Upscaling Effects of Soil Improvement by Microbially Induced Calcite Precipitation by Surface Percolation

Liang Cheng[1], *Ralf Cord-Ruwisch*[2]

[1]Research fellow, School of Biological Science and Technology, Murdoch University, 90 South Street, Murdoch, 6150 Western Australia

[2]Senior lecturer, School of Biological Science and Technology, Murdoch University, 90 South Street, Murdoch, 6150 Western Australia

*Corresponding author: Liang Cheng: L.cheng@Murdoch.edu.au

Abstract

This study has contributed to the technology of soil stabilization via biocementation based on microbially induced calcite precipitation. The newly described method of *in-situ* soil stabilization by surface percolation to dry soil under free draining environment is tested for its up-scaling potential. 2 m columns of one-dimensional trials indicated that repeated treatments of fine sand (< 0.3 mm) could lead to clogging closed at the injection end, resulting in limited cementation depth of less than 1 m. This clogging problem was not observed in 2 m coarse (> 0.5 mm) sand columns, allowing strength varying between 850 to 2067 kPa along the entire 2 m depth. Three-dimensional fine sand cementation trials indicated that relatively homogenous cementation in the horizontal direction could be achieved with 80% of cemented sand cementing to a strength between 2 to 2.5 MPa and to a depth of 20 cm.

A simple mathematical model elucidated that the cementation depth was dependent on the infiltration rate of the cementation solution and the *in-situ* urease activity. The model also correctly predicted that repeated treatments would enhance clogging close to the injection point. Both experimental and simulated results suggested that the surface percolation technology was more applicable for coarse sand.

Key Words: soil stabilization, biocementation, microorganisms, calcium carbonate, surface percolation

Introduction

From recent investigations of biological techniques of biocement/biogrouting for ground reinforcement (Chu et al. 2011; DeJong et al. 2010; Ivanov and Chu, 2008; Whiffin et al. 2007), it is understood that the biological activity is used to generate carbonate and elevate the pH within the soil, creating a continued slightly supersaturated calcium carbonate solution leading to controlled precipitation rates and in turn crystals sufficiently large to enable bridging between soil particles. Microbiologists and engineers have used this principle to explore microbially induced carbonate precipitation (MICP) as a soil improvement method (Whiffin et al. 2007; van Paassen et al. 2010). Controlled precipitation of calcite crystals in the pore spaces of soil with a subsequent change of mechanical properties of the soil matrix is a new innovative approach in soil geotechnics with significant scope for development (Whiffin et al. 2007).

Most studies with regard to soil improvement use bacteria containing the urease enzyme to catalyze the hydrolysis of urea into ammonium and carbonate (Eq. 1).

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

In the presence of dissolved calcium ions (e.g. $CaCl_2$), the previously produced carbonate ions will precipitate and form calcium carbonate crystals (Eq. 2).

[2] $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\ (s)$

When sufficient calcium carbonate crystals are precipitated in the pore spaces, desired mechanical properties of soil can be achieved.

While an improvement in material strength is possible by inducing MICP, the treatment often leads to limited injection depth (in the order of centimeters) associated with a major reduction in permeability, causing full clogging near the injection point (Stocks-Fischer et al. 1999). In order to prevent clogging near the inlet, sequential pumping of bacteria (or enzyme) and cementation solution ($CaCl_2$ and urea) has been developed (Kucharski et al. 2006; Al-Thawadi and Cord-Ruwisch, 2012), and described of reaching cementation depths of more than 2m (Whiffin et al. 2007).

Current exploitations of MICP technology have been mostly tested in water logged soils via using submersed flow (saturated flow) (DeJong et al. 2006; Whiffin et al. 2007; van Paassen et al. 2009a, 2009b; van Paassen et al. 2010), requiring heavy machinery and hydraulic injection of the cementation solution and physical extraction of waste solution. The feasibility of such water-saturated treatment method has been demonstrated in 100 m^3 large scale experiment, which indicated varied strength of products from loosely cemented sand to moderately strong rock with unconfined compressive strengths of 0.7 – 12 MPa (van Paassen et al. 2010).

A method of applying MICP specifically to unsaturated sandy soil by using surface percolation has been developed and demonstrated in the scale of 1 m depth (Cheng and Cord-Ruwisch, 2012). In comparison to the saturated flow MICP application, the unsaturated zone created in the surface percolation MICP treatment provided environmental conditions more conducive to the formation of effective crystals (Cheng et al. 2013).

The surface percolation technique of MICP could be applied in the geo-engineering practice of the stabilization of unsaturated soil, such as dry sand. However, under surface percolation, significant strength improvement (in the order of MPa) in larger

scale (> 1 m), and also the three-dimensional homogeneity of bio-cemented has not been investigated. The aims of this paper are to evaluate:

- The feasibility and limitation of MICP via surface percolation to reach depths of more than 1 m as it is relevant for subgrade reinforcement applications
- The 3-D surface application by simple batches of surface irrigation as it can be applied by spray trucks for dust control by cementing of loose sand grains of the topsoil.

Materials and Methods

Ureolytic bacteria and MICP Reagents

The urease active strain of *Bacillus sphaericus* (MCP-11) (DSM 23526, available from DSMZ, Germany), which was isolated from a previous study (Al-Thawadi and Cord-Ruwisch, 2012), was used in current experiments. Cultivation of strain MCP-11 was conducted under sterile aerobic batch condition in a medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate and 0.1 mM nickel chloride, at a starting pH of 9.25. After 24 hours of incubation at 28°C, the culture was collected and stored at 4°C prior to use. The urease activity of the culture was approximately 10 mM urea/min. Cementation solution (MICP reagents) consisted of 1 M calcium chloride and 1 M urea.

Sand Column and Container Setup

Four PVC columns (55 mm in diameter and 2000 mm in length) were positioned vertically and packed with either fine or coarse-grained silica sands (Cook Industrial,

Minerals Pty. Ltd. Western Australia) to a dry density of 1.62 (fine sand with porosity of 38.9 %) and 1.63 g/cm^3 (coarse sand with porosity of 37.9%) respectively.

An approximately cylindrical container (450 mm in diameter and 500 mm in height) was filled with two layers of soil. The bottom layer (thickness = 300 mm) consisted of raw sandy soil obtained from the campus of Murdoch University, Perth, Western Australia. At the bottom of the container, 5 outlets of the drains were designed for the excess of solution to drain out. The top layer (thickness = 200 mm) consisted of the pure fine silica sand, which was going to be treated by MICP via surface percolation method. The density of the fine sand layer was about 1.60 g/cm^3 with porosity of approximately 40.8 %.

MICP Process

Two-Meter Sand Columns

A summary of the treatment procedure is given in Table 1. All solutions were applied to the top of sand columns and allowed to percolate by gravity and capillary forces. Excess solution was allowed to drain from the bottom. All solutions were continuously applied into the column to form about 5 cm high ponding on the top surface of the sand until the percolation was complete.

For fine sand columns, the fixation of bacteria into the sand prior to applying the cementation solution was conducted, as described previously (Cheng and Cord-Ruwisch, 2012), by introducing 6 or 12 layers of alternating bacterial suspension and cementation solution (Figure 1) followed by 24 hours of incubation at room temperature (25±1°C). Then the sand columns were percolated with cementation solution only followed by 24 hours of reaction time at room temperature (25±1°C).

Repeated treatments of bacterial placement (supplied at day 1, 5, and 8) and cementation solution were applied in order to achieve significantly improved strength. For the coarse sand column, the process was similar to the fine sand column (6 layers) (Figure 1). Because of the lower water retention capacity (Table 1) more repeated treatments of bacterial placement (supplied at day 1, 5, 8, 11, 14, 17, and 20) and cementation solution were applied with the aim to achieve similar significantly improved strength to the fine sand. Unless specified the overall percolation treatment procedure described here was shown as follow (Figure 1 and Table 1).

Cylinder Container

In an attempt to strengthen the fine silica sand layer, a round and shallow depression with size of 35 cm in diameter and 2.5 cm in depth was formed on top of the silica sand layer to retain the MICP solutions (bacterial suspension/cementation solution) during the treatment. Under free-draining environment, the infiltration rate of the solution was dependent on gravity and capillary force.

3 L of bacterial suspension was introduced into the shallow depression via watering can forming a puddle. After the infiltration of the bacterial suspension was completed, 6 L of cementation solution was introduced followed by 24 hours of incubation of the container at room temperature. Then cementation solution (6 L) was supplied every 24 hours.

