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Microbial Carbonate Precipitation as a Soil Improvement Technique

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In order to evaluate MCP as a soil strengthening process, a five meter sand column was treated with bacteria and reagents under conditions that were realistic for field applications. The injection and reaction parameters were monitored during the process and both bacteria and process reagents could be injected over the full column length at low pressures (hydraulic gradient < 1; a flow rate of approximately 7 m/day) without resulting in clogging of the material. After treatment, the column was subjected to mechanical testing, which indicated a significant improvement of strength and stiffness over several meters. Calcium carbonate was precipitated over the entire five meter treatment length. Improvement of the load bearing capacity of the soil without making the soil impermeable to fluids was shown with microbial carbonate precipitation, and this is a unique property compared to alternative soil treatment methods that are currently available for use in the subsurface.

Keywords Microbial carbonate precipitation, soil improvement, strengthening, urease

INTRODUCTION

Microbial carbonate precipitation (MCP) has experienced an increased level of interest in recent years, for applications such as restoration of calcareous stone materials (Tiano et al. 1995; Castanier et al. 2000; Stocks-Fisher et al. 1999; Rodriguez-Navarro et al. 2003), bioremediation (Ferris 2003; Fujita et al. 2000; Warren et al. 2001), wastewater treatment (Hammes et al. 2003), strengthening of concrete (Ramachandran et al. 2001) and selective plugging for enhanced oil recovery (Ferris and Setehmeir 1992; Gollapudi et al. 1995; Nemati and Voordouw 2005). From a geotechnical perspective, the potential of MCP has been identified as a means of adapting soil properties to suit desired land-uses. Controlled precipitation of minerals in the pore space in such a way as to change macro-soil properties or so-called "pore-space engineering," is a new innovative approach in soil geotechnics with significant scope for development.

MCP can occur via a variety of processes whereby microbial activities result in the generation of carbonate in a calcium-rich environment (Castanier et al. 1999). The resulting $CaCO_3$ precipitation is governed by four key parameters: (i) calcium concentration, (ii) carbonate concentration, (iii) pH and (iv) the availability of nucleation sites (Hammes and Verstraete, 2002). Many biological reactions can result in the production of carbonate or carbonate species. Because of its simplicity and the lack of an excess proton production, the most commonly studied system of applied MCP to date is urea hydrolysis via the enzyme urease, in a calcium-rich environment.

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$
$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow$$

Other studies have concluded that an improvement in material strength is possible by inducing MCP, but usually the treatment has limited injection depth (in the order of centimetres) and it is often associated with a major reduction in permeability. In most cases bacteria and reagents are mixed with the granular material, sprayed on a surface or injected together under high velocity or pressure. Injection of bacteria and reagents together at low flow rates can result in full clogging of the system near the injection point (Stocks-Fischer et al. 1999). For the purposes of soil improvement, the reduction of permeability is an undesired characteristic. Lowering permeability in the treated area will

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promote redirection of natural groundwater flow paths. This can result in an increase in pore pressure in the soil, which increases the risk of soil failure. The retention of soil permeability offers an additional advantage in that it also allows the additional application of further strengthening treatments and thus control over the amount of strengthening that is finally achieved. These desirable characteristics have been successfully implemented into a process design whereby MCP occurs under conditions such that all components can be applied without soil disturbance (i.e., soil mixing/replacement), soil strengthening is achieved and permeability is largely maintained (Whiffin 2004; Kucharski et al. 2006). This design has been tested under laboratory conditions to penetration depths of less than 0.5 m.

Other MCP investigations to date have focussed on microbial carbonate precipitation for applications other than soil improvement, thus the important parameters for this specific application are yet to be evaluated (e.g., significant penetration depth (in the order of meters) at low hydraulic gradients, retention of permeability, and the correlation of mechanical parameters with calcium carbonate content). The objective of this paper is to make a first correlation of these parameters and evaluate the feasibility of MCP for use as a soil improvement technique.

