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Improving sand with microbial-induced carbonate precipitation

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Microbial-induced carbonate precipitation using urea hydrolysis is a relatively new improvement technique for granular soils. An important factor in achieving uniform calcite deposition (and hence consistent improvements in geomechanical properties) throughout the treated soil mass is the protocol adopted for injecting the reagents of ureolytic bacteria, urea and calcium. This paper reports a laboratory study investigating a technique to treat two loose medium quartz sands using different injection strategies. Staged injection including retention periods, and with a pressure head applied during injection of the bacterial cell solution, proved most effective. Sand specimens were treated using different concentrations of bacterial cell and urea–calcium chloride solutions and for a single injection cycle. Measured strength and stiffness values from unconfined compression tests ranged from 50 to 240 kPa and from 6 to 56 MPa, respectively. Permeability coefficient values were reduced by up to approximately one order of magnitude. Hence, a single injection cycle adhering to the proposed treatment method did not significantly affect the drainage capacity of the sand media. Greater improvements in stiffness and strength were achieved for lower bacterial cell and higher cementation solution concentrations, with a higher molarity of urea (non-equimolar solutions) proving even more effective. These findings were confirmed by scanning electron microscope observations.

1. Introduction

Traditional grouting methods for ground improvement employ particulate (cement/bentonite) or chemical grouts that can be rather expensive and environmentally unfriendly (Ivanov and Chu, 2008). Recently, novel grouting techniques (e.g. see DeJong *et al.*, 2006, 2010; Khatami and O'Kelly, 2013; Stabnikov *et al.*, 2011; Whiffin *et al.*, 2007) have been developed to treat unsaturated coarse-soils by stimulating natural processes. One of these methods, termed biogrouting, has shown some promise in soil cementation via microbially induced carbonate precipitation (MICP). This approach mimics natural processes by depositing calcite ($CaCO_3$) on the soil grains, thereby increasing the material's stiffness/strength and reducing its erodibility. The microbiological process relies on ureolytic (non-pathogenic) bacteria such as *Sporosarcina pasteurii* or *Bacillus pasteurii* to hydrolyse urea in the presence of calcium ions, resulting in the precipitation of calcite crystals.

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

$$2.\quad Ca^{2+} + CO_3^{2-} \xrightarrow{\text{yields}} CaCO_3(s)$$

The application of the MICP technique has shown promise in various fields, including improvement in the stiffness/strength of sandy soil (Rong *et al.*, 2012; van Paassen, 2009; Whiffin *et al.*, 2007); reductions in foundation settlement (DeJong *et al.*, 2010) and soil permeability (Dennis and Turner, 1998; Seki *et al.*, 1998); liquefaction mitigation (DeJong *et al.*, 2006; Montoya *et al.*, 2012); strengthening of concrete and remediation of cracks (Achal *et al.*, 2010; Bang *et al.*, 2001; Ramachandran *et al.*,

2001); microbially enhanced oil recovery (Nemati *et al.*, 2005); dust control (Meyer *et al.*, 2011); and wastewater treatment (Hammes *et al.*, 2003).

In ground treatment applications for sandy soil, the deposition of calcite over the grain surfaces and around the grain contacts (the latter produces more direct benefit to the geomechanical properties through interparticle cementation) creates a sandstone-like material. In principle, MICP treatment protocols can be tailored to produce a more targeted deposition of calcite around the grain contacts, with the porosity decreasing by less than 10% (Viganotti, 2014). Hence, significant improvements in the geomechanical properties can be achieved while maintaining permeability. Alternatively, for high concentrations of component chemicals, the calcite precipitation can occur as more concentrated in the pore voids as well as over the full surface area of the grains, producing significant reductions in the volume of the pore space and the sizes of the pore throats. In this case, more dramatic reductions in permeability can be achieved (Yasuhara *et al.*, 2011).