In order to maintain sufficient *in-situ* urease activity, additional bacterial suspension (3 L) was supplied at day 3, 5, 8, 11, and 14. Total injected bacterial suspension and cementation solutions were 18 and 96 L respectively. Throughout the experiment, the cylinder container was covered with plastic cover to reduce evaporation.

Monitoring Methods

Monitoring of the Urease Reaction.

The bacterial urease activity was monitored by following the concentration of its end-products, ammonia and carbonate over time. Ammonia was determined by the Nessler method (Greenburg et al. 1992). Calcium carbonate content was measured by determining the produced CO_2 gas volume (Whiffin et al. 2007).

Liquid Infiltrate Rate

The liquid infiltration rate is defined as the rate (cm/min) at which the solution penetrates into the sand column. It is calculated by dividing the inflow rate of the percolated solution (cm^3/min), by the cross section area (cm^2) and then by the porosity of the sand column.

Unconfined Compressive Strength (UCS)

In our experiments, soluble salts were removed from the cemented samples by flushing with tap water prior to strength measurement. In situ, that without removing the soluble salts, more strength will be obtained. However this is only transient strength, which would vanish over time and was deliberately not considered in the manuscript. After dismantling of the columns, the consolidated sand columns were cut into several sections to give a selected diameter to height ratio of 1:1.5 to 1:2. The unconfined compressive strength tests (UCS) were conducted on cemented samples with a constant load rate of 1.0 mm/min (conforming with Australian standard 1012.9-1999).

Local Strength of Cylinder Sample

After dismantling of the container, the consolidated sand soil was cut along the vertical middle plane. The vertical cross-section surface was divided into equal sections of 25 mm in length and 20 mm in width by applying a uniform grid. The local strength of each of the grid sections of the cemented cylindrical container was measured as rebound hardness by using the "Schmidt Rebound Hammer" test, also known as a rebound hammer test.

Local strength measurements were conducted at least 4 times at different spots within each grid section to gain an average value. With proper laboratory calibration, it is possible to relate the rebound hardness to the elastic properties of the masonry or compressive strength (Noland et al. 1982). In this study, a linear correlation of the rebound hardness value (x in the range of 10 to 30) and the UCS of bio-cemented samples were determined and expressed as the following equation (Eq. 3).

[3] $$q_{ucs}(MPa) = 0.0806 \times x\ (\text{rebound reading}) + 0.2745 \quad (R^2=0.9034)$$

Results

Cementation of 2 m Columns

Surface percolation associated with bacterial placement with multiple layers has been demonstrated in short columns (Cheng and Cord-Ruwisch, 2012). However, for large scale application depths of more than 1 m may need to be achieved. In this study, fine and coarse silica sand were packed in 2 m PVC columns to test whether homogenous cementation of non-saturated sand could be achieved to the depth of 2 m. The particle size distribution of the fine and coarse sands used in this study is shown in Table 2.

Fine Sand Columns

The previous research (Cheng and Cord-Ruwisch, 2012) demonstrated that after primary treatments (2 flushes) a homogenous strength could be achieved in the 1 m fine sand column with 6 layers of bacterial placement. As discussed previously (Cheng and Cord-Ruwisch, 2012), with increasing column length a larger number of alternating layers of bacterial suspension and cementation solution for bacterial immobilization are needed. Therefore, the 2 m column was cemented by using 12 of such layers. After 4 percolation batch applications with cementation solution, $CaCO_3$ precipitation was obtained over the entire 2 m column, while minimum detectable strength (UCS approximately 100 kPa) of the cemented sand was only achieved over the top 1000 mm (Figure 2).

An identical column with a total of 10 treatments indicated that significant cementation of more than 500 kPa was restricted only to the top 500 mm (Figure 2). The $CaCO_3$ accumulation to more than 0.1 g/g was obtained in the top part of the column, indicating that clogging had occurred preventing sufficient penetration of the cementation solution to deeper layers. This suggested that with the number of repeated treatments used, the risk of local clogging in the top of sand columns increases.

To solve the problem of cementation being restricted only to the top 500 mm (clogging), fewer layers for bacterial immobilization (6 layers of alternating bacteria) were used. According to previous results (Cheng and Cord-Ruwisch, 2012), this is expected to help deeper cementation as the risk of accumulation of the urease active bacteria in the top layers could be diminished. The column with 6 layers resulted significant cementation (>500 kPa) up to 1000 mm depth (Figure 3 and Figure 4).

Coarse Sand Column

Fine sand has less permeability leading to low liquid infiltration rate, which increases the risk of local clogging. Compared to fine sand, coarse sand has higher permeability due to the large pore spaces, allowing higher liquid infiltration rates making the column less susceptible to clogging.

The entire coarse sand column was successfully cemented by the repeated treatments to a strength varying between 850 to 2067 kPa (UCS). A significant strength improvement greater than 1000 kPa was obtained down to the bottom of the column (Figure 5). This showed that sufficient urea/$CaCl_2$ was transported to a depth of 2 m, which was confirmed by the profile of the precipitated calcium carbonate through the column (Figure 5). In contrast to the $CaCO_3$ profile of the fine sand columns, the $CaCO_3$ content in the coarse sand column was relatively constant from the surface to the depth of 1500 mm and then increased gradually with depth. Clearly, the local clogging was avoided in the coarse sand columns.

Permeability and Infiltration Rate

Whiffin et al. (2007) suggested a high infiltration rate of cementation solution is desired to avoid crust formation (local clogging) and to achieve significant cementation at greater depth in soils. The fact that cementation of the fine sand columns was restricted to the top part (Figure 2) might be caused by the low infiltration rate of the cementation solution. In order to clarify the relationship between infiltration rate of the solution and cementation depth, the liquid infiltration rates during the previous series tests were calculated.

The lowest infiltration rate of the cementation solution was detected in the 12 layers fine sand column, where the most serious clogging occurred. The non-clogged coarse sand column presented approximately 10-fold higher liquid infiltration rate than that obtained in the fine sand columns. Liquid infiltration rate also decreased with the number of treatments in all sand columns, which was the result of the decrease in permeability due to the calcium carbonate crystals precipitated in the pore spaces (Figure 6).

At low infiltration rates of about 0.25 cm/min (12 layers fine sand column), approximately 90% (1800/2000 mM ammonium) of the cementation solution had already reacted by the time it reached the bottom (Figure 7). Evidently this leads to low $CaCO_3$ precipitation in the bottom section relative to the top section of the column. By comparison, in the coarse sand column, the high liquid filtration rate (7-28.5 cm/min), representing fast movement of the cementation solution in the column, led to more than 80% of the un-reacted cementation solution reaching to the bottom (Figure 7).

Theoretical Analysis of Heterogeneity of 1-D Cementation

Assumptions of Mathematical Model

- The urease activity is homogeneously distributed over the entire sand column.
- Cementation solution is injected from the top of the sand column after bacteria are immobilized. After the cementation solution is fully loaded into the sand column, the sand column is undisturbed to allow the cementation reaction to be completed.

- The downward infiltration of cementation solution has little dispersion and diffusion through the column (Van Wijngaarden et al. 2012).

Mathematical Model Building

In order to simulate $CaCO_3$ content over the entire distance, kinetic models were developed, which is presented as follows:

a) The length of the sand column is *L* (cm), the column is divided into N layers and thickness of each layer is *L/N*;

b) The urea hydrolysis rate (r_{urea}) can be described by Michaelis-Menten kinetic:

[4] $$r_{urea} = r_0 \times (\frac{C_{urea}}{k_m + C_{urea}});$$

r_0: maximum urease hydrolysis rate (here, equals to the urease activity of the injected bacterial suspension), C_{urea}: urea concentration and k_m: constant parameter;

c) During the infiltration of cementation solution, in each layer (e.g. layer N) the concentration of inflow and outflow cementation solutions were $C_{N\text{-}in,}$ and $C_{N\text{-}out}$ respectively. Accordingly, the concentration of outflow cementation solution at layer N is equal to the concentration of inflow cementation solution in layer N+1:

[5] $$C_{N-out} = C_{(N+1)-in};$$

d) In each layer (e.g. layer N), the concentration of outflow cementation solution is subtraction of consumed cementation solution ($C_{N-consumed}$) during the

infiltration from the inflow C_{N-in}, and the consumed cementation solution depends on the urea hydrolysis rate (r_{urea}) and retention time (t):