MATERIALS AND METHODS

Column Parameters and Sampling

The 5-meter-long PVC tube (internal diameter 66 mm) was positioned vertically and packed with 125–250 μm Itterbeck sand (grain size characteristics: $d_{10} = 110\mu$m (10% of the grains have a diameter of this size or lower); $d_{50} = 165\ \mu$m; $d_{90} = 275\mu$m) to a dry density of 1.65 g/cm^3 (porosity of 37.8%). The column was positioned vertically with downward flow direction to avoid any settling of the packing material and generation of preferential flow paths that may occur if the column was positioned horizontally. Each end of the column was fitted with filter material consisting of 3 layers of scouring pad (Scotch Brite) at the outside and approximately 8 cm of filter gravel on the inside, next to the sand (Figure 1). Packing of the sand column was conducted under water to the required density to avoid the inclusion of air pockets.

Five water pressure transducers were fitted to monitor water pressure inside the column at 0, 0.5, 1, 2 and 3 m from the top of the column. In addition to these, the column was fitted with 10 pore fluid sampling ports (0.25, 0.5 and thereafter at 0.5 m intervals until reaching 4.5 m). Fluid reservoirs containing the injected fluids (water, bacteria, $CaCl_2$, Urea etc.) were connected at the top of the column. A pump was installed at the bottom of the column to regulate the outflow rate and hydraulic head between free gravity flow of 1 L/h at a hydraulic head of 5 m when the pump was fully open and zero when the pump was fully closed. During the experiments the flow rate was kept constant at approximately 0.35 L/h.

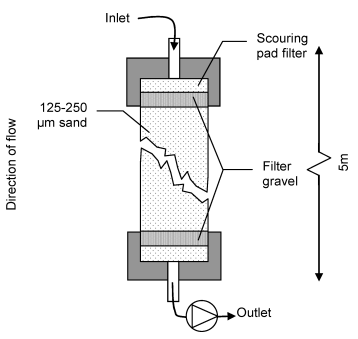

FIG. 1. Schematic of column filter setup.

Before any experiments were conducted, tap water was flushed through the column to test the pressure transducers and check the flow regulation of the pump. This information was used to define the initial hydraulic conductivity of the column (2×10^{-5} m/s). All subsequent experiments were performed at a constant injection flow rate of approximately 0.35 L/h and ambient temperature of 18°C ± 2°C.

During the course of the experiment, samples were taken from the sampling ports positioned along the column length and with an in-line feed from the effluent stream to an automated fraction collector. Immediately after collection the samples were centrifuged and the supernatant transferred to a clean tube, which was frozen at −18°C awaiting analysis. Samples were tested for urease activity, ammonium and calcium concentrations as required.

Microorganism

The urease positive microorganism used was *Sporosarcina pasteurii* (DSMZ 33). Cultivation of the organism was conducted under aerobic batch conditions in a medium containing 20 g/L yeast extract and 10 g/L NH_4Cl, at a pH of 9. The organism was grown to early stationary phase before harvest (i.e., all readily available nutrients were consumed from the medium), and stored at 4°C for 48 hours prior to use.

MONITORING METHODS

Urease Activity

In the absence of calcium ions, urease activity was determined by a conductivity method. The urease reaction involves the hydrolysis of non-ionic substrate urea to ionic products thus generating a proportionate increase in conductivity under standard conditions. Then 1 ml of bacterial suspension was added to 9 ml of 1.11 M urea (reaction concentration 1 M urea) and the relative conductivity change was recorded over 5 minutes at 20°C ± 2. The urease activity was then calculated taking the dilution into account.

This method was not suitable for determining urease activity in the presence of calcium ions (due to precipitation of calcium carbonate particles and the dampening effect of the counter ion on the solution conductivity), thus in these cases urease activity was determined from the ammonium production rate (see later). Both methods for determining urease activity were independently calibrated and correlated with each other.

