of bio-slurry treated sand samples with 8 flushes of cementation
567 solution.

568 **Fig. 10.** Effect of flushes of cementation solution on the mechanical response of bio-slurry
569 treated sand: (a) UCS; and (b) stress-strain curves.

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Fig. 2. Specific urease activity, total amount of bio-slurry and total amount of urease activity of the bio-slurry produced from 100 mL of raw bacterial culture using varied concentrations of urea and CaCl₂.

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