In-Situ Soil Cementation with Ureolytic Bacteria by Surface Percolation

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Abstract

The possibility of using microbiological processes to improve the mechanical properties of soil by undisturbed in-situ application has gained attention over recent years. This paper describes a new variation of in-situ soil reinforcement technology based on Microbially Induced Carbonate Precipitation (MICP), which involves both the hydrolysis of urea by soil bacteria enzyme and calcium carbonate precipitation in the presence of dissolved calcium ions. In contrast to other previously published approaches, the current work uses surface percolation for in-situ placement of bacteria and cementation solution. Bacteria could be immobilised over the full length of a 1 m column by surface percolation. To accomplish this it was necessary to percolate alternate solutions containing either bacteria or fixation solution containing calcium ions.

The biologically triggered cementation resulted in homogeneous cementation over the entire length of the 1-meter sand column. The efficiency of calcite crystals to form strength was found to be related to the pore water content of the continuously drained column with less water content enabling more efficient strength formation. Scanning electron microscopy supported the idea that lower water contents lead to selective positioning of crystals at the bridging points between sand grains. These findings imply that the cost of MICP technology can be reduced by optimizing the conditions for effective crystals precipitation. This is expected to make this technology more readily acceptable for large scale applications

KEYWORDS: Biocementation, Microbially induced carbonate precipitation (MICP), Calcium carbonate, soil improvement, unsaturated soil, soil liquefaction

1 Introduction

Microbial induced calcium carbonate precipitation (MICP) by urea hydrolysis has been a topical subject of research in recent years (Ivanov and Chu, 2008; De Muynck et al., 2010). Enabled by interdisciplinary research at the confluence of microbiology, geochemistry, and civil engineering (DeJong et al., 2010), MICP technology has been applied to relevant applications, such as wastewater treatment (Hammes et al., 2003), and calcareous stone restoration (Stocks-Fisher et al., 1999; Castanier et al., 2000). In addition, this process has been explored for the improvement of the strength and stability of soft and poorly consolidated sand soil (Whiffin et al., 2007; van Paassen et al., 2009). Compared to chemical or cement grouting techniques, which are usually harmful to the environment, MICP has been proposed as an environmentally friendly method (Le Metayer-Levrel et al., 1999).

The microbial method of soil improvement generally involves three steps:

1. Urea is hydrolyzed by microbial urease to form ammonium and carbonate ions (Eqn. 1).

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

2. The produced carbonate ions react with calcium ions and precipitate as calcium carbonate crystals (Eqn. 2).

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

3. Sand grains are bound together by the calcium carbonate crystals.

As the bacterial cells are distributed throughout the solution, or sand core, the reaction of generating an oversaturation of dissolved calcium carbonate happens uniformly throughout the sample and at a controlled rate. This is in contrast to reactions resulting from mixing calcium solutions with carbonate solutions.

Current exploitations of the bacterial capacity to release carbonate in situ in the presence of calcium ions has been limited to using water logged soils (DeJong et al., 2006; Whiffin et al., 2007; van Paassen et al., 2009), requiring heavy machinery and hydraulic injection of the cementation solutions. In order to induce MICP in the water saturated soil subsurface, a method of two-phase injection of bacterial suspension and cementation solution for bacterial immobilisation has been developed (Whiffin et al., 2007; van Paassen et al., 2010). This method makes use of the effect of ionic strength, on microbial fixation to sand particles. Increased ionic strength, in particular calcium ion concentration, encourages bacterial adsorption onto the surface of sand particles (Scholl et al., 1990; Torkzaban et al., 2008). Initially, a solution of suspended bacteria is injected into a soil matrix. This is followed by the addition of a fixation solution consisting of 50 mM $CaCl_2$.

For sufficient bacterial adsorption, a slow flow rate of fixation solution is required which will enable adequate intermixing between the bacterial suspension and fixation solution (Harkes et al., 2010).

The cementation of non-water saturated sandy soils, as they are encountered above the groundwater table, and in areas such as sand dykes, road or train embankments, and sand dunes etc., has not been studied and described extensively. This is most likely due to the less control of flow that can be exercised in the non-saturated soil environment.

The aims of this paper are to:

- develop a bacterial immobilisation process that works for unsaturated sand
- allow the in-situ soil cementation of non saturated soils by using a simple surface percolation application, and
- understand calcite crystal formation in a soil matrix under a non-water saturated condition.

2 Materials and methods

2.1 Bacterial culture and cementation solution

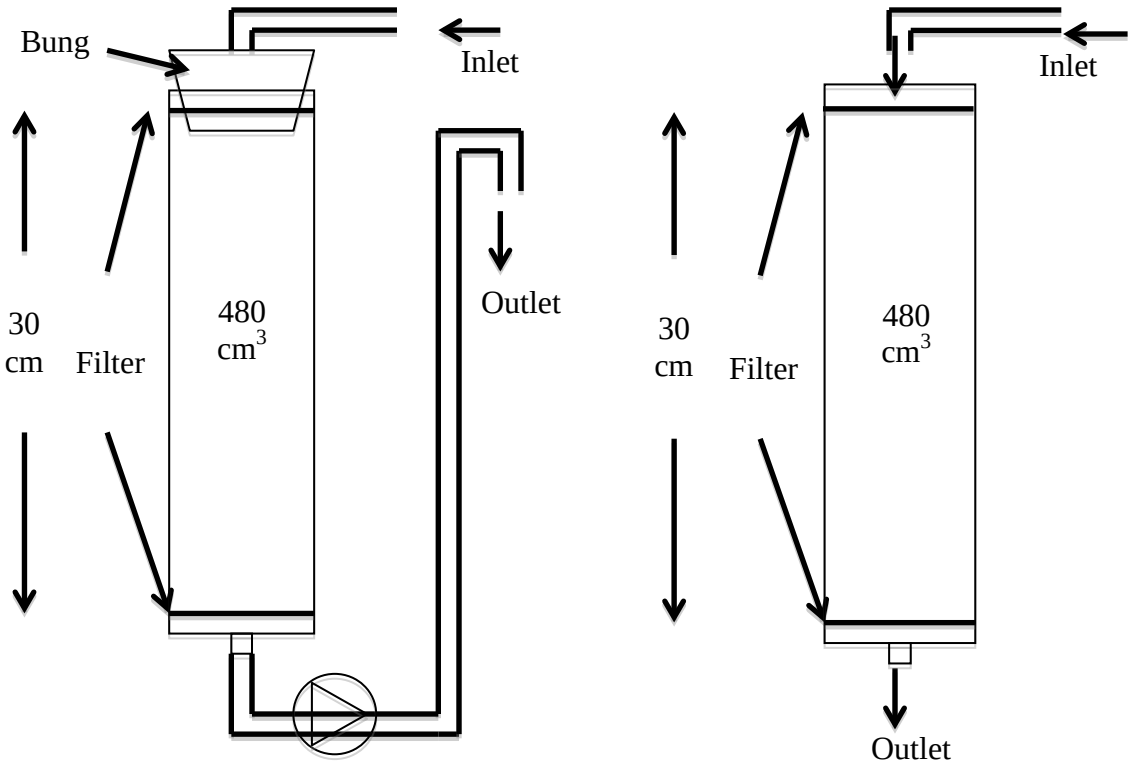
The urease active bacteria named as MCP-11 (*Bacillus sphaericus*, available now from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany) have been isolated from local soil samples in the Laboratory of Biological and Biotech Science of Murdoch University, WA (Al-Thawadi, 2008). For the current study, samples of the isolated strain (MCP-11) were cultivated under sterile aerobic batch conditions in a medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate and 0.1 mM $NiCl_2$, at pH of 9.25. After 24 hours incubation at 28 ^oC, the culture was collected and stored at 4 ^oC prior to use. The optical density (OD_{600}) of harvested culture varied between 1.5 to 2 and the urease activity was approximately 10 U/ml (1 U=1 μmol urea hydrolyzed per min). Cementation solution consisted of 1 M $CaCl_2$ and 1 M urea.