Ammonium Concentration

Ammonium concentration was determined by a modified Nessler method (Greenburg et al. 1992). The sample was diluted with deionized water to be in the range of 0–0.5 mM, using a volumetric flask. The 2 ml of sample was added to a cuvette and mixed with 100 μl of Nessler reagent (Merck, Germany), and allowed to react for exactly 1 min. The sample was then read in a spectrophotometer at 425 nm. Absorbance readings were calibrated with several NH_4Cl standards measured under the same conditions.

Calcium Concentration

Calcium concentration was determined with a commercial cuvette test for water hardness (LCK 327 – Hach Lange, Germany). Samples were diluted with deionized water to be within the concentration range of 5–100 mg Ca/L.

Calcium Carbonate Content

The calcium carbonate content of the cemented samples was measured with a U-tube manometer, under standard conditions (298 K, 1 atm). A 1–4 g sample was weighed into a glass vial, 2 ml of 2 M HCl was added in a separate compartment and the vial was sealed. The initial gas volume in the manometer was recorded, and then the 2 compartments were allowed to mix, resulting in acid dissolution of the sample and evolution of a proportionate amount of carbon dioxide gas. Samples were blanked against untreated sand and the method was calibrated with analytical grade $CaCO_3$.

Water Pressure

The water pressure was measured using water pressure transducers (Model PDCR 10F – Druck Ltd, UK) that were numbered 1 to 5 (top to bottom) and were placed at 0, 0.5, 1, 2 and 3 m along the length of the column.

Flushed Volume

The flushed volume (excluding the effluent and port samples) was collected in a container. The weight of the container was continuously monitored using a weight sensor (Model U2A - S/N 51759, maximal range 0–40 kg–HBM, Germany). The overall column flow rate was calculated from the total flushed volume (liquid in container plus effluent and port samples) versus time.

Strength, Stiffness, Porosity, and Permeability

After dismantling of the column, the column was cut into 25 cm sections and compressive strength (q) and stiffness (E_{50}) of the sections were determined by single-stage confined drained triaxial tests with a confining pressure of 50 kPa (conforming with Dutch standard NEN-5117). Porosity was determined from the wet and dry densities of the samples after strength testing. Permeability (K) was measured by a constant head test (conforming with Dutch standard NEN-5123).

RESULTS

Placement of Bacteria Prior to Cementation

In order to immobilize bacteria in the column for use in subsequent cementation, a 2-phase injection was conducted. First bacteria were injected to fill the column volume. When bacteria were detected in the outlet by visually observing an increase in turbidity (confirmed by microscopic analysis), one pore volume of 50 mM calcium chloride solution (6.1 L) was injected to immobilize the bacteria in a moving reaction front in the column (European patent pending; EU05077869.5). A summary of column injections is given in Table 1.

Injection of Urea and Calcium for Cementation

Immediately after the bacterial placement step, 1.1 equimolar urea and calcium chloride solution was injected to initiate cementation (Table 1). Under the constant flow conditions during injection, the movement of the front of reaction fluid (1.1 M urea/calcium) could be followed in the column. The first appearance of ammonium at each of the sampling ports along the column length was measured and matched with the residence time that the fluid had been present in the column (Figure 2).

The linear slope of the ammonium versus retention time line suggested that the ammonium was continuously being produced during injection and that the production rate was relatively constant throughout the column (Figure 2). According to the ammonium production at the reaction front, 75–80% of injected urea was used in the top of the column (up to 2.85 m) leaving only 20–25% for bottom section column and thus only the possibility of significantly lower $CaCO_3$ precipitation in this section relative to the top of the column.