[6] $C_{N-out} = C_{N-in} - C_{N-consumed} = C_{N-in} - r_{urea} \times t = C_{N-in} - r_{urea} \times (\frac{L}{N \times v})$;

v: infiltration rate (cm/min), N: layer number;

e) In each layer (e.g. layer N), the total precipitated $CaCO_3$ during the cementation solution ($W_{N\text{-}infiltration}$) infiltration is calculated by the summation of the reacted cementation solution in this layer, which also can be calculated by multiplying the total volume of cementation solution flowed through layer N (V_N) by the difference of concentration between inflow and outflow in this layer:

[7] $W_{N-infiltration} = V_N \times C_{N-consumed}$;

f) For each layer (e.g. layer N), the total volume of passed though solution (V_N) can be expressed by:

[8] $V_N = V_{total} - V_{solution} \times (N - 1)$;

V_{total}: total volume of injected cementation solution and $V_{solution}$: the volume of retained cementation solution in each layer, which is same for all layers;

g) After infiltration of cementation solution is completed, the unreacted cementation solution in each layer (e.g. layer N) will result in further precipitation of $CaCO_3$ crystals ($W_{N\text{-}stationary}$), which is expressed by:

[9] $W_{N-stationary} = V_{solution} \times C_{N-in}$;

h) Total precipitated crystals ($W_{N\text{-}total}$) in each layer is expressed by:

[10] $$W_{N-total} = W_{N-infiltration} + W_{N-stationary};$$

i) Content of $CaCO_3$ in each layer (e.g. layer N) is expressed by:

[11] $$P_N = \frac{W_{N-total}}{W_{N-sand}} = \frac{W_{N-total}}{\rho \times V_{N-sand}};$$

$W_{N\text{-}sand}$: weight of each sand layer, ρ: density of sand column, and $V_{N\text{-}sand}$: volume of each sand layer;

For all simulations, the parameters of the model are set and presented as follows:

- L=200 cm and N=100;
- The diameter of the column is 5.5 cm and content of pore void volume is 39%. Therefore, the volume of each sand layer is $V_{N\text{-}sand}$ = 47.51 cm^3 and pore void volume of each layer is about 18.53 cm^3;
- k_m=55 mM (Hammes et al. 2003);
- Initial cementation solution consists of 1000 mM urea and 1000 mM $CaCl_2$, which is the same as the concentration of the inflow cementation solution in the first layer (C_{1-in});
- ρ =1.625 g/cm^3.

Effect of *In-situ* Urease Activity and Infiltration Rate

A simple model was established that could calculate from the rate of urea infiltration and the rate of urea hydrolysis in the soil, how much $CaCO_3$ precipitation would be

present at each depth of the sand column. To simplify the calculation, the sand column was assumed to be fully saturated.

For a fixed infiltration rate of 5 cm/min, equivalent to fine sand, the model predicted the cementation potential ($CaCO_3$ precipitation) with respect to depth (Figure 8a) after one void volume of cementation solution was injected and completely reacted. The predicted $CaCO_3$ distribution is more homogeneous in the presence of lower urease activity in the soil. Hence, in principle the observed lack of cementation in deeper layers of fine sand columns could be alleviated by placing less urease active bacteria in the soil.

Figure 8b shows the model predictions of the effect of infiltration rates of cementation solution on $CaCO_3$ distribution in the presence of constant infiltration rate of 5 cm/min. The predicted trend is that the $CaCO_3$ distribution is more homogeneous at higher infiltration rates. The lowest infiltration rate (1 cm/min) enables about 60 cm depth of cementation.

Effect of Numbers of Treatment

As discussed previously, the infiltration rate plays a major role in the biocement process, which can be demonstrated from the experiments (Figure 2) as well as model predictions (Figure 8b). A further complication is that the infiltration rate will decrease during the repeated treatments as the porosity and permeability decrease, which is a result of the precipitation of $CaCO_3$ crystals.

In order to simulate this phenomenon, the previous model is expanded by including the effect of $CaCO_3$ precipitation on infiltration rate. An empirical relationship between the permeability (intrinsic permeability) and the porosity that is commonly

used in ground water flow modelling is described by the following equation (Eq. 12) (Bear, 1972):

[12] $$k = k_x = k_y = k_z = \frac{(d_m)^2}{180} \times \frac{\theta^3}{(1-\theta)^2};$$

In this equation, k is the permeability; d_m is the mean particle size of the subsurface medium, and θ is the porosity, which is expressed by the following equation (Eq. 13):

[13] $$\theta = \frac{V_{pore} - V_{crystals}}{V_{total}} = (V_{pore} - \frac{m_{crystals}}{\rho_{crystals}})/V_{total} = \theta_0 - \frac{C_{crystals}}{\rho_{crystals}};$$

In this equation, V_{pore}, $V_{crystals}$ and V_{total}, are volume of initial total pores, volume of precipitated crystals, and volume of sand matrix respectively. $m_{crystals}$ and $\rho_{crystals}$ are mass of precipitated crystals (g) and density of crystals (i.e. 2.71 g/cm^3), θ_0 and $C_{crystals}$ are initial porosity and content of precipitated crystals (g/cm^3). As the local permeability varies along the sand column, this model is derived under the assumption that the liquid infiltration rate in the sand columns is proportional to and depending on the lowest local permeability.

Figure 9 shows a model of bio-cemented columns with starting infiltration rate of 2.5 cm/min and porosity of 39% cemented by different numbers of treatment. Considering a decreasing infiltration rate during the repeated treatments (real situation), the loss of porosity at the top part of the column where most calcite precipitates (in fine sand) appears to be a self-enhanced phenomenon.

Comparison Between Experimental and Modelling Results

In order to evaluate the mathematical model, the experimental data and the mathematical model results were compared. In this case, the decrease in infiltration

rate was obtained from the experimental results (Figure 6), which is a function of the number of treatments. For the fine sand column with 12 layers, the correlation is described by the following equation (Eq. 14):

[14] $y = 50.367 \times e^{-0.284x}$; R^2=0.9969

In this equation, y is infiltration rate and x is number of treatment. In this mathematical model, the total injected volume of cementation solution for each treatment was 660 mL, which was the same as that used in experiments.

Figure 10 indicates that the results of the simulation are in accordance with the experimental data, suggesting that this mathematical model is possible to be used to predict the $CaCO_3$ precipitation in-situ providing the infiltration rate and urease activity are known.

To a large extent, the results of simulation are in accordance with the experimental data, however, the measured $CaCO_3$ content in the bottom layer is about 3 times higher than the simulated results. This is likely due to the simplified calculation, which assumes the solution is homogenously distributed over the entire column. This is in contrast to the real soil application, where more solution is retained at the bottom of the column (Cheng and Cord-Ruwisch 2012) leading to the formation of more crystals than predicted by the model.

Three Dimensional Cementation

Procedure of Treatment

The measurement of the ammonium concentration in the effluent indicated that at least 90% of the total injected urea (96 L of 1 M) was hydrolyzed (data not shown).

After the treatment was completed, the container was flushed with about 200 L of tap water removing all soluble salts and the cemented sand was removed from the container (Figure 11). Opening the container showed that the top layer of fine silica sand was successfully cemented. Further excavation indicated that the cemented sand body, about 70 L became clearly visible and stretched over about 40 cm depth.

Strength Profile (Local Strength)

The Schmidt Hammer tests showed that the bio-cemented sand body had considerable strength between 1 and 4 MPa (Figure 12). Below the silica sand layer, part of the raw sandy soil was also cemented. Under the surface ponding, a reasonably homogeneous cementation of the silica sand layer (300 mm in diameter and 200 mm in depth) was achieved (dashed rectangle in Figure 12). In this area the strength distribution indicated that about 80% of the cemented sand had strength between 2 to 2.5 MPa.

Discussion

Cementation in the Vertical Direction

Overall it was found that the well described (Whiffin et al. 2007; van Paassen et al. 2010) phenomenon of clogging of MICP application is linked to the problem of reaching insufficient depth of cementation. Theoretical considerations and simple modeling attempts lead to the explanation that the amount of urea hydrolyzed and hence calcite formed at the injection end (here top) is determined by the urease activity present and duration over which the cementation solution is exposed to the area. This means that conducive to clogging is:

- The immobilization of high bacterial numbers at the injection end

- Fine sand slowing down the infiltration rate
- Prior excessive biocementation lowering the pore volume and limiting infiltration rate

The increased cementation at the inflow end leaves subsequently insufficient urea and calcium ions to reach the deeper levels of the column. This could be shown in results (Figure 2 to 4) and also simple model predictions (Figure 8 to 10).