2.2 Sand column setup and sampling

Pure silica sand (Cook Industrial, Minerals Pty. Ltd. Western Australia) was used (>0.3mm: 1.13%, 0.212-0.3 mm: 63.39%, 0.15-0.212 mm: 29.59%, and <0.15 mm: 5.89%) for all experiments. All columns used in this study were made of Poly Vinyl Chloride (PVC) tubing (internal diameter of 4.5 cm, length of 30 cm and 1 m). Sand columns were packed with dry silica sand under continuous vibration to give an even density of 1.61~1.63 g/cm^3 (porosity 37.3~38.5%). The top and bottom of column were covered with a layer of scouring pads (porous plastic pad) as filters**.**

Under surface percolation and fully drained conditions, the water retention capacity of 30 cm and 1 m sand columns was determined to be about 180 mL and 360 mL respectively. The retained water was characterized as adsorbed water, that is the water had been adsorbed onto the sand grain surface, and capillary water, the water found between the grains (Baker and Frydman, 2009).

In this study, the sand columns were treated under two different water saturation conditions. (1) Water fully saturated condition: sand columns were treated by the submersed flow method**.** The setup and treatment process were based on the method that was described by Whiffin et al. (2007) (Figure 1A), except for the flow rate, which was kept constant at 220 mL/h. (2) Water unsaturated condition: sand columns were free drained and treated by surface percolation, as described below.



A： Submersed Flow Treatment　　B： Surface Percolation Treatment

Figure 1. Comparative scheme of submersed flow treatment (A, saturated (Whiffin et al., 2007)) and surface percolation treatment (B, unsaturated) of the biocementation process.

2.3 Percolation method of cementation

For biocementation under unsaturated conditions the percolation method was used. Sand columns were positioned vertically with top and bottom fully open. Reagents (bacterial suspension and cementation solution) were introduced from the top of the columns (Figure 1B). The transport of liquid was the result of gravity and capillary forces.

Unless otherwise stated for specific experiments, the percolation method consisted of the following 4 steps:

1) Percolation of bacterial suspension (50% of the water retention capacity of the sand columns (90 mL for 30 cm columns, 180 mL for 1 m columns))

2) Percolation of fixation solution (same amount as aforementioned bacterial suspension), which was identical to the cementation unless otherwise specified.

3) Incubation for 12 hours at 25±1^{o}C, allowing the added layers to diffuse into each other.

4) Percolation of cementation solution (100% of the water retention capacity of the sand columns) and allowing the reaction process (urease hydrolysis and calcite precipitation) to occur for 12 hours.

The separate addition of bacterial suspension (BS) and fixation solution (FS) could also be in more than two layers, for example by adding 25% of the water retention capacity of BS + FS + BS +FS to accomplish 4 layers (Figure 2). By alternately injecting smaller volumes of bacterial suspension and cementation solution multiple layers (2-12 layers) were created in each of four sand columns.

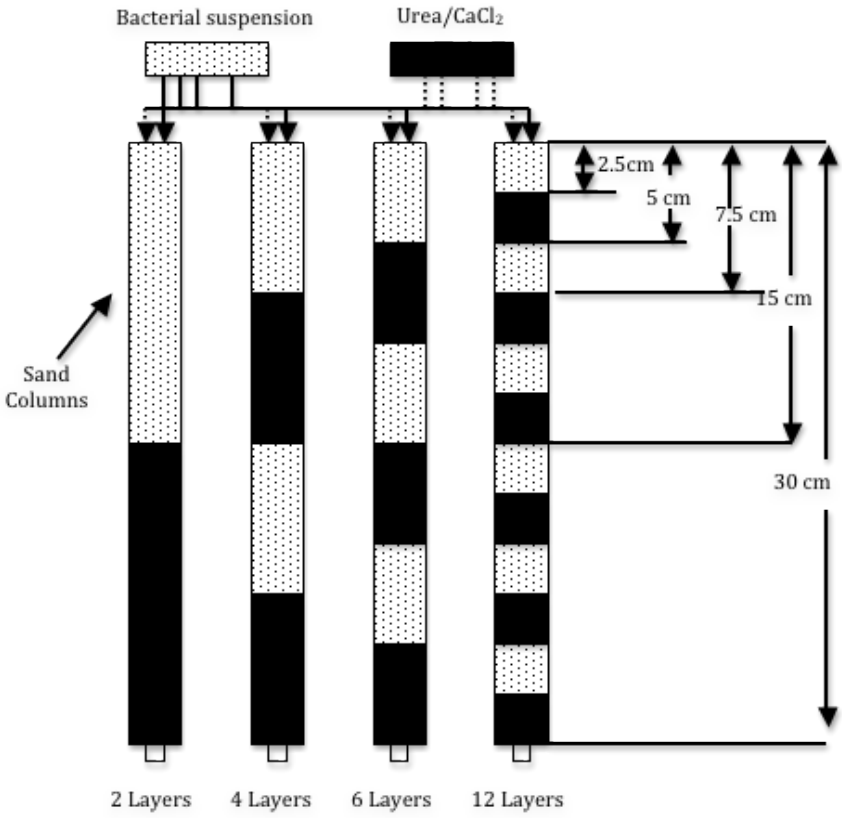


Figure 2. Diagram of bacteria placement by introducing different numbers of alternating layers (bacterial suspension / $CaCl_2$ + urea)

During the course of all experiments, samples were taken from the outlet and immediately tested for urease activity and ammonium concentration. By recording the urease activity in the effluent, the bacterial activity lost from the columns was determined. The bacterial urease activity retained in the column was calculated by subtraction from introduced activity.

2.4 Monitoring methods

2.4.1 Total ammonia nitrogen concentration

Total ammonia nitrogen concentration was determined by the Nessler method (Greenburg et al., 1992).

2.4.2 Biomass measurement (OD_{600})

Biomass concentration was determined by measuring optical density of bacterial suspension with a spectrophotometer (Pharmacia Biotech NovaspecII, Cambridge, England) at a wavelength of 600 nm (Harkes et al., 2010). If required, samples were diluted to stay in the absorbance range of 0.2 to 1.

2.4.3 Urease activity

In the absence of calcium ions, urease activity was determined via solution conductivity (Whiffin, 2004). 1 mL of bacterial suspension was added to 5 mL of 3 M urea and 4 mL of DI water (reaction concentration 1.5 M urea) and the relative conductivity change was recorded over 5 min at 25 ± 1 ^{o}C. The urease activity was then calculated taking the dilution into account. In the presence of calcium ions, urease activity was determined from the ammonia production rate. Urease activity for both methods was determined independently found to correlate well (R^2=0.992) with each other. One unit of urease activity is defined as the amount of enzyme that degrades 1 μmol of urea per minute at 25 ^{o}C.

Urease activity retained in the column was determined by subtracting the urease activity present in the effluent from the amount of urease introduced. As the culture was

collected at the stationary phase of growth (i.e. all readily available nutrients were consumed from the medium), no further growth of bacterial cells in the sand column during the incubation time could occur.