TABLE 1
Summary of column injections - OD_{600} is the injected biomass concentration (measured by optical density at 600 nm); Act is the urease activity

Phase	Description	Duration (h)	Flow rate (L/h)	Volume (L)	Details
Rinse	water flush	30.7	0.35	10.75	Tap water
Placement	Bacterial injection	18.1	0.35	6.34	OD_{600}: 1.583 Act: 0.23 mS/min
	$CaCl_2$ injection	17.1	0.35	5.99	0.05 M $CaCl_2$,
Cementation	Reaction fluid injection	24.9	0.35	8.72	1.1 M Urea and $CaCl_2$
	No flow—reaction	102	0	0	—
Rinse	water flush	23.7	0.35	8.30	Tap water

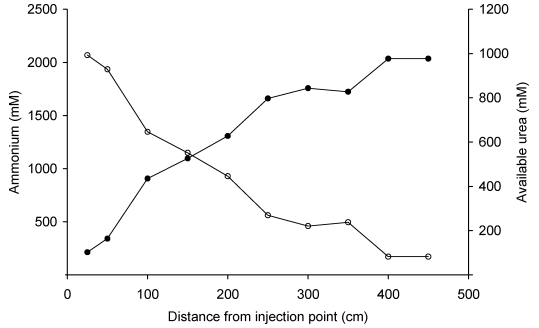

FIG. 2. Movement of reaction front (estimate of front 250 ml of fluid) in column during the injection phase (0-17 hours). Ammonium concentration (●) and calculated available urea concentration (○) from the known molar ratio (2 NH_4^+ : 1 urea).

In an attempt to extend the injection distance, the injection of urea and calcium chloride solution was continued beyond one pore volume. During this time the ammonium concentration in the column effluent decreased, indicating that less urea was hydrolysed during the time in the column. No bacteria were observed in the effluent. These observations suggested that the urease activity in the column had decreased over time.

In order to allow some cementation at the end of the column, the injection was continued at the same flow rate until less than 1.5 M ammonia was measured in the effluent, which indicated that at least 0.25 M unreacted urea had reached the end of the column. The total amount of calcium/urea injected was equivalent to 1.43 times the pore volume of the column (8.7 L).

The ammonium production rates were directly calculable: during the flow phase as the accumulation of ammonia between 2 sampling points and during the stationary phase as the accumulation in time at each particular sampling point of the column. The average ammonium production rate during stationary phase (27–53 h) was approximately a third of the rate observed at the reaction front during the injection phase (0–24 h) (180 mM NH_4^+/h during the injection phase versus 60 mM NH_4^+/h in stationary phase (Figure 3)).

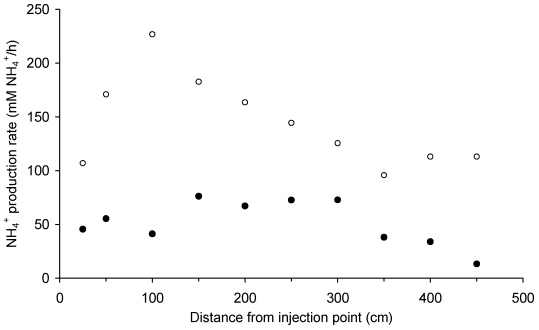

FIG. 3. Ammonium production rates at each port along the column length at the first appearance of the urea/calcium (○) (during continuous flow) and at beginning of the stationary phase (●) (these rates were calculated between 27–53 hours, during which the r^2 values were greater than 0.93).

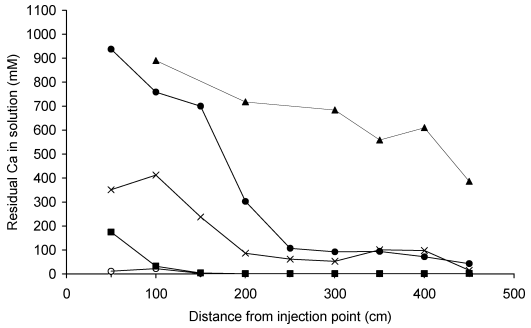

FIG. 4. Calcium concentration in the pore fluid (i.e. not yet precipitated) after 24 h (▲) (at the end of the injection phase), 32.5 h (●), 53 h (×), 96 h (■) and 124 h (○). Injected calcium concentration was 1.1 M.

Calcium concentrations were measured in the pore fluid of the column at the end of the injection phase (24 h) and over several intervals until 124 hours. After 124 hours no soluble calcium was detected in the pore fluid and the precipitation reaction was complete (Figure 4).