The surface percolation is suitable for field application without disturbing the soil structure. This simple method could reduce the costs of required machinery (wells, liquid pumping machine, etc.) and labor. However, The current presented results of the large laboratory scale experiment and mathematical model indicated that the $CaCO_3$ content and consequent geotechnical properties were not as homogeneously distributed as desired. Especially, vertical profiles of the cemented fine sand columns illustrated the $CaCO_3$ content and strength decreased with depth. The heterogeneity of cementation leading to clogging at the inflow end and insufficient strength at the deep levels could be explained as follows:

Cementation Reaction

The lack of cementation depth by the surface percolation method is likely due to the reaction of cementation solution during the infiltration leading to less reagents moving to deeper areas. Whiffin et al. (2007) suggested fast flow rates would move the cementation reagents further into the column. However, for the surface percolation technique, a high flow rate can only be obtained in the coarse sand due to the high permeability. The results of the coarse sand cementation with relative homogeneous strength distribution demonstrated the technical feasibility of

biocement for coarse sand reinforcement at large distance up to 2 meters (Figure 5). This relatively homogeneous $CaCO_3$ content and strength distribution achieved in the coarse sand columns was likely due to a) the more uniform distribution of pore solution (here, cementation solution) under free draining conditions (Lu and Likos, 2004), and b) the higher permeability and shorter retention times which prevent excessive urea conversion in the top part of the column allowing more reagents to move to deeper area (Cheng, 2012). The maximum attainable cementation distance did not appear to be limited to 2 meters and it may be possible to extend this distance further. To apply surface percolation to low permeable soil, pumping or vacuuming to enhance the flow rate of cementation solution within soils might be required.

Surface clogging can also be self-enhanced by increased crystals precipitation and decreased porosity. In the clogging areas, the decreased permeability led to an increase in hydraulic retention time of the cementation solution during the infiltration, resulting in more reagents being converted as crystals and precipitated in the clogging areas.

Urease Activity Distribution

Apart from the factors discussed previously, the urease activity distribution may also play a significant role on the heterogeneity of cementation. During the infiltration, the amount of precipitated $CaCO_3$ at a specific location depends on the amount of available urea catalyzing bacteria (van Paassen, 2009). More reagents are converted and precipitate as $CaCO_3$ crystals in the areas where the ureolytic bacteria are accumulated.

Compared to the 12 layers column, the 6 layers column with less activity accumulation at the top of the column (Cheng and Cord-Ruwisch, 2012), would allow

reagents to infiltrate further. Accordingly, the clogging could be partly avoided (Figure 4 and Figure 5). However, fewer layers would also lead to less immobilization of bacteria (Cheng and Cord-Ruwisch, 2012). Therefore, at different lengths of sand columns (same as depth of soil in field), optimization of the number of layers is essential to achieve homogeneous cementation.

As Al-Thawadi (2008) suggested, urease activity inhibitor could be introduced to reduce the urease activity around the injection point, such as hydroxyurea, hydroxamic acids, phosphoroamide compounds, phosphate, etc. This would reduce urea conversion rate in the top part of sand column allowing more cementation solution to move further.

Cementation in the Horizontal Direction

At free-draining environment, the liquid infiltration driven by gravity force has preferential flow paths, where the flow resistance is lower than other areas. Therefore, along these preferential flow paths, soils continuously receive reagents, leading to reasonably higher content of $CaCO_3$ and strength than in other areas. The $CaCO_3$ crystals precipitated in pores will decrease the porosity of soil and also the permeability. This will cause an increase in the flow resistance, leading to development of new preferential flow paths of liquid (van Paassen, 2009). The new preferential flow paths will bring bacteria and cementation solution to the areas, where fewer crystals precipitated previously, and enhance the cementation. This suggests that although the liquid infiltration flow paths cannot be controlled in the surface percolation method, the self-adjustable flow paths might favor homogeneous cementation.

Conclusions

Laboratory larger scale experiments presented in this study have shown that strengthening (consolidation) of unsaturated soil by using the novel surface percolation method of MICP is technically feasible.

The results of $CaCO_3$ content and mechanical properties of the bio-cemented samples demonstrated that the surface percolation technique is particularly suitable for porous granular materials with high permeability (e.g. coarse sand, gravel, etc.). This type materials allows for the unobstructed flow of the MICP solution permitting large depths (more than 2 meter) consolidation. However, for fine sand (< 0.3 mm) the slow infiltration rate (0.25-4.8 cm/min) resulted in the cementation being restricted to 1 m depth.

A simple mathematical model has demonstrated that the cementation depth is dependent on the infiltration rate of cementation solution and immobilized urease activity. Higher infiltration rate and lower urease activity will result in deeper cementation. Repeated treatment will enhance the sand clogging close to the injection point.

In the case of fine sand cementation by surface percolation, the 1-D application demonstrated the heterogeneity of cementation in vertical direction, while the 3-D application indicated that homogenous cementation in horizontal direction could be achieved.

Acknowledgement

The authors would like to acknowledge Deltares (the Netherlands) and University of Murdoch (Australia) for the financial support given to this project. The authors would also like to thank Atticus Dekuyer for providing assistance in the UCS measurements.

References

Al-Thawadi SMJ. 2008. High Strength In-situ Biocementation of Soil by Calcite Precipitating Locally Isolated Ureolytic Bacteria. Ph.D. Thesis. School of Biological Sciences and Biotechnology, Murdoch Univ., Perth, Australia.

Al-Thawadi SMJ, Cord-Ruwisch R. 2012. Calcium Carbonate Crystals Formation by Ureolytic Bacteria Isolated from Australian Soil and Sludge. J Adv Sci Eng Res 2:13-26.

Bear J. 1972. Dynamics of fluids in porous media. Dover Publications, New York. p 119–194.

Cheng L. 2012. Innovative ground enhancement by improved microbially induced $CaCO_3$ precipitation technology. Ph.D. Thesis. School of Biological Sciences and Biotechnology, Murdoch Univ., Perth, Australia.

Cheng L, Cord-Ruwisch R. 2012. In-situ soil cementation with ureolytic bacteria by surface percolation. Ecol Eng 42:64-72.

Cheng L, Cord-Ruwisch R, Shahin MA. 2013. Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Can Geotech J 50:1-10.

Chu J, Stabnikov V, Ivanov V. 2011. Microbially induced calcium carbonate precipitation on surface or in the bulk of soil. Geomicrobiol J 29:544-549.

Lu N, Likos WJ. 2004. Unsaturated soil mechanics. John Wiley & Sons, Inc. New Jersey.

DeJong JT, Fritzges MB, Nusslein K. 2006. Microbially induced cementation to control sand response to undrained shear. ASCE J Geotech Geoenviron Eng 132:1381–1392.

DeJong JT, Mortensen BM, Martinez BC, Nelson DC. 2010. Bio-mediated soil improvement. Ecol Eng 36:197–210.

Greenburg AE, Clesceri LS, Eaton AD. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington.

Hammes F, Boon N, De Villers J, Verstraete W, Siciliano SD. 2003. Strain-specific ureolytic microbial calcium carbonate precipitation. Appl Environ Microbiol 66:4901-4909.

Ivanov V, Chu J. 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev Environ Sci Biotechnol 7:139–153.

Kucharski ES, Cord-Ruwisch R, Whiffin VS, Al-Thawadi SMJ. 2006. Microbial biocementation. World Patent WO/2006/066326, June 29.

Noland J, Atkinson R, Baur J. 1982. An investigation into methods of nondestructive evaluation of masonry structures. National Technical Information Service Report PB

82218074. National Science Foundation.

Stocks-Fischer S, Galinat JK, Bang SS. 1999. Microbiological precipitation of $CaCO_3$. Soil Biol Biochem 31:1563–1571.

Van Paassen LA. 2009. Biogrout, ground improvement by microbial induced carbonate precipitation. Ph.D. thesis. Department of Biotechnology, Delft University of Technology, The Netherlands.

Van Paassen LA, Ghose R, Van der Linden TJM, Van der Star WRL, Van Loosdrecht, MCM. 2010. Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. ASCE J Geotech Geoenviron Eng 136:1721–1728.

Van Paassen LA, Harkes MP, Van Zwieten GA, Van der Zon, W.H., Van der Star WRL, Van Loosdrecht MCM. 2009a. Scale up of biogrout: a biological ground reinforcement method, p. 2328-2333. *In* Hamza M, Shahien M, EI-Mossallamy Y (ed), Proceedings of the 17th international conference on soil mechanics and geotechnical engineering. The Academia and Practice of Geotechnical Engineering, Egypt.