2.4.4 Calcium carbonate content

Calcium carbonate content of the consolidated samples was determined by adding 2 mL of 2 M HCl solution into a 1-2 g dry sample and then measuring the volume of CO_2 gas with a U-tube manometer under standard conditions (25^o C, 1 atm) (Whiffin et al., 2007). The calibration was made with analytical grade $CaCO_3$ powder.

2.4.5 Strength measurement

In order to measure the local strength (AI-Thawadi, 2008), the consolidated sand columns were cut into 3cm sections and strength determined by using a pocket penetrometer. Several stainless steel tips of different diameters were chosen depending on sample strength. The tip was applied on the centre of the cross section (centre of circle). The penetrometer was calibrated with dead weights (0.5-7 kg) prior to use. The reading of loaded weight was recorded when the sample was penetrated completely (breakage). The local strength was calculated according to the following equations (Eqns. 3 and 4).

Local strength P (N.cm^{-2})= W (kg)×g (m×s^{-2})/S (cm^2) (Eqn. 3)

Where, W (kg): the reading obtained from the pocket penetrometer, g: gravitational acceleration, S: surface area of tip.

S (cm^2) =π×(D/2)^2 (Eqn. 4)

Where, *D* is the diameter of the pocket penetrometer tip.

To determine the entire strength of the cemented column, it was cut into 9 cm sections to give a length/diameter ratio of 2, and compressive strength (q) was determined by single-stage unconfined compression strength (UCS) tests (conforming with Australian standard 1012.9-1999).

2.4.6 Scanning electron microscope (SEM)

Fractions of cemented samples, taken from different parts of 1-meter sand column, were prepared and examined by scanning electron microscope (PHILIPS XL20 Scanning Electron Microscope, Eindhoven, The Netherlands).

3 Results

3.1 Method of submersed flow application is not suitable for surface percolation treatment in dry sand.

For successful bio-cementation it is necessary that firstly bacteria are introduced into the soil and immobilised, followed by the application of cementation solution, containing urea and calcium chloride. The urease activity of the bacteria retained in the soil then generates carbonate from urea hydrolysis, which in the presence of excess calcium ions precipitates as calcium carbonate. This method has been developed and patented (Kucharski el al., 2006) and described in detail for water saturated soils in which liquid transfer is accomplished by pumping solutions from an injection point to a recovery point (Kucharski et al., 2006; Whiffin et al., 2007; van Paassen et al., 2009).

There is a clear potential for the use of in-situ stabilisation of soils for non-saturated conditions such as sand dykes, train embankments, mine-site slopes and road pavements. For a good stabilisation of loose sand sufficient bacterial cells inside of sand column need to be retained. The addition of calcium salts has been shown to enhance bacterial retention in sand columns (Whiffin et al., 2007; Harkes et al., 2010).

For the submersed and the percolation treatment the bacterial fixation method by Harkes et al. (2010) was used, which uses 25% of bacterial suspension and 25% fixation solution consisting of 50 mM of $CaCl_2$. This method resulted in the fixation urease activity of about 85% (15% lost) and 30% (70% lost) for submersed and percolation treatment respectively (top and bottom lines in Figure 3). In order to improve bacterial fixation for percolation treatment lower percolation rates and higher calcium concentrations were tested but did not result in effective fixation.

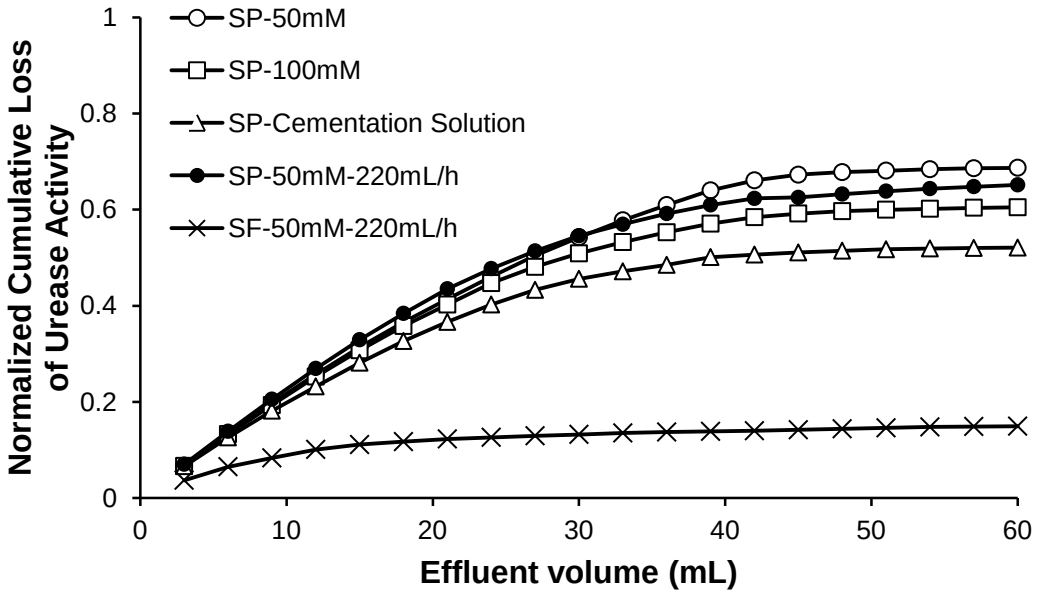


Figure 3. Normalized cumulative loss of urease activity from sand columns by flushing-out by different fixation solutions (45 mL), flushed after a 0.25 pore voids volume of bacterial suspension (45 mL) under surface percolation treatment (SP) without flow rate control: (◊) 50 mM $CaCl_2$; (□) 100 mM $CaCl_2$; (Δ) cementation solution; and with flow rate control (220mL/h) (• 50mL $CaCl_2$). Submersed Flow (SF) with flow rate control (220mL/h) was operated as control: (× 50mL $CaCl_2$).

Local strength analysis of the above samples showed that the strength of such percolation treatment was significantly inferior to the strength obtained from submersed flow (saturated) treatment (Figure 4). Low local cementation (average local strength <1000 $N \cdot cm^{-2}$) was obtained in the percolation application while the sand column treated by the previously described method using submersed flow developed an average local strength level of 2500 $N \cdot cm^{-2}$. The fact that less strength was formed via surface percolation suggested that the calcium carbonate formation reaction was not completed. This was evident by the urea conversion, which was only 40% in the percolation method compared to 80% in the submersed flow method. This was probably due to less immobilized bacterial cells.