$CaCO_3$ Profile along the Column

After cementation was complete, the column was flushed with excess water, the filter material was removed and the remaining sand column was cut with a saw into 25 cm sections for evaluation of the mechanical properties. An average calcium carbonate content value was determined from at least 3 samples for each column section (Figure 5).

Effect of Cementation on Porosity

Porosity was determined from the wet and dry densities during strength testing. The presence of calcium carbonate had a clear effect on porosity of the material and a reasonably linear relationship between the two parameters was observed. At the maximum calcium carbonate content (105 kg/m^3 $CaCO_3$) the column porosity was decreased to 90% of the untreated material (Figure 6).

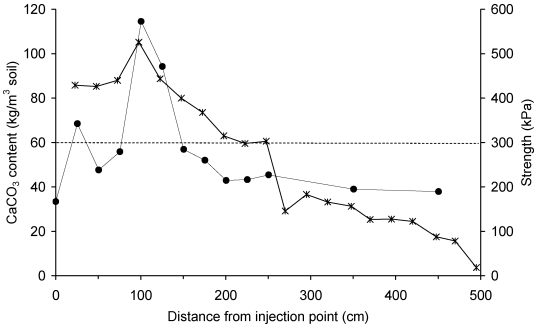

FIG. 5. Calcium carbonate (∗) and strength (●) profiles along the column length. The column was injected with 8.715 L of 1.1 M urea/calcium which can react to produce an average overall column value of 59.2 kg $CaCO_3$/m^3, indicated by dashed line.

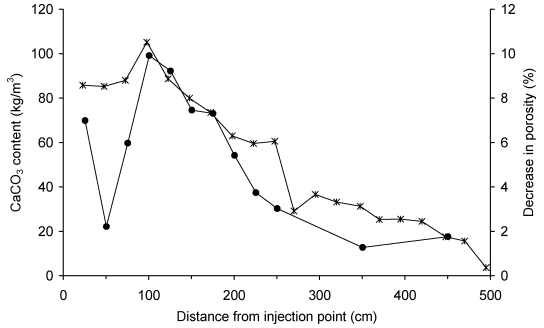

FIG. 6. Relative decrease in porosity (•) versus calcium carbonate content (∗) over the length of the column.

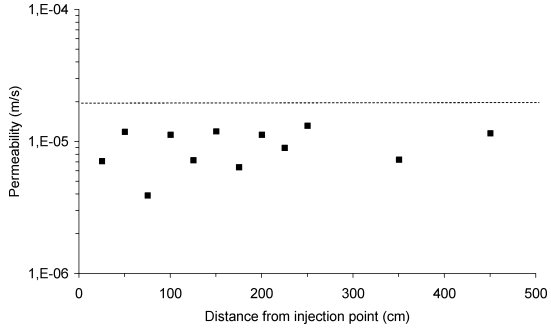

FIG. 8. Permeability over the core length determined after treatment during the triaxial test. Permeability was determined by constant head test. Initial material permeability before treatment is indicated by a dashed line (1.92×10^{-5} m/s).

Effect of Cementation on Strength, Stiffness, and Permeability

In order to determine the effect of calcium carbonate precipitation on the mechanical properties of the treated material, the permeability and strength results were correlated with the calcium carbonate content of each of the tested samples (Figure 7). Low calcium carbonate concentrations (below 60 mg/cm^3) did not significantly improve the strength of the samples. At higher calcium carbonate contents there was a significant improvement in strength relative to untreated sand. The highest strength in the column was 570 kPa, which was measured at the same location as the maximum amount of $CaCO_3$, at approximately 1 m from the injection point (Figure 5). An apparent minimum calcium carbonate content of 60 kg/m^3 was required for a measurable strength improvement in the material under the testing conditions (Figure 7).

Residual strength was also determined after failure and the residual strength values were comparable with unconsolidated sand, irrespective of the amount of calcium carbonate present (Figure 7). This indicated that once the bonds were broken, the strength improvement of the material was almost completely lost. The low residual strength attribute of the material highlights the importance of careful sample handling prior to evaluation.