Van Paassen LA, Van Lossdrecht MCM, Pieron M, Mulder A, Ngan-Tillardm DJM, Van der Linden TJM. 2009b. Strength and deformation of biologically cemented sandstone. proceedings of the ISRM Regional conference EUROCK 2009-Rock engineering in difficult ground conditions- Soft rocks and karst, 29-31 October 2009, Dubrovnik, Croatia, p 405-410.

Van Wijngaarden WK, Vermolen FJ, Van Meurs GAM, Vuik C. 2012. A mathematical model and analytical solution for the fixation of bacteria in biogrout. Transp Porous Med 92:847-866.

Whiffin VS, Van Paassen LA, Harkes, M.P. 2007. Microbial carbonate precipitation as a soil improvement technique. Geomicrobiol J 24:417-423.

Table 1
Summary of treatment procedure by percolating solution into sand column

Sand Columns	Fine sand		Coarse sand
Rinse (tap water, mL)	2000		2000
Water retention capacity (mL)	620		380
Bacterial placement (mL of each layer and number of layers)	55 mL 12 Layers	110 mL 6 Layers	70 mL 6 Layers
Incubation (hours)	24		24
Cementation (mL)	CS (660)		CS (420)
Reaction (hours)	24		24
Total injected solution (mL)	BS: 990 and CS: 5610		BS: 1470 and CS: 7770

BS: Bacterial suspension; CS: Cementation solution.

TABLE 2

Grain size distribution curves for the fine and coarse sand used

Type of sand	Grain size (mm) distribution							
	1.18-1	1-0.71	0.71-0.6	0.6-0.5	0.5-0.3	0.3-0.21	0.21-0.15	0.15-0.05
Fine	-	-	-	0.02%	1.11%	63.39%	29.59%	5.89%
Coarse	0.78%	40.82%	38.28%	16.68%	3.44%	-	-	-

Figure 1

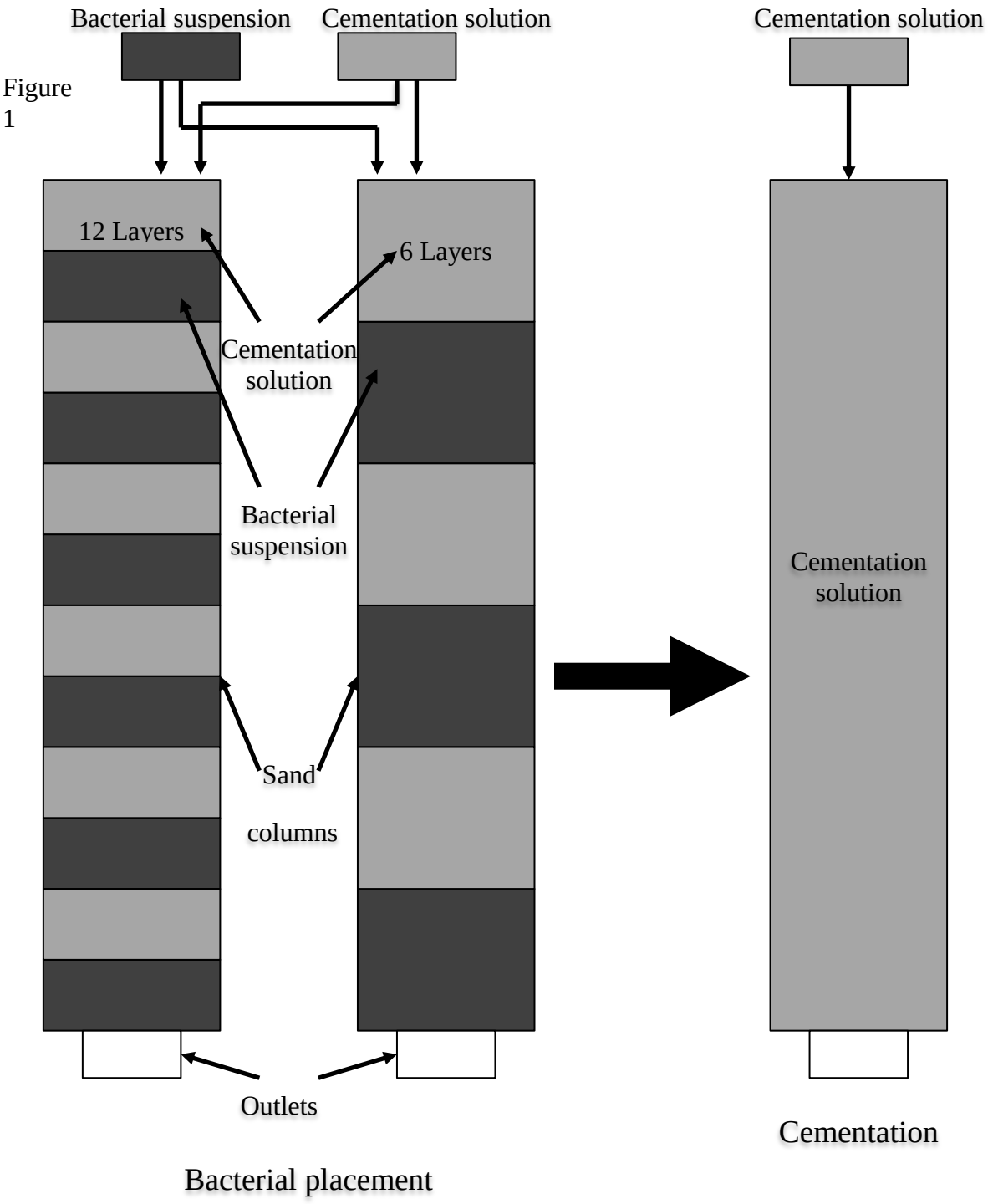

FIG. 1. Schematic diagram of bacterial placement by introducing different numbers (6 and 12) of alternating layers (bacterial suspension / cementation solution).

Figure 2

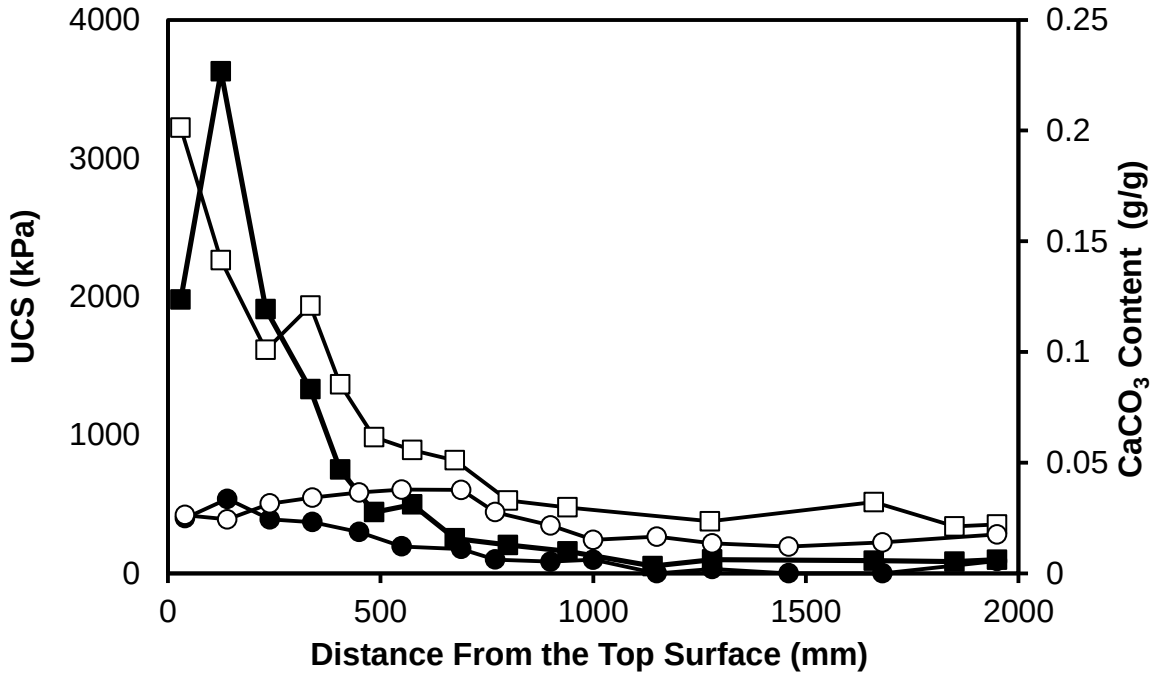

FIG. 2. $CaCO_3$ content and strength profiles of the 12 layers fine sand columns after 4 and 10 treatments (■: UCS (10 treatments); □: $CaCO_3$ (10 treatments); ●: UCS (4 treatments); ○: $CaCO_3$ (4 treatments))