Table 1 summarises previous experimental laboratory studies on MICP soil treatment reported in the literature. The technique has also been successfully trialled at full scale on a gas pipeline installation project in the Netherlands (van Paassen, 2011). In advance of this project, laboratory testing was performed on MICP-treated soil (gravel) columns to estimate the strength improvement possible. A summary of these test results is included in Table 1. From the limited available data, key factors governing the degree of strength improvement achievable by the MICP technique include environmental circumstances (i.e. temperature, pH, oxygen level, degree of saturation, chemical composition of resident pore fluids), the soil grading and surface characteristics of the constituent solids, the concentration of the urea–calcium chloride ($CaCl_2$) cementation solution and the number of treatment cycles/injections. For instance, a rise of 10°C over the temperature range 5–35°C causes the urease activity to increase by a factor of 2·4 (van Paassen, 2009). No urease activity was observed for a soil temperature below 5°C. Microbiologically induced carbonate mineral precipitation occurs for a pH range of 8·3–9·0, for which urease activity remains high (Stocks-Fischer *et al.*, 1999). For a similar calcite content, higher soil strength can be achieved when the MICP treatment is performed at a low degree of saturation (Cheng *et al.*, 2013). The degree of permeation of the bacterial cell solution over the soil treatment depth is dependent on the minimum pore size: more specifically, the relative dimensions of the bacteria and pore voids/throats.

Several combinations of urea and $CaCl_2$ solution concentrations have been investigated for the production of calcite precipitate. For instance, Okwadha and Li (2010) investigated different combinations of 0·33 and 0·67 M urea and 0·0025, 0·025 and 0·25 M calcium (Ca^{2+}) solution concentrations. They reported that for the same bacterial cell concentration, greater amounts of calcite were deposited for higher urea and Ca^{2+} concentrations, with the optimum combination from those investigated reported as 0·67 M urea and 0·25 M Ca^{2+}. In order to determine the maximum concentration of urea and Ca^{2+} that can be effective for MICP performed in a single treatment application, Whiffin (2004) investigated several equimolar urea–Ca^{2+} concentrations in the range 0·5–3·0 M. Her results indicated that, up to 1·5 M, the amount of precipitated calcite increased in direct proportion to the urea–Ca^{2+} solution concentration. Above 1·5 M, the amount of precipitated calcite was found to reduce. In the study by Al-Thawadi (2008), equimolar urea–Ca^{2+} solution concentrations in the range 0·125–2·0 M were continuously flushed up through sand columns. The results showed that the greatest amount of precipitated calcite and the highest mobilised strength occurred for the 0·5 M cementation solution. Al Qabany *et al.* (2011) also investigated different equimolar urea–$CaCl_2$ concentrations in the range 0·1–1·1 M, in order to study the effect of chemical concentration on the calcite deposition patterns. The observed patterns indicated that the use of lower chemical concentrations applied over multiple injections produced more uniform cementation. Yasuhara *et al.* (2011) reported that the

Reference	Soil type	Cementation concentration: M	Number of injection cycles	UCS: kPa	Permeability reduction: %
Whiffin *et al.* (2007)	Itterbeck sand, $D_{50} = 0{\cdot}165$ mm	1·1	1	0–500	22–75
Yasuhara *et al.* (2011)	Sand	0·5, 1·0	4–8 for UCS 1–4 for permeability	373–1500	60–70
van Paassen (2011)	Gravel	NR	1–4	15–40	NR
Palmén (2012)	Quartz sand, $D_{50} = 0{\cdot}85$ mm	NR	1 5	200 2600	NR
Soon *et al.* (2013)	Sand, $D_{50} = 0{\cdot}52$ mm	0·25	8	50	90

NR, not reported; UCS, unconfined compressive strength.

Table 1. Some MICP studies reported in the literature

cementation solution concentration is a key factor in modifying the strength and permeability of MICP-treated sand. They investigated 0·5 and 1·0 M (equimolar) urea–$CaCl_2$ solutions, with the 1·0 M solution producing a greater increase in strength and reduction in the permeability coefficient for the test sand. Although many research papers have been published on the MICP process, the current authors' review of the literature indicated that none of these studies specifically considered the effect of the bacterial cell solution concentration on the strength and hydraulic properties of the treated soil.