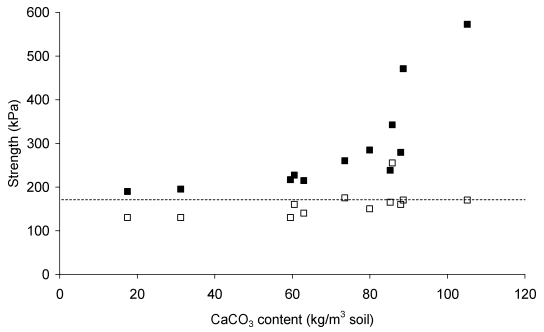

FIG. 7. Confined compressive strength (■) and residual strength of material after failure (□) versus calcium carbonate content. Confining pressure was 50 kPa. Under the same consolidation conditions, untreated sand of the same density gave a strength value of 167 kPa and a residual strength of 130 kPa.

After the column was dismantled, permeability was directly measured on each section prior to triaxial testing. Permeability was slightly reduced over the entire column after treatment but the effect was constant and did not appear to be related to calcium carbonate content (Figure 8).

DISCUSSION

Precipitated calcium carbonate was detected over the entire length of the column (Figure 5), indicating that bacteria and reactants were present at all locations. However, the profile of calcium carbonate was not homogeneous over the column length. A gradient was evident with relatively high amounts of calcium carbonate at the injection end (top) that decreased over the length (Figure 5).

Under the low-flow conditions that are suitable for field applications (i.e., in order to avoid soil fracturing), it is clear that the cementation process had already begun during the injection phase. This was evident by production of ammonium during injection (Figure 2) and lower concentrations of calcium in the pore fluid at the end of the column compared to the beginning (Figure 4). Because of the high conversion and slow flow rates, the top of the column closest to the injection port was exposed to significantly more reactants than the bottom and the profile of calcium carbonate reflected this (Figure 5). In order to produce a more homogeneous result, the balance between supply and conversion needs be shifted. For example faster flow rates will move the cementation reactants further into the column allowing less time for reaction along the path, and similarly lower conversion rates will leave more reactants in the fluid, also resulting in further infiltration distances.

The capability of bacteria to degrade urea appeared to decrease during the reaction time (Figures 3, 4). During the injection phase (0–24.5 h) the average ammonium production rate was 180 mM NH_4^+/h compared to 60 mM NH_4^+/h during the stationary phase (24–48 h). Possible reasons for this reduction in activity could be related to:

- *A reduction of the pore volume*, caused by precipitation of calcium carbonate in the pore spaces. This narrowing

of the pore volume resulted in an increase in flow velocity and a consequential decrease in residence/reaction time.
- *Increase in the amount of $CaCO_3$ solids precipitated*—resulting in a diffusion barrier around the microorganisms and limiting access to substrate or removal of by-products.
- *Decrease in urea concentration*—this is not a very likely possibility according to the Michaelis Menten kinetics ($K_m = 18.5$ mM; $V_{max} = 200$ mS/min/OD_{600})
- *Degeneration of bacterial viability in cementation conditions*—this could be related to conditions or possibly the formation of a diffusion barrier around the cell surface.

Effect of Cementation on Engineering Parameters

Lower concentrations of calcium carbonate (below 60 kg/m^3 or 3.5% w/w) had no significant effect on strength or stiffness properties relative to untreated sand (Figure 7). At calcium carbonate contents above this value, a clear improvement was evident that was proportional to the amount of precipitate present. After the initial strength measurement, the residual strength after failure was also determined and in all samples this value approximated the strength of untreated sand (Figure 7). This indicated that any strength improvement given by the treatment was lost after failure and thus the material was more characteristic of rock than soil. In future experiments it would be useful to extend the upper range of calcium carbonate precipitated, to give a broader understanding of the relationship between strength/stiffness and calcium carbonate content.