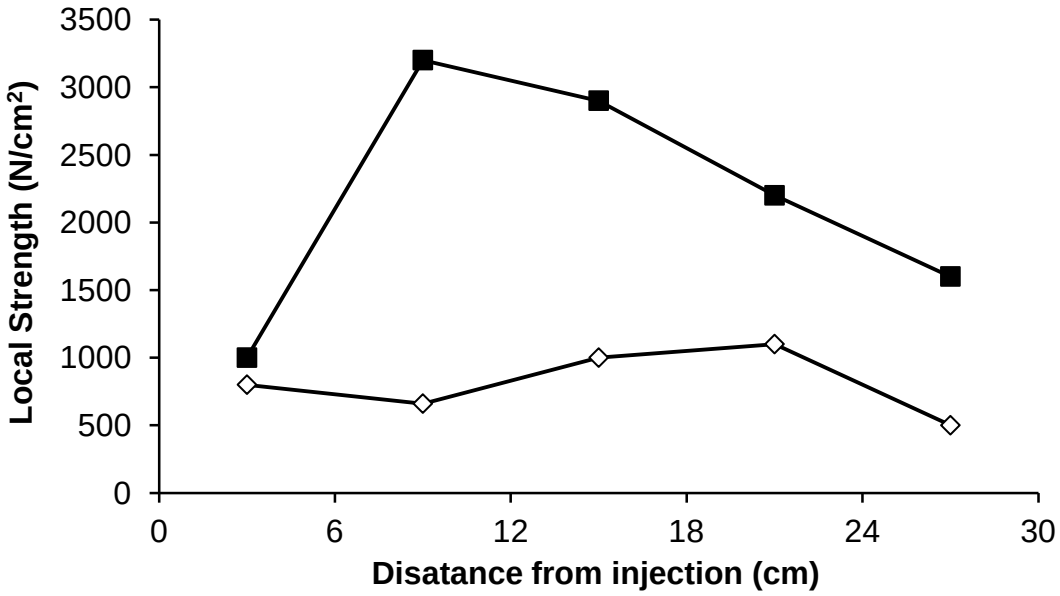


Figure 4. Local strength profiles of cemented columns treated by percolation (◊) and submersed flow (■) with 50 mM $CaCl_2$ fixation solution and 220 mL/h flow rate. The columns were injected with 45mL of a ureolytic (10 U/mL) bacterial suspension. For cementation 3 batches of 1 M urea/calcium cementation solution (3×180 mL) were used.

3.2 Improving bacterial retention by incubation in the presence of cementation solution

The reason why fixation method did not work well in the percolation treatment above (Figure 3), could be inadequate mixing caused by the percolation application, preventing the access of high ionic strength solution to the bacteria suspension. To achieve better intermixing of the bacterial and cementation solution an incubation period was used after the sequential loading of 90 mL (50% void volume) bacteria solution and 90 mL cementation solution. After these solutions were fully loaded into columns, the columns were incubated for 0, 6, 12 and 24 hours at 25±1 ^{o}C respectively.

Therefore, the column had two layers of solution present. It was anticipated that incubation process could allow the Ca^{2+} ions to diffuse to the bacterial suspension, improving their immobilisation. After the incubation, further cementation solution was added, pushing out those bacteria that were not fixed to the sand (Figure 5). The retention of bacteria in the sand was greatly aided by the duration of incubation. A minimum of 24 hours of incubation was needed to improve bacterial activity retention from 30% (70% lost) to approximately 80% (20% lost) (Figure 5).

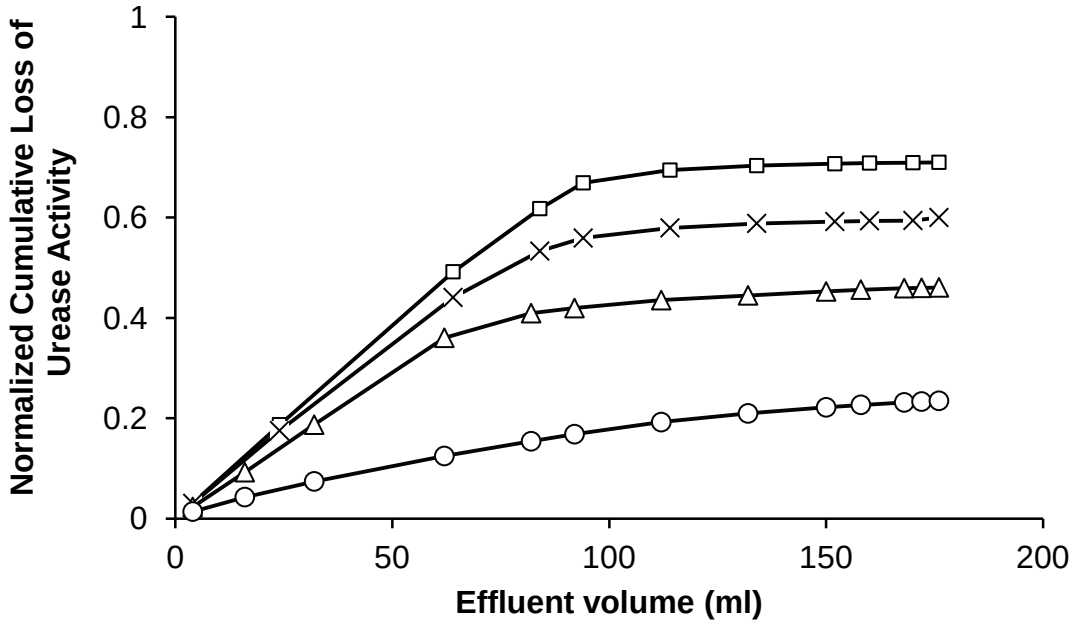


Figure 5. Normalized accumulative loss of urease activity of the columns injected with half pore voids volume (90 mL) of each bacterial suspension and cementation solution with different incubation times allowed prior to flushing further cementation solution into the column. (Without incubation (□), 6 hours of incubation (×), 12 hours of incubation (Δ), and 24 hours of incubation (○))

3.3 Improving bacterial retention by using multi-layers of bacterial suspension and cementation solution

In the previous experiments, better mixing between bacteria and cementation solution was reached by allowing an incubation time for diffusion between the different layers of solution. Alternatively, thinner layers would also be expected to enhance diffusion. To evaluate the effect of thinner layers on bacteria retention, the 30 cm columns were injected with layers of different thickness resulting in 1 to 12 layers of alternating content of bacterial suspension/cementation solution. A clear trend was obtained showing that the bacterial retention increased with the number of layers used (Figure 6).

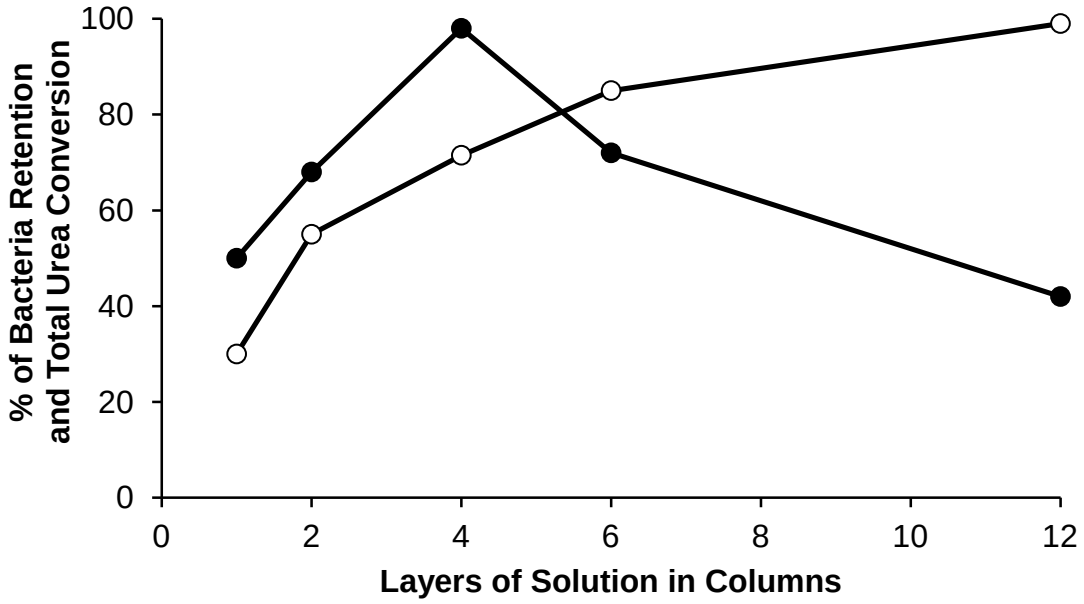


Figure 6. Effect of number of layers of alternating bacteria/cementation solution on total bacteria retention (○) (measured by recording bacterial activity in the outflow) and urea conversion (●) (after 12h) in the 30 cm sand columns.