Figure 3

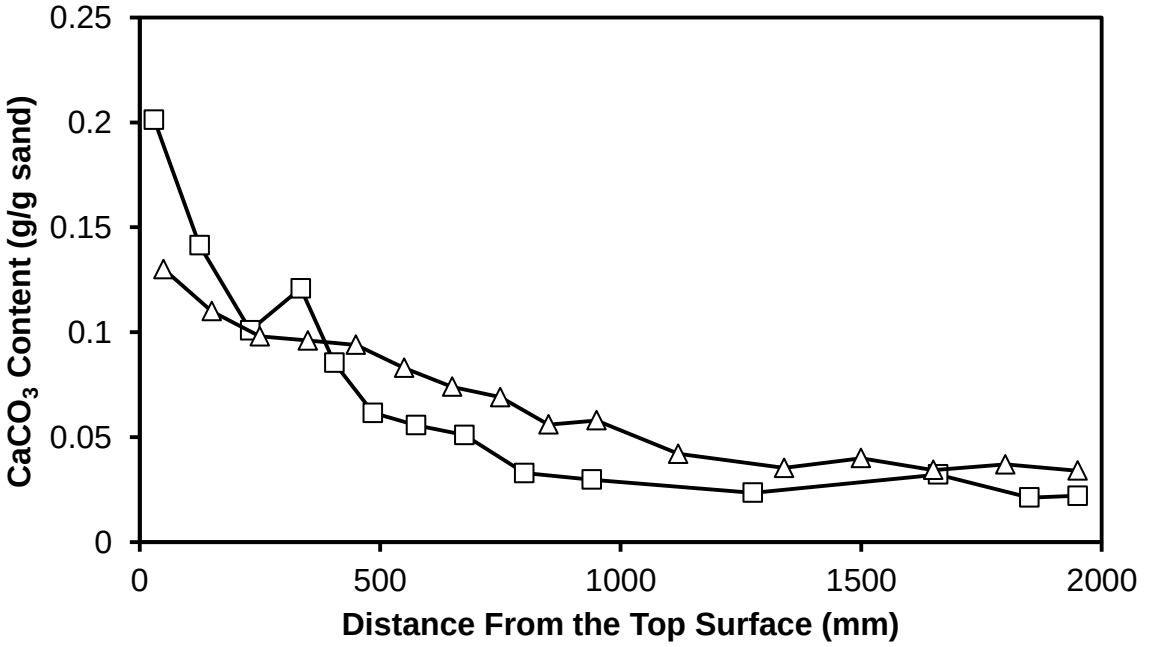

FIG. 3. Effect of using 6 (△) or 12 (□) layers for bacterial immobilization on $CaCO_3$ content profiles of the 2 m fine sand columns (6 and 12 layers) after 10 treatments.

Figure 4

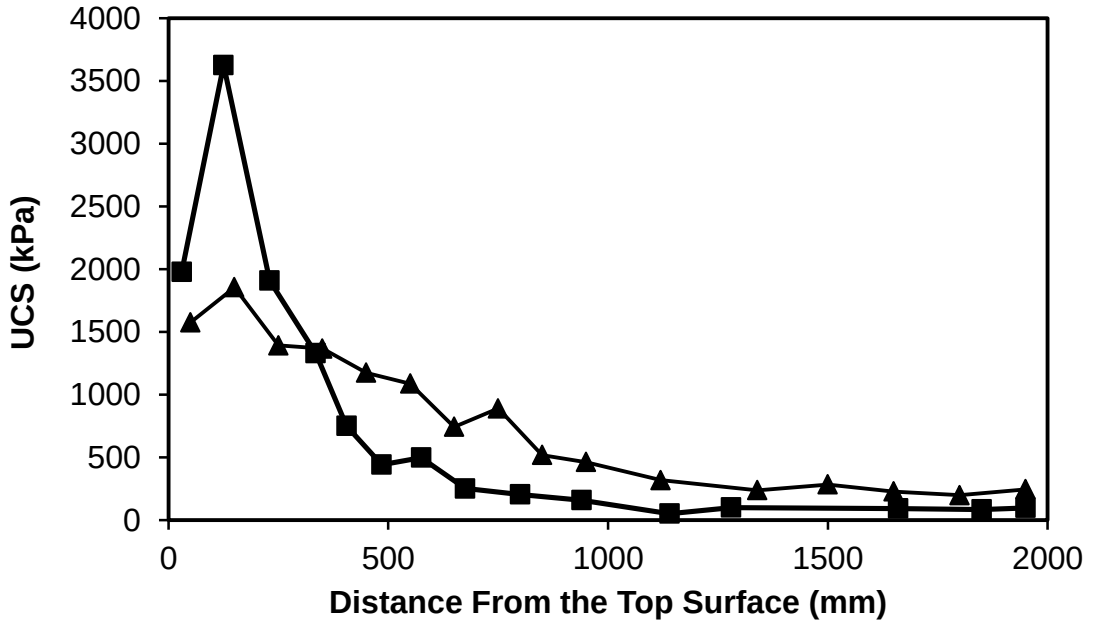

FIG. 4. Effect of using 6 (▲) or 12 (■) layers for bacterial immobilization on UCS profiles of the 2 m fine sand columns (6 and 12 layers) after 10 treatments.

Figure 5

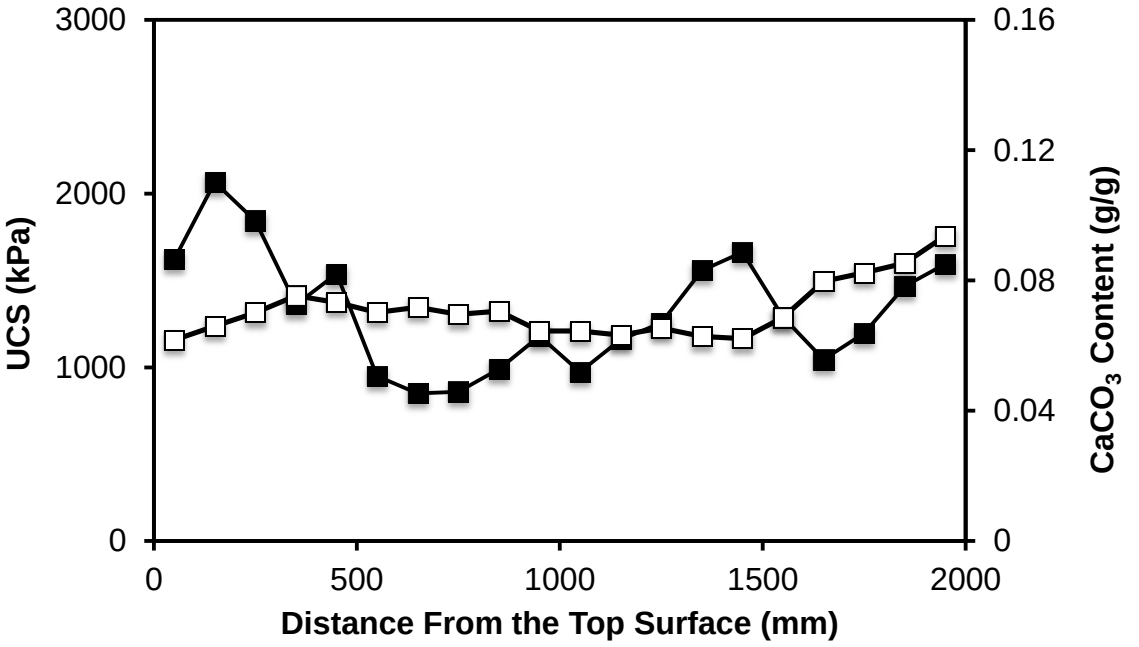

FIG. 5. Mechanical properties of cemented 2-m coarse sand column after 22 treatments. $CaCO_3$ content (□) and UCS (■) profiles along the coarse sand column.