Upscaling biomediated soil improvement from laboratory element tests to field scale is generally more complex than for other grouting methods, and an integration of geotechnical, ecology, microbiology and geochemistry knowledge is required (Ivanov and Chu, 2008). Practical challenges include creating favourable/optimum environmental and hydrological conditions in situ for the MICP process, ensuring calcite deposition occurs over a wide volume of ground and not just adjacent to reagent injection points, maintaining the necessary retention periods for reagents within the ground mass under treatment and also the recovery/recycling of unused reagents. Potential limitations for the practical application of MICP for ground improvement include the production of undesirable by-products, especially ammonia (see Equation 1), and the reversibility of the process (Ivanov and Chu, 2008). Also, improvements in the geomechanical properties and (or) permeability coefficient reductions achievable by MICP generally occur at slower rates compared with other grouting methods.

The aims of this experimental laboratory study are to investigate

- a suitable method of injecting the reagents to achieve uniform calcite precipitation throughout columns of loose sand
- the effect of injecting different concentrations of bacterial cell and cementation solutions on the stiffness, strength and hydraulic properties of the treated sands.

For the latter, the authors investigated the effects of equimolar and non-equimolar urea–$CaCl_2$ cementation solutions on the properties of the treated sand at an ambient laboratory temperature of 20 ± 2°C. The effects were assessed in terms of measured unconfined compressive strength (UCS), stiffness and permeability coefficient values, and also from scanning electron microscope (SEM) observations. These results are discussed and linked to the current understanding in the literature.

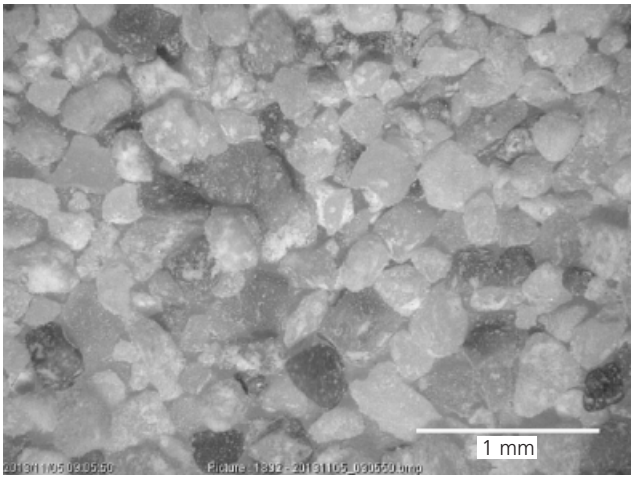
2. Materials

2.1 Soil type

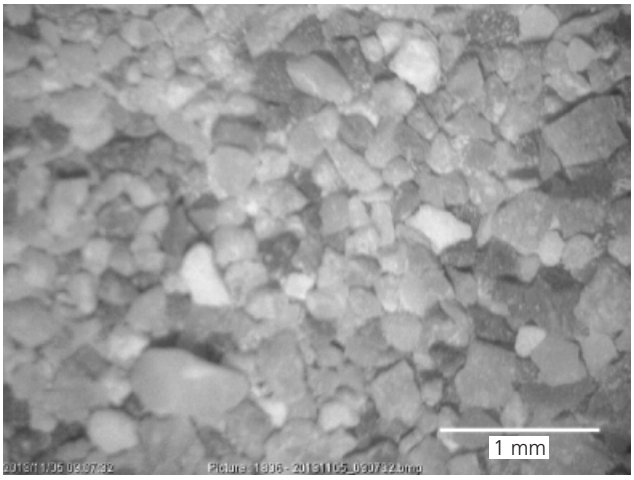
Compatibility between the soil grain characteristics and bacteria size is an important factor for MICP treatment. The soil pores should be of sufficient size to allow the transportation of bacteria 0·5–3·0 μm in length (Mitchell and Santamarina, 2005), with 50–400 μm reported as the most favourable soil particle size range for bacterial activity in the pores (Rebata-Landa, 2007). In the present study, two clean medium sands were investigated (test materials A and B in Figures 1 and 2 and Table 2); both comprised angular to sub-angular quartz grains. Sand B was slightly coarser than sand A, although the grain-size distributions for up to 40% passing were approximately similar (see Figure 2).