Thinner layers of alternating bacteria/cementation solution (i.e., increased numbers of layers) in the column improved the bacterial retention (Figure 6). However, the total urea conversion did not improve with the level of bacteria fixation in the thinnest layers (6 and 12 layers) (Figure 6). This was caused by an obvious crust formation at the top of the column, where bacterial cells were filtered out and accumulated close to the injection point. The crust formation can be demonstrated by a high local strength layer at the top of the column (Figure 7). In contrast to the columns with 12 and 6 layers where crust formation was observed, the strength was uniform to the full depth of the column when 4 layers were used. Using less than 4 layers resulted in more strength formation in the lower layer (Figure 7). The effect of using different layers could be useful to customize biocementation, aiming at either uniform strength or targeted strength (either upper or lower layers).

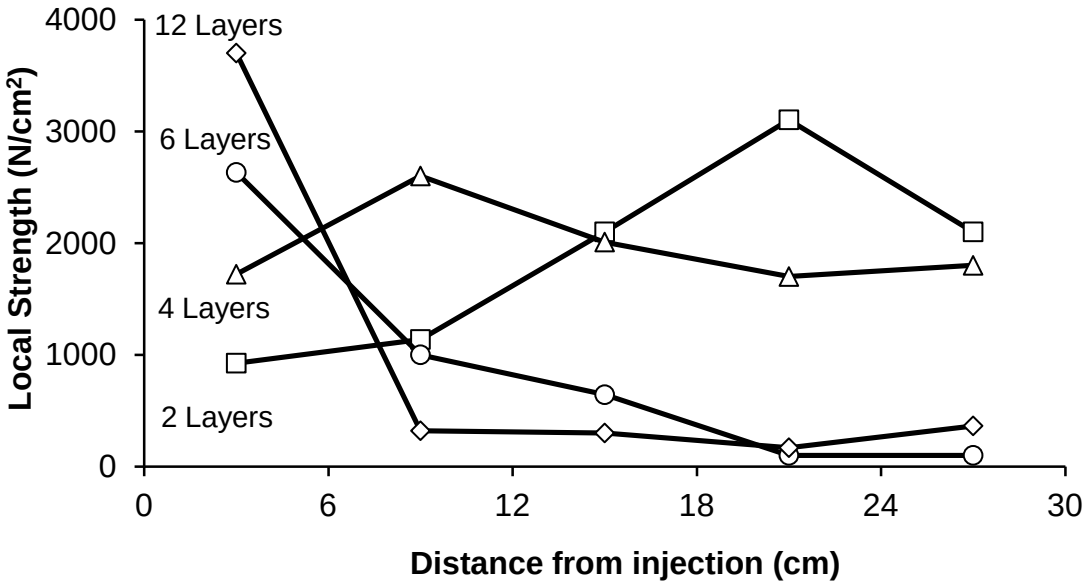


Figure 7. Effect of number of layers of bacteria/cementation solution on strength profiles along the column length. (Columns percolated with 2 layers (□), 4 layers (△), 6 layers (○) and 12(◇) layers of alternating bacteria/cementation solution). In this experiment step 4 of the percolation treatment was carried out twice.

Controlled bacterial placement as described above, enabled successful consolidation of sand columns treated with surface percolation as measured by local strength. While local strength measurements give a good relative indication of strength distribution, geotechnical engineers require UCS data as an established measure. UCS tests showed that strength of sand columns treated by surface percolation were similar to columns treated with the more established submersed flow application (Table 1). In both cases similar amounts of cementation solution and bacterial solution were used. This shows that in the case described here, the simpler percolation treatment could enhance soil strength in much the same way as the traditional submersed method.

Table 1. Comparison of consolidated sand columns treated with surface percolation and submersed flow methods, both with high water content. 30 cm sand columns were treated and the middle part (9 cm long) was used for UCS and $CaCO_3$ tests. 90 mL of bacterial suspension and 800 mL of cementation solution were used.

Sand Samples	Bacterial Placement	Volumetric Water content (%)	Bacteria Retention	Urea Conversion	$CaCO_3$ Content (g/cm^3)	UCS (KPa)
Surface Percolation (Funicular Regime)	4 Alternating Layers	35.9-37.1	75%	86%	0.12	390
Submersed Flow (Saturated Regime)	50 mM $CaCl_2$ Fixation Solution	38.5	82%	92%	0.14	340

3.4 Treatment of longer sand columns with surface percolation and submersed flow methods.

Pratical and engineering applications of soil stabilisation will need deeper consolidation. In order to test the effect of multi-layered bacterial placement on consolidation in longer of sand columns, two 1 m sand columns were treated by surface percolation. In each of the columns bacteria were placed by using 2 and 6 layers of alternating bacteria/cementation solution respectively, after 12 hours followed by 2 injections of cementation solution. The local strength determination of the treated sand column showed that deep consolidation of sand could be obtained by using surface percolation application (Figure 8).

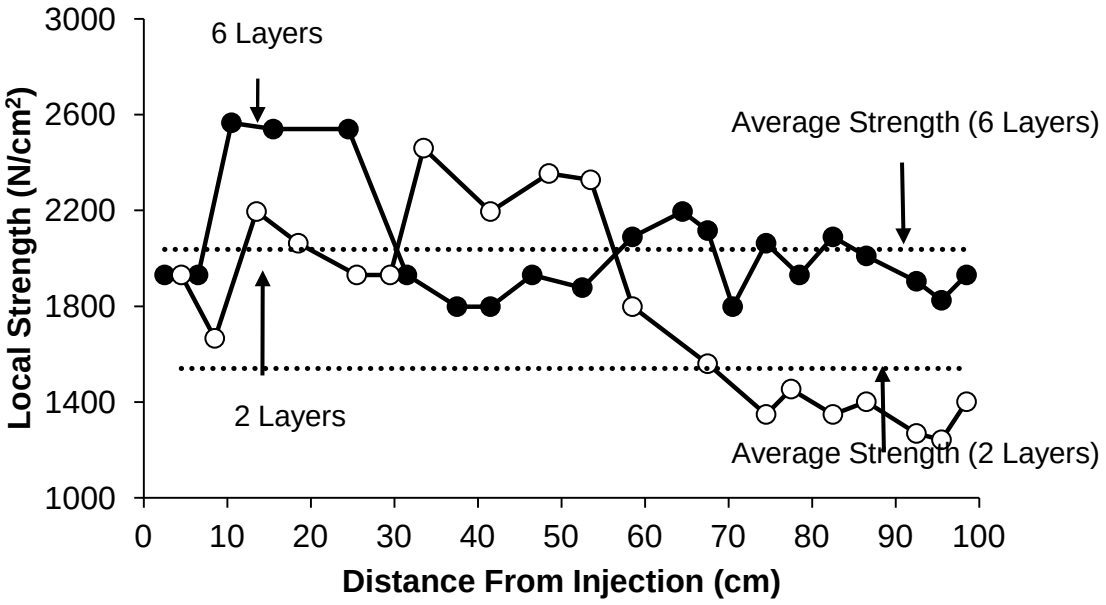


Figure 8. Effect of the number of layers of bacteria/cementation solution on the local strength profile of 30 cm sand column consolidation (surface percolation). 180 mL of bacterial suspension and 900 mL of cementation solution were used for the column.