Figure 6

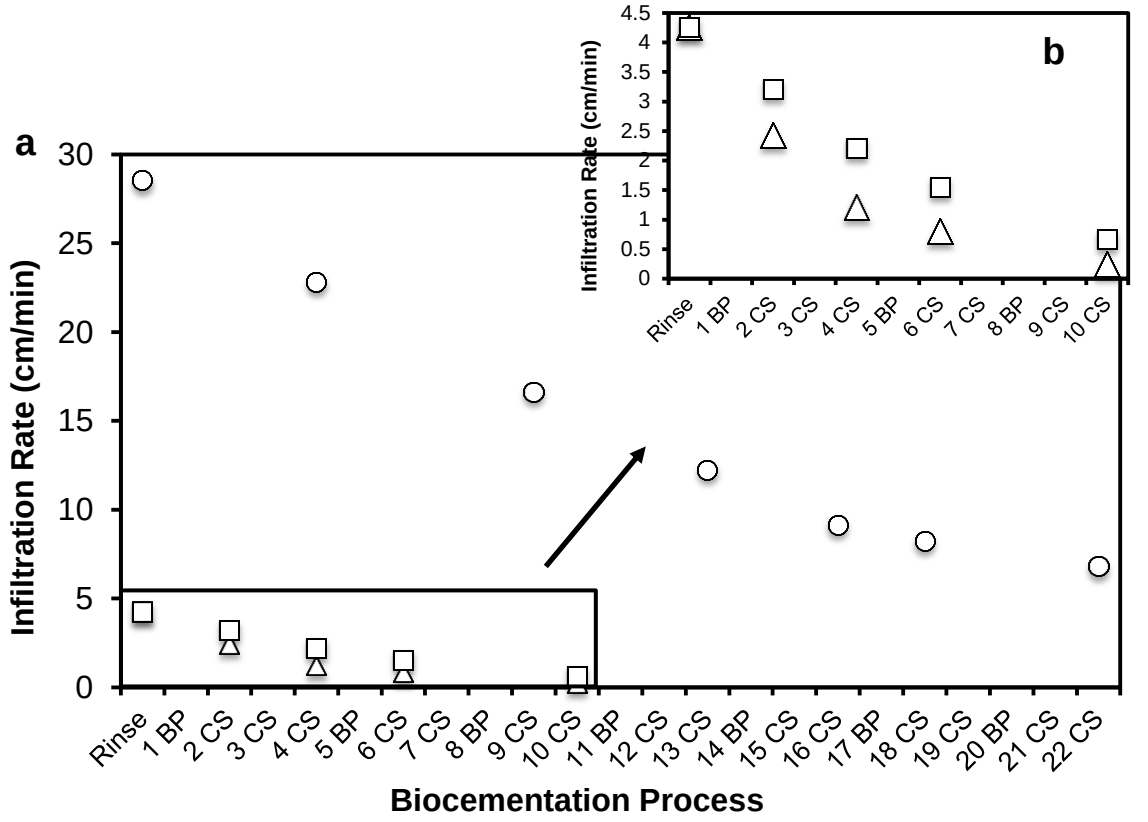

FIG. 6. Infiltration rate of the introduced solutions into the sand columns (☐: 6 layers fine sand; △: 12 layers fine sand; ○: 6 layers coarse sand) throughout the biocementation process (a: overall process; b: details of the two fine sand columns) (BP bacterial placement; CS: cementation solution).

Figure 7

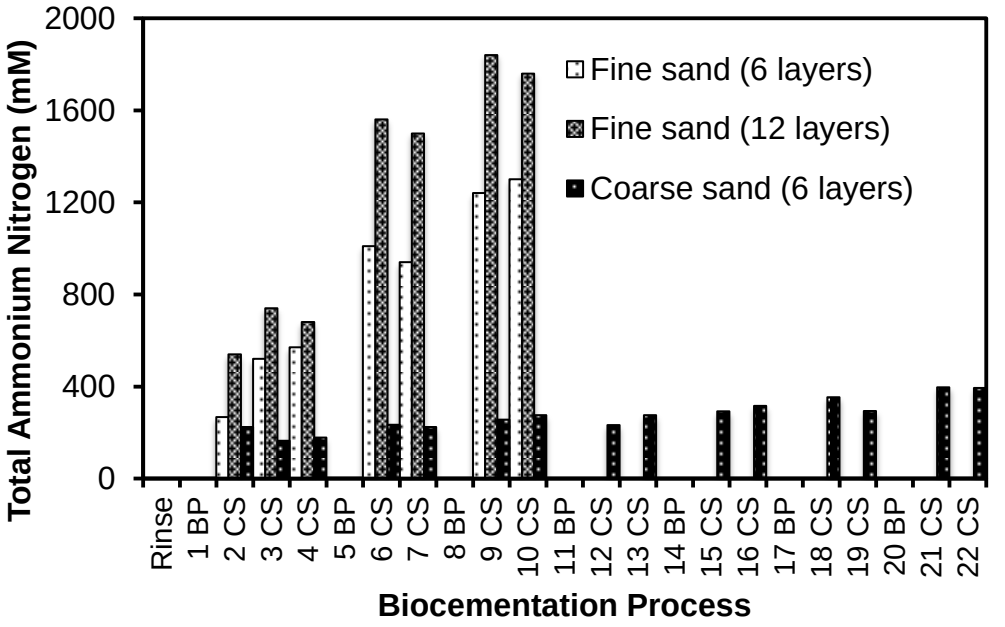

FIG. 7. Ammonium concentration of the injected cementation solutions when they reached the bottom of the columns. 2000 mM of ammonium concentration represented 100 % conversion of urea (1 M) (BP bacterial placement; CS: cementation solution).

Figure 8

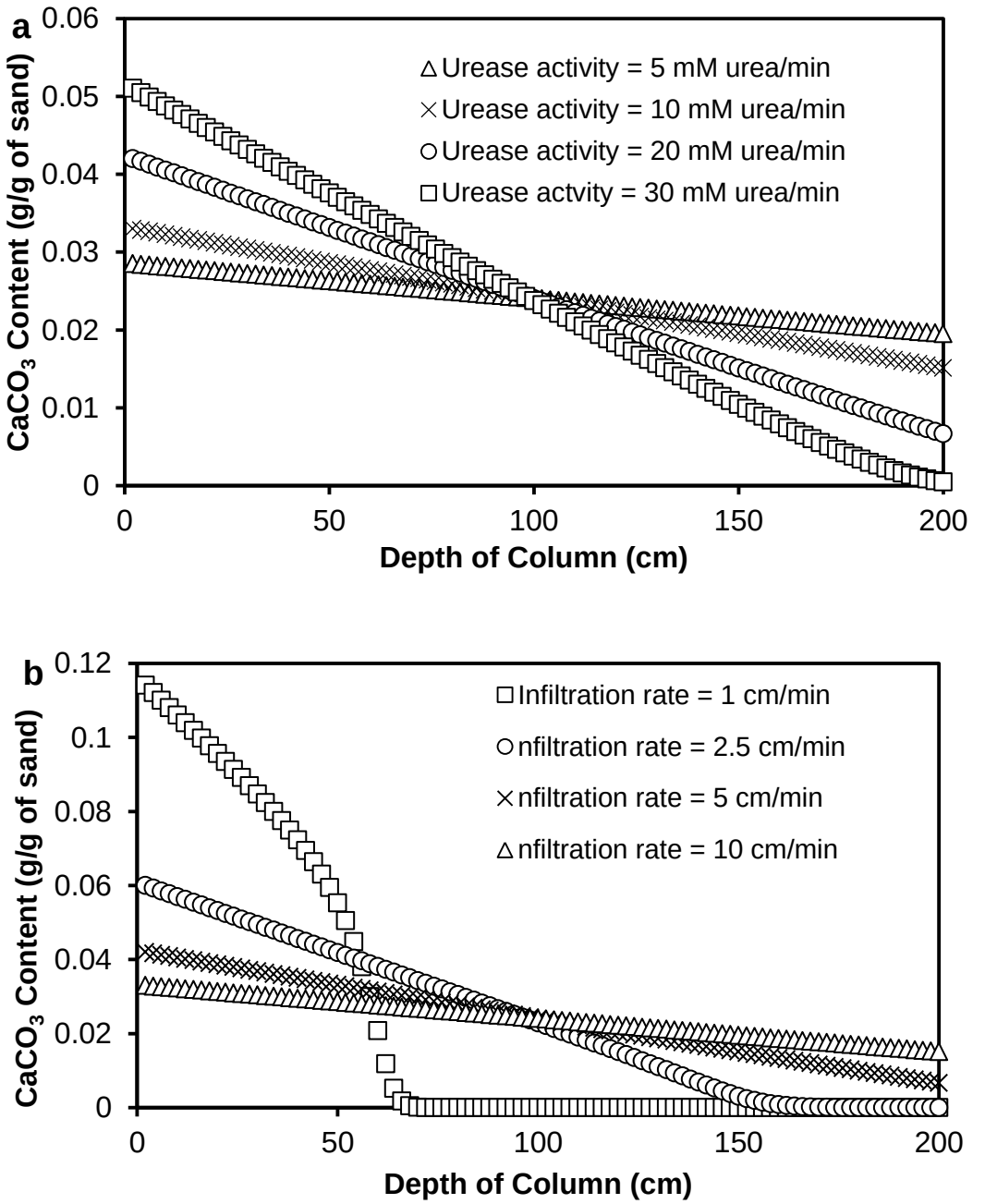

FIG. 8. The total amount of calcium carbonate precipitated as a function of depth (cm) under a: specified urease activities with constant infiltration rate of 5 cm/min, b: specified infiltration rates with constant urease activity of 20 mM urea/min.