2.2 Micro-organism and culture medium

For the MICP process, *Sporosarcina pasteurii* (PTCC 1645) was used as the urease-positive bacterium. Following the work of Whiffin *et al.* (2007), an ammonium yeast-extract medium comprising 10 g of ammonium chloride and 20 g of yeast extract per litre of deionised water was selected for bacterial growth. For optimum urease activity, the pH value of the medium was adjusted to pH 8·5 (Stocks-Fischer *et al.*, 1999) by slowly adding 4 M sodium hydroxide prior to inoculation. The culture medium was sterilised by autoclaving at 121°C for a 15 min period.



(a)



(b)

Figure 1. Particle shape characteristics: (a) sand A; (b) sand B

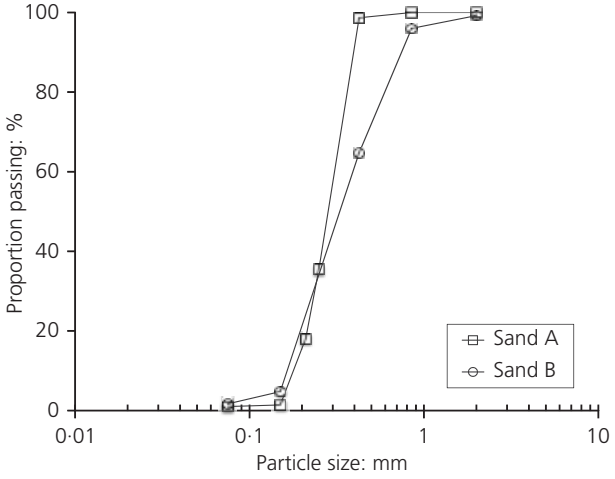


Figure 2. Grading curves for test sands

Soil property	Sand material	
	A	B
G_s	2·66	2·65
D_{10}: mm	0·18	0·16
D_{50}: mm	0·28	0·34
D_{60}: mm	0·31	0·41
D_{90}: mm	0·39	0·72
Coefficient of uniformity	1·72	2·56
Coefficient of curvature	1·03	0·88
Maximum void ratio	1·01	0·98
Minimum void ratio	0·63	0·60
Density index: %	33	23

Table 2. Some physical properties of the test sands

2.3 Preparation of the bacterial cell solution

A seed culture was produced by transferring a small amount of the *S. pasteurii* lyophilised culture into 100 ml of the culture medium and allowing culture growth to occur at 30°C over a 24 h period. This mixture was then cooled and stored at 4°C prior to its use. The main culture medium was inoculated with the seed culture (10% v/v) and incubated aerobically under agitation at 30°C over a 48 h period, by the end of which the cells had reached maximum population (i.e. a stationary phase). After the required bacterial growth had occurred, the cells in the culture medium were harvested at a temperature of 4°C by centrifugation for 10 min at 5000***g***. The harvested cells were then washed twice in sodium phosphate buffer 0·1 M (pH 7) to remove metabolic waste and any metabolism produced during the bacterial growth phase. 'Metabolism' refers to all chemical reactions that occurred within the bacterial cells. Metabolic wastes are substances that cannot be used by the bacterial cells (i.e. surplus or have lethal effect) and must be excreted. Bacterial cells at the desired concentration were resuspended in nutrient broth (NB)–urea solution comprising 3 g of NB, 20 g of urea, 10 g of ammonium chloride and 2·12 g of sodium bicarbonate per litre of deionised water. The pH value of the bacterial cell solution was adjusted to pH 6 using 4 M hydrochloric acid prior to autoclaving. To investigate the effect of the bacterial cell concentration on the properties of the treated sands, solutions were prepared in the laboratory at OD_{600} values of 0·8, 1·5 and 3·0, where OD_{600} is the optical density of bacteria measured by a spectrophotometer at a wavelength of 600 nm. Using Equation 3, the cell concentrations (Y, in ml^{-1}) corresponding to these OD_{600} values were estimated at approximately 6×10^7, 1×10^8 and 4×10^8 cells/ml, respectively (Ramachandran *et al.*, 2001).