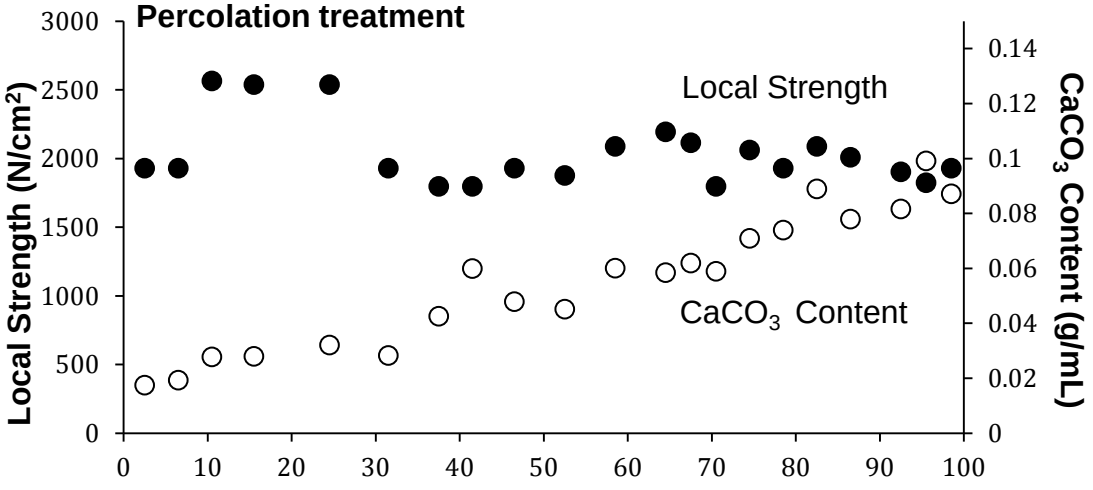
The 6 layered column reached a higher average strength and more homogeneous strength distribution compared to the 2 layered column (Figure 8). This can be explained by the higher bacterial retention (85% compared to 60%) and the greater amount of urea hydrolyzed (92% compared to 77%) (Table 2).

Table 2. Urease activity fixation and urea conversion in 1 m sand columns

Sand Samples	Bacterial Placement	Bacteria Retention	Urea Conversion
Surface Percolation	2 Alternating Layers	60%	77%
	6 Alternating Layers	85%	92%

For submersed flow application of biocementation (biogrout) where sand is fully water-saturated, van Paassen et al. (2010) have established that there is a correlation between calcium carbonate precipitated and mechanical properties of the treated sand (UCS, permeability, shear modulus, deformation behavior, etc.). The current study confirmed this trend for the submersed cementation where increased local strength was observed at the top and bottom of the column where also the calcium carbonate concentration was highest (Figure 9).

In contrast to the submersed cementation method, the surface percolation method (non-saturated condition) resulted in higher local strength and showed no correlation between strength and the amount of calcium carbonate that had precipitated. Comparison of local strength with the amount of calcium carbonate precipitated in each layer of the percolation treated 1 m column showed that while calcium carbonate content increased with the depth of the column, the local strength of samples did not increase propotionally (Figure 9). A relatively constant local strength was obtained overall the sand column, while three times more of calcium carbonate crystals were precipitated at the bottom of column compared to the top part.



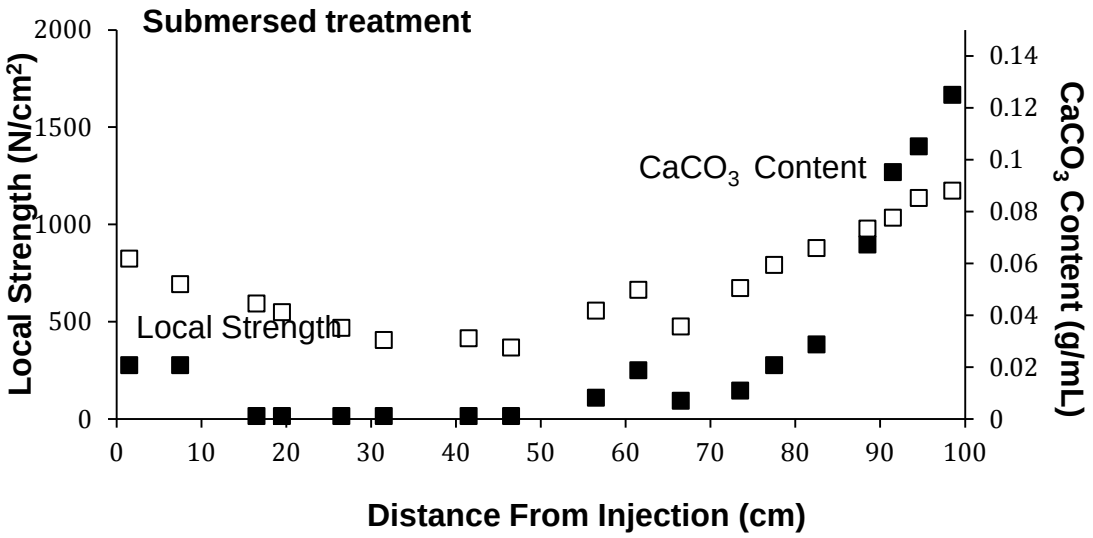


Figure 9. Profiles of local strength and $CaCO_3$ content of consolidated sand columns (1 m length) treated by the surface percolation (6 layers) and submersed flow methods. Both methods used the same amount of bacterial suspension (180 mL), and urea/calcium (0.9 moles). The cementation step (step 4) was carried out twice. The submersed treatment required a larger volume of cementation solution, which was accordingly diluted. The total amount of calcium carbonate formed in the columns was similar (82 g (surface percolation) and 78 g (submersed flow)) as expected due to same total amounts of urea and calcium added.

In the sand columns treated by the submersed flow method, calcium carbonate contents of cemented sand less than 0.05 g/cm^3 resulted in non-measureable local strength (Figure 9), which is in line with the previous literature observations (Whiffin et al., 2007). To obtain higher strength, repeated applications are necessary. It was surprising that the sand column treated by the surface percolation method (6 layers) showed a high local strength of 2500 N/cm^2 (10 to 30 cm) at low $CaCO_3$ of only 0.03 g/cm^3 (Figure 9). This rather strong effect of significantly increased strength with similar or even less amounts of $CaCO_3$ formed is interesting in both scientific and applied aspects.

The finding of highest strength with least calcium carbonate formed, obtained at the top layer of sand column treated by the percolation method (Figure 9), implies that the

calcium carbonate crystals formed in these particular area were specifically effective. Further SEM analysis of those cemented sand samples was carried out.

3.5 Effect of water content differences in the percolated column

The surface percolated sand columns showed a gradient of volumetric water content from 7% at the surface, where, according to unsaturated soil mechanics theory (Figure 10 b), the solution exists primarily in the form of disconnected menisci, to about 39 % at the bottom where the sand matrix is completely saturated and filled with solution (Figure 10 a). The phenomenon of higher water content towards the bottom end of the columns is a known phenomenon explained in detail in Lu and Likos (2004). As crystals can only form inside the cementation solution, crystal formation in the top of the column were restricted to form precisely where the menisci were situated, which is the bridging points between sand grains, the preferred location for crystal formation that provide strength.