Figure 9

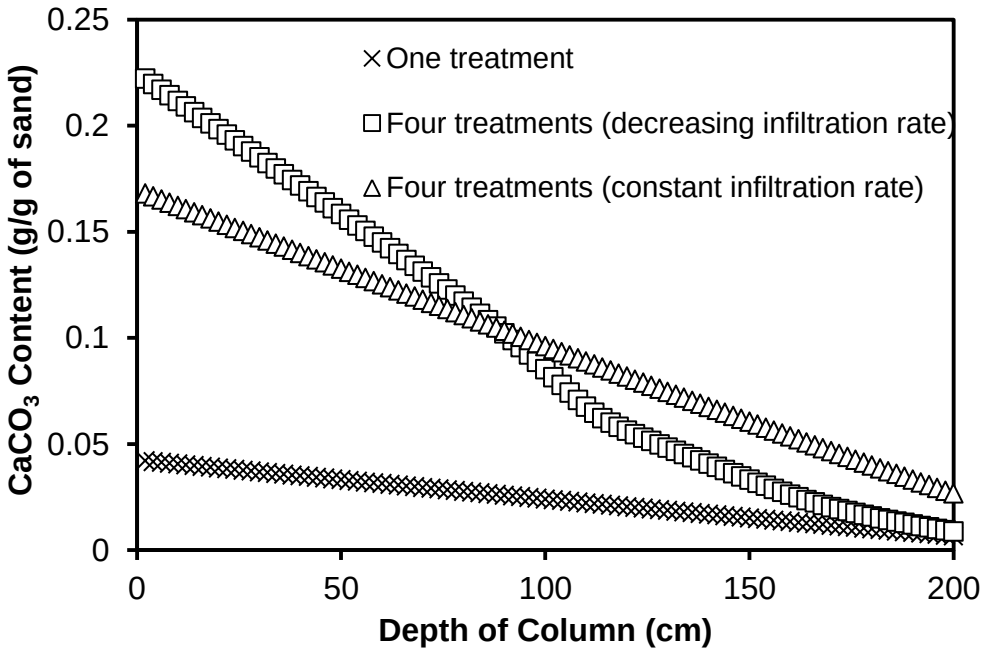

FIG. 9. Predicted effect of repeated treatments gradually slowing the infiltration rate on calcite precipitation in fine sand columns, in which the $CaCO_3$ was presented as a function of depth (cm) after one and four treatments. The urease activity rate was assumed to be 10 mM urea/min.

Figure 10

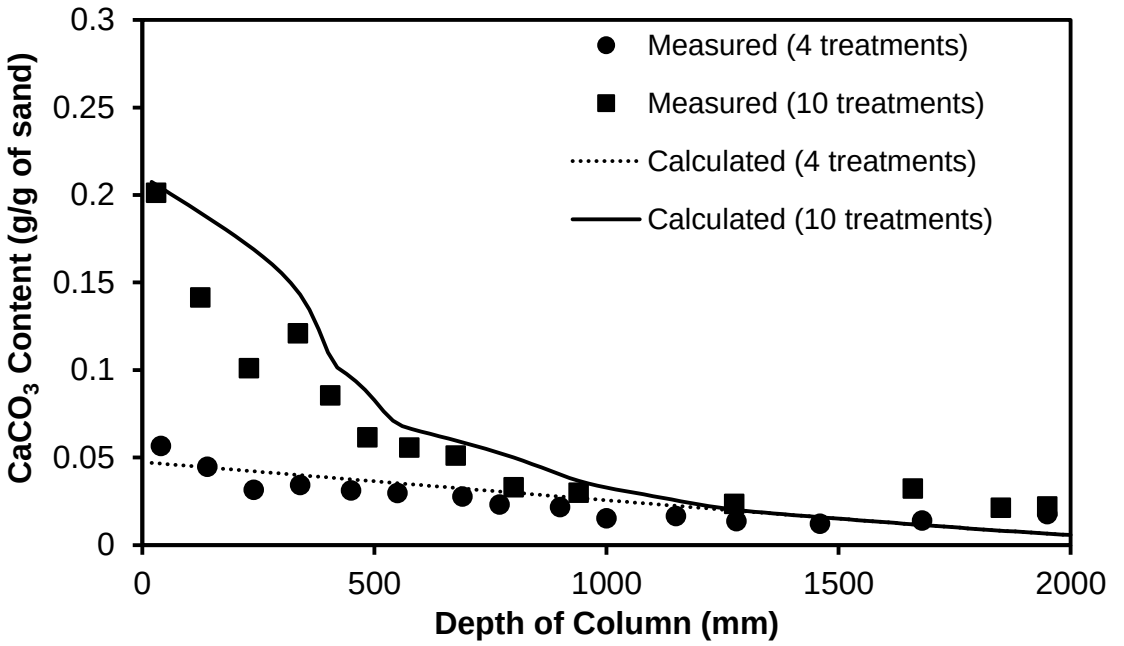

FIG. 10. Comparison of model predictions and experimental data on calcite precipitation over column depth. The urease activity was 10 mM urea/min.

Figure 11

FIG. 11. Results or biocementation of a 3-D sample of 80 L of sandy soil topped with about 32 L of fine silica sand. (a: cemented sample in cylinder container, b: dismantled cemented sample, c: cemented sampled was hosed with tap water, d: vertical cross section along the longitudinal centerline.

Figure 12

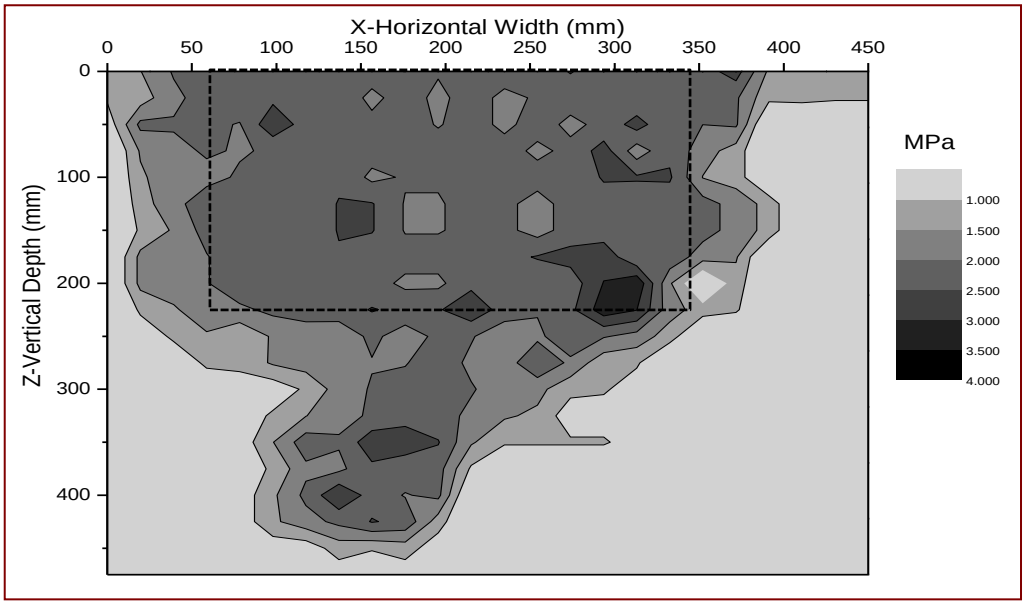

FIG. 12. Local strength measurement on the middle cross-section through the cemented sand body.