$$Y = 8.59 \times 10^7 \times OD_{600}{}^{1.3627} \quad (3)$$

Stocks-Fischer *et al.* (1999) reported that bacterial cell solutions that have OD_{600} values in the range 0·8–1·2 produced higher urease activity. The lower-bound value of this range, along with approximately two and four times this value (i.e. $OD_{600} = 1{\cdot}5$ and 3·0, respectively), were investigated in the present study. Approximate estimates of the total microbial population present per gram of in situ soil near the ground surface are of the order of 10^6–10^9 (Mitchell and Santamarina, 2005; Pichan and O'Kelly, 2014) and 10^8–10^{10} (Ehrlich, 1996). The microbial population generally includes *Bacillus*, *Arthrobacter* and *Pseudomonas* bacteria, with their concentrations decreasing with depth below ground surface level. The preparation in situ or under laboratory conditions of solutions having $OD_{600} > 3$ is very difficult, if not impossible. Hence, bacterial cell solutions having such high OD_{600} values were not investigated in the present study.

2.4 Preparation of cementation solutions

Six different combinations of urea and $CaCl_2$ concentrations (Table 3) were investigated as cementation solutions for sand A. The non-equimolar urea–$CaCl_2$ cementation combinations 3 and 6 listed in Table 3 were prepared by dissolving the same masses of urea and $CaCl_2$ in distilled water. Considering that the molecular weights of anhydrous $CaCl_2$ and urea are 111 and 60 g/mol, respectively (i.e. molecular weight ratio of 1·85), for these cementation solutions the urea molarity was 1·85 times that of the $CaCl_2$. Hence, to produce cementation combination 6 (1·85 M urea–1 M $CaCl_2$), 111 g of urea and 111 g of $CaCl_2$ was dissolved per litre of distilled water. The same approach was used to determine the required masses of urea and $CaCl_2$ in producing cementation combinations 4 and 5 reported in Table 3.

3. Experimental methods

3.1 Specimen preparation

Test specimens of sands A and B were prepared for MICP treatment followed by strength testing using a method adopted from Ismail (2000). The test specimens were formed using PVC split moulds (50 mm inner diameter by 170 mm long), with the

Cementation combination	Urea: M	$CaCl_2$: M	Stiffness: MPa	UCS: kPa	Permeability coefficient: m/s	Reduction of permeability: %
1	0·10	0·10	6·5	50	$2·1 \times 10^{-4}$	50
2	0·25	0·25	5·6	75	$1·8 \times 10^{-4}$	57
3	0·46	0·25	30	110	$1·4 \times 10^{-4}$	67
4	0·25	0·50	9·7	80	$1·7 \times 10^{-4}$	60
5	1·00	0·50	49	180	$8·1 \times 10^{-5}$	81
6	1·85	1·00	56	240	$2·6 \times 10^{-5}$	94
Control (untreated)	0	0	–	–	$4·2 \times 10^{-4}$	–

Table 3. Some properties of MICP-treated sand A using a bacterial concentration of 1×10^8 cells/ml

two halves of these moulds held together during the preparation and curing stages using hose clamps. A rubber membrane was fitted in contact with the inner wall surface of each assembled mould. A layer of filter paper was placed at the bottom of each mould. Then, 385 g of dry sand was air pluviated into each specimen mould in three layers of equal thickness. The sand was slowly poured from a container positioned 100 mm vertically above the top of each mould. After deposition, each sand layer was individually compacted by gently tapping around the outer wall of the mould. After performing numerous trials, a consistent methodology was established to produce 140 mm-long sand specimens. This specimen preparation method produced a loose sand state (density index values of 33% and 23% for sands A and B, respectively) having a dry unit weight of 14 kN/m^3.

A 30 mm-deep gravel filter was placed above each sand specimen prepared as described above. For the injection process, each mould was fitted with end caps, which were sealed using tape, and positioned vertically. For the strength tests, the clamps were released, and the two halves of each split mould separated to free the treated specimens, which remained laterally enclosed by their rubber membranes. For the permeability tests, the same specimen preparation method was used except that intact PVC tubes of the same dimensions were employed instead of split moulds. The permeability tests were performed on the treated specimens while still contained in these tubes. To check reproducibility, test specimens were prepared in triplicate for each experimental condition investigated in this study.