The apparent minimum calcium carbonate content for strength may also partly be attributable to other characteristics of the process (e.g., sand type) or perhaps sample handling after treatment. For example, the concentrations of the crystal precursors (calcium and carbonate ions) are known to play a role in speed and hence type of crystals that are formed. Given the changes in concentration over the course of the reaction, it is possible that qualitative factors (i.e. factors other than simple quantity) may also contribute to lower strength crystals. In addition, lower strengths are more difficult to preserve with sample handling (e.g., sawing of the column into sections, removal and handling during testing). It is possible that lower strengths are compromised during these procedures.

Two essential parameters for understanding fluid movement in soils are porosity and permeability. Porosity is the proportion of non-solid (void) volume relative to the total volume of the material. It is a measure of the fluid volume that a soil body can hold without increasing in total volume. Permeability indicates how easy fluids flow through porous media. It is a measure of how the voids in the soil are interconnected. Porosity and permeability are related to each other. If the porosity is high and the pores are well connected, permeability will be high. If the porosity is low or the pores are badly connected the permeability will be low.

As porosity is the proportion of non solid volume relative to the total volume of the material, the precipitation of calcium carbonate in the pore spaces will result in a reduction of the pore space volume. As expected, the decrease in porosity was proportional to the amount of calcium carbonate present at a given location (Figure 6). Even in the section with the highest calcium carbonate content, the porosity changed from 41% before treatment to 31% after treatment (i.e. 75% of original porosity was retained). This correlated with the expected loss of porosity given the volume of calcium carbonate, indicating that the loss of porosity due to space occupied by the bacteria was insignificant. At other locations with less calcium carbonate the porosity decrease was proportional and the treatment did not result in clogging at any location in the column.

After treatment, the permeability was slightly reduced over the entire column, irrespective of calcium carbonate content (Figure 8). This suggested either that the reduction of permeability was more affected by the nature of the treatment (the flowing of fluids though the material) than by the cementation process itself or that the method of evaluation was not sensitive enough to detect the small changes caused by the process. The average permeability over the column after treatment was 9×10^{-6} m/s compared to the original material permeability of 2×10^{-5} m/s.

CONCLUSIONS

The following conclusions can be drawn from this work:

- Bacteria were placed in the column over the entire 5 meter length to a reasonable degree of homogeneity at a low injection rate and with no associated clogging.
- The maximum attainable injection distance did not appear to be limited to five meters and it may be possible to extend this distance further.
- There was an effect of declining bacterial activity over the injection time.
- A significant strength increase was demonstrated after the treatment. The strength improvement was higher at the top compared to the bottom of the column, however this is largely related to the supply of cementation reactants versus the bacterial activity in the column.
- An apparent minimum calcium carbonate content was required for a measurable increase in strength (60 kg/m^3). This could be linked to over saturation concentrations of the crystal pre-cursors (calcium and carbonate) and/or possible handling limitations of low strength samples.
- The flow conditions in this experiment were limited to 1-D by the column walls. In a real application flow will have 3-D properties and fluid density and transport times will play a more significant role.
- A continuous cementation process is more realistic of field conditions and may be necessary in a 3-D fluid flow situation. Control under these conditions will be essential for real applications.

This work presents a new application for MCP as a ground improvement technique. Precipitation of calcium carbonate by microbial methods made a significant improvement in soil strength without a major reduction in permeability. For ground improvement requirements, it is desirable to achieve this result at low injection pressures, which are acquired with relatively low flow rates (<10 meters per day). This study was conducted under such conditions and successful soil strengthening was achieved. In addition a clear critical aspect of this process has been identified. Balancing the rate of urea hydrolysis in the column with the delivery of reactants via the flow rate is essential to precipitate calcium carbonate at locations where strengthening is desired. When these two parameters are out of balance, a non-homogeneous result will be attained with higher strengths near the injection point. This work demonstrated that microbial carbonate precipitation can be applied for large-scale soil improvement work and further development of the technique for this application area is warranted.

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