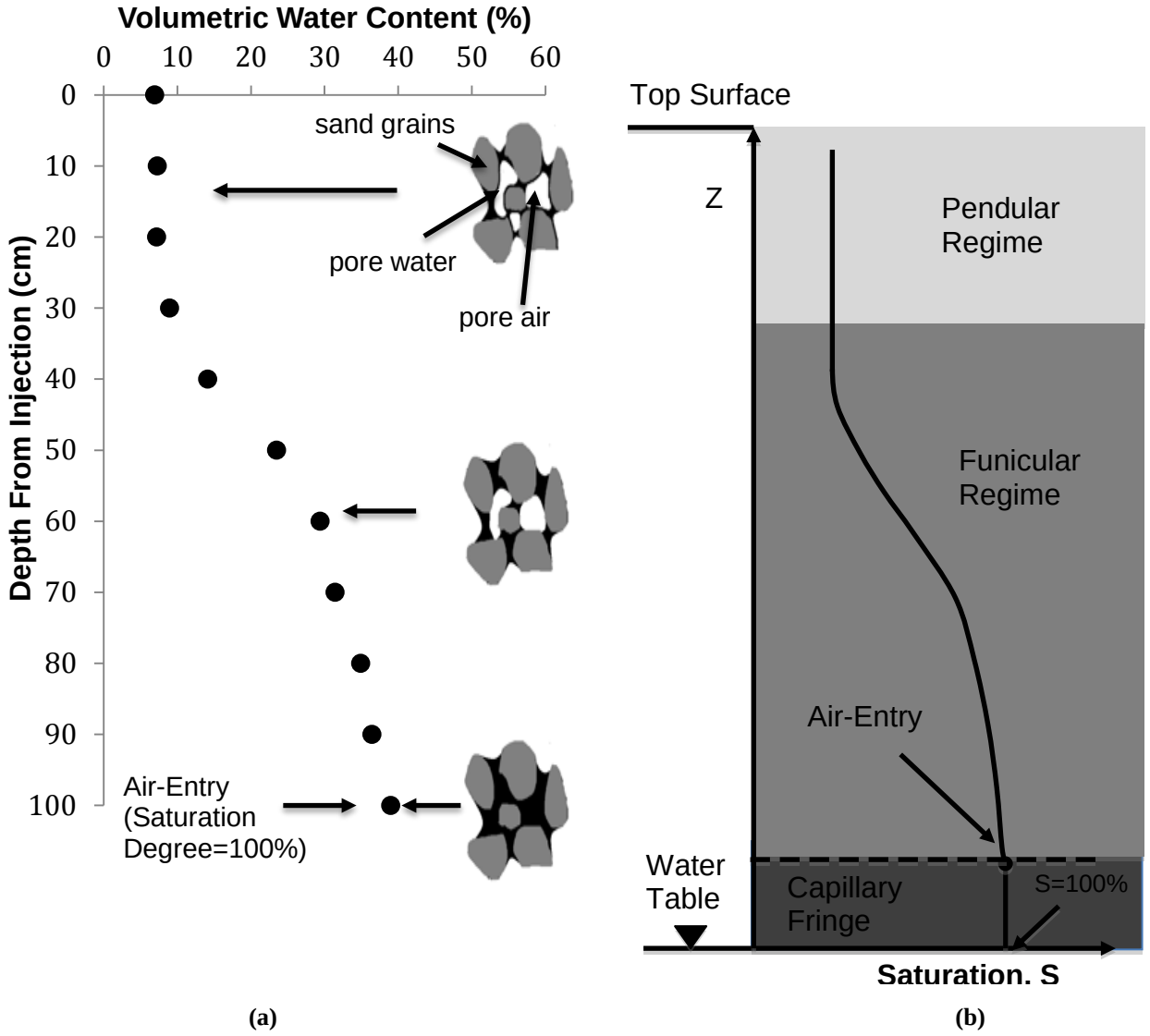


Figure 10. (a) Profile of volumetric water content in a 1 m sand column under surface percolation (unsaturated) conditions (porosity 37.7%) and (b) conceptual illustration of saturation in the unsaturated soil zone and water regime of the unsaturated soil zone (modified from Lu and Likos, 2004).

To test the above hypothesis of preferred crystal formation at the bridging points between sand grains at low water levels, scanning electron microscopy (SEM) was used to inspect crystal numbers and positions. The SEM images of cemented samples taken from top and bottom of percolated column (6 layers) showed that the bridging crystals formed in the gaps of sand grains were predominant in the top of column (Figure 11).

By counting the number of crystals in the image, more than 80 % of total crystals were precipitated in the gaps and acted as a bridge to bind sand grains. In contrast, in the bottom of the column less than 10% of crystals were precipitated in the conjunctions, but a larger number of total crystals was present.

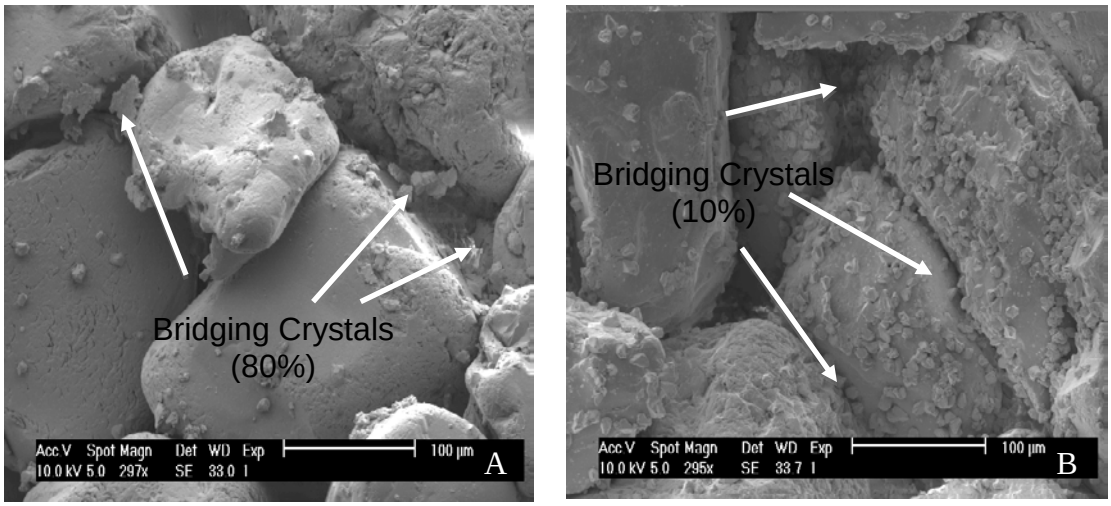


Figure 11. SEM images of cemented sand samples taken from top (a) and bottom (b) of the percolated column (6 layers). Arrows indicate the location of "bridging crystals". Percentage values give the percentage of bridging crystals for each section. The porosity restriction caused by numerous crystals coating sand grains in the bottom part of the column can be visualized.

4 Discussion

The results of this paper show that the biocementation process, based on ureolytic bacteria induced calcium carbonate precipitation, not only works in water saturated sand by the submersed flow method, but also in unsaturated sand zone by surface percolation. The use of the percolation method is not only easier but also produced higher strength than the submersed flow method. The increased strength is not attributed to the different type of operation but to the lower water content in free draining soil compared to

submersed soil as this paper shows that in the same column more strength is obtained where the water content is lower.