The specimens' length-to-diameter (aspect) ratio of 2·8:1 was greater than the standard 2:1 ratio used in triaxial compression testing (BS 1377: BSI, 1990). In maintaining a test specimen diameter of 50 mm, a decision was made to adopt the larger aspect ratio of 2·8:1 for the purpose of investigating the degree of success achievable for different injection strategies in uniformly transporting the reagents over greater distances. In triaxial compression testing, the standard aspect ratio of 2:1 provides a zone of near-uniform strain/stress within the mid-third of the specimen length (i.e. ~33 mm gauge) (Jardine *et al.*, 1984; O'Kelly and Naughton, 2008), within which shear failure generally occurs. The larger aspect ratio of 2·8:1 adopted in the present investigation provided a longer central gauge length (zone of uniform strain/stress) of ~73 mm. In other words, the larger aspect ratio would not have adversely affected (and may have enhanced) the measurement of the material's shear resistance.

3.2 Injection strategies

Successful MICP treatment in sand requires the injection of the bacterial cell and cementation solutions followed by their permeation through the entire sand specimen/bed. The most important factor in achieving an even deposition of precipitated calcite throughout the sand mass is the uniform distribution/fixation of the bacterial cells. In the literature, different MICP injection strategies have been investigated for sand, including the following scenarios.

(*a*) Mixing the bacterial cell and cementation solutions together before injection into the sand. However, the reagents flocculate immediately, and while this approach may be considered for coarser soils (Le Métayer-Levrel *et al.*, 1999), rapid clogging of the pore voids generally occurs for fine/medium sand (Whiffin, 2004), rendering the treatment ineffective.
(*b*) Two-phase injection, in which the bacterial cell solution is injected first, followed by the cementation solution (Whiffin *et al.*, 2007).
(*c*) Staged injection (Tobler *et al.*, 2012), with or without retention periods between the injection phases. Using this approach, excessive crystal accumulation close to the injection point can be prevented from occurring and a more uniform distribution of calcite crystal formation can be achieved over a greater distance in the sand specimen/bed. More effective MICP treatment is achieved using retention periods between injection phases that allow more bacteria to be fixed into the pore void space. Retention periods also facilitate greater numbers of reactions to occur between the bacterial cell and cementation solutions (Al Qabany *et al.*, 2011; Rong *et al.*, 2012).

A fourth scenario that was examined in the present study but,

to the authors' knowledge, investigations employing the following approach have not been reported in the literature.

(*d*) Single-phase injection (i.e. reagents injected simultaneously into the sand).

In the present study, preliminary tests were performed to investigate all four injection strategies for the 140 mm-long sand specimens. The relative success achieved for the different approaches was similar to that reported by previous researchers, with staged injection (scenario (*c*)) proving the most effective. For scenario (*a*), the reagents flocculated immediately on mixing, and any attempt to permeate the mixture through the sand specimens rapidly caused clogging of the pore voids next to the injection points. For single-phase injection (scenario (*d*)), the bacterial cell and cementation solutions were simultaneously injected via separate delivery tubes into the tops of the sand specimens. Again, rapid clogging of the pore voids next to the injection points prevented any significant permeation of the mixture, which was evidenced by only the portions of the specimens nearest the injection points gaining toughness. For two-phase injection (scenario (*b*)), rapid flocculation and clogging of the pore voids were not observed but the toughness of the treated specimens did not significantly increase away from the injection points. A possible explanation is that during subsequent injection of the cementation solution, its downward permeation may have leached/flushed bacterial cells from the specimens via their outflows. Scenario (*c*) (staged injection) was investigated further as the most promising approach to achieving greater fixation of the injected bacterial cell solution in the pore voids.

The next series of tests considered both upward and downward injections through the sand specimens as well as investigating different injection rates and retention periods. It was observed that uniform strength improvement could not be achieved over