Bacterial fixation

The problem of bacteria washout (Figure 3) at high percolation rates could be overcome by using a method in which the bacterial cells were incubated in the presence of cementation solution (Figure 5). This can be accomplished by using discrete alternations of the two different solutions, bacterial suspension and cementation solution. This method of fixing bacteria in a sand column by using alternating layers of bacterial suspension and fixation solution is based on diffusion principles and may also be useful for the fixation of non-urease active bacteria in the soil. Future work could test whether this method can be applied for purposes other than biocementation, for example bioremediation of contaminated soils.

The success of this method to fix bacteria in the sand column could be explained by one or all of the following effects: (1) Time-dependent bacterial attachment, usually mediated by extracellular polymers (Fletcher et al., 1973; Marshall 1985; Morris et al., 1989). (2) Time-dependent calcium ion diffusion from the cementation solution increases the ionic strength around the bacterial cells, which is known to encourage bacterial attachment (Scholl et al., 1990; Torkzaban et al., 2008). (3) Calcium carbonate crystals, by acting as filter, prevent bacterial cells from washing out.

For calcium ions to reach the bacteria they need to diffuse through the interface of the cementation solution and bacterial solution. Hence by using multiple thinner layers of the two solutions will improve the access of calcium to the bacteria encouraging fixation. In order to achieve homogeneous strength, a thickness of the supplied layers

needs to be chosen that is suitable for the desired depth of cementation. The aim is to prevent both crust formation and insufficient cementation. Using many thin layers (e.g. < 3 cm thickness) caused crust formation due to bacterial accumulation close to the surface. By contrast, thicker layers of bacterial suspension enable cells to be transported deeper into the column. For example, in the 1-meter column with a layer thickness of 10 cm, the bacterial suspension successfully reached the bottom where it was immobilized as evidenced by high levels of urease activity and $CaCO_3$ (Figure 9). However layer thickness of 30 cm or more resulted in bacteria washout and insufficient cementation (Figure 8).

Effect of water content on effective crystal formation

Generally, the soil located above the underground water table can be subdivided into three regimes: 1) capillary fringe (saturated), 2) funicular regime (unsaturated with continuous water phase), and 3) residual or pendular regime (unsaturated with isolated water phase) (Figure 10b). These regimes are principally characterized by different water content and water pressure (Lu and Likos, 2004). In this study where the 1 m column was freely drained during the experiments only the funicular and the pendular regime were present. This was evident by the profile of volumetric water content (Figure 10).

The successful consolidation of the entire sand column showed that the surface percolation method could effectively cement the sand of different water contents. This fact could be useful for treating loose sandy soil to extending depth (i.e. reaching funicular zone).

In the 1 m column treated by the surface percolation method, less $CaCO_3$ crystals formed in the pendular regime compared to the funicular regime. This is expected, as there was 4 times less liquid and hence 4 times less calcium available for calcite precipitation. The difference in calcite content (3 times less in the top (pendular regime)) was approximately reflected by the difference in water content (4 times) present during the cementation reaction. Despite the higher amount of crystals observed in the funicular regime (bottom layer), no increased in strength was found, as the strength was relatively uniform. With other words, the strength per amount of calcite present (specific strength) had decreased from the pendular regime to the funicular regime suggesting that more specific strength is obtained with lower water content in the sand.

In the top layer of a 1-meter column, the residual water after drainage is known to accumulate largely at the contact points between sand grains as menisci (Figure 10) due to capillary forces (Lu et al., 2004). Hence the crystals precipitation will be restricted to these precise locations and crystal formation will occur, at what could be considered the optimum position for bridging of sand grains and developing strength (Figure 11). Crystals at junctions are likely to be strength providing crystals (Paraskeva et al., 2000; Ismail et al., 2002), while crystals that form on the sand grain or in the pore space, as expected at higher water content cannot be seen to add to strength.

In general, biocementation at the high (> 30%) water content conditions (short 30 cm percolated column and the bottom part of 1-meter percolated column) led to similar strength as the submersed application (Table 1 and Figure 9) suggesting that it is the water content rather the method of application that determines the degree of soil

solidification obtained. The specific strength (local strength/calcite content) of those high water content biocementations could not reach the strength obtained for the low water content areas (pendular regime).

Potential practical limitations of the method

This study is limited to one-dimensional vertical cementation in which the flow conditions were confined by the column walls. In a real application the liquid flow will be three-dimensional. In this case the fluid density and porosity of the sand matrix will influence the local water content and also the flow dynamics of the different layers by the different layers. However, the precipitated crystals may also play a role in controlling the liquid flow. It would be expected that as the initial opted pathway of solutions becomes restricted due to crystal formation, the liquid flow would change to alternative pathways with a lower permeability resistance. To what extent homogeneity can be reached when allowing a 3-dimensional flow, will be described in a subsequent paper.

Potential application of biocementation by surface percolation

The described method opens up new potential applications for the process of biocementation using ureolytic bacteria. While the submersed method is suited for in-situ soil stabilisation of submersed or submarine soils / sediments, the current method enables the simple process of surface percolation by – for example – spray irrigation onto dry, free draining ground, such as dunes or dykes. Because of up to three times higer strengths reached with the same amount of chemicals the method offers also a potentially cheaper way of ground improvement than previously described submersed application. However before this method can be widely used, practical problems (e.g.

soil inconsistencies, water repellency, etc.) need to be explored and solved with large scale applications. Further research is needed to investigate whether this method can be used for coarse sands and and silty soils and whether the results shown can be confirmed with unconfined strength measurements.

Overall the results suggest that MICP by surface percolation has the potential of cementing soil grains to strength, similar to conventional grouting techniques (e.g. jet grouting, (Warner, 2004) or permeation grouting (DeJong et al., 2010)). The increased strength is often required to prevent unacceptable deformations (e.g. soil liquefaction) caused during construction and operation activities or natural phenomena such as earthquakes. The specific advantage of the surface appliation method described here is that it is non invasive. The surface percolation of MICP is most suitable for granular soils with high permeability, which allows for the unimpeded flow of the MICP solution permitting greater depths consolidation.

Despite the strengthening capabilities of groutings, constituents of the conventional grout mixtures have come under close scrutiny due to their negative environmental impacts. Recent research indicates that all chemical grouts except sodium silicate are toxic and/or hazardous (DeJong et al. 2010). To what extent the MICP via the urease reaction is environmentally more acceptable remains to be seen. On the one hand the endproduct, calcium carbonate, is a common natural, stable and non-toxic mineral, on the other hand the by-product ammonia is not acceptable to many ground environments and may require expensive remediation processes. Further, more applied investigations relating to reducing soil liquefaction and land sliding, enhancing slope stability, or increasing bearing capacity for shallow foundations have not been carried out yet.

5 Conclusions

- This work presents a new application for MICP as a consolidation technique for unsaturated ground by using an easily applicable surface percolation method.

- Bacteria could be immobilized in the column over 1 m length at high percolation rate by applying multiple alternating layers of bacterial suspension and fixation solution followed by incubation. The srength improvement of sand column reached a reasonable degree of homogeneity without crust formation at the surface.

- At low water content (pendular regime), about 3 times higher local strength was reached per mass of calcite formed than in samples with higher water content (funicular regime), indicating that costs per strength can be reduced 3 times by using surface percolation compared to submersed application (highest water content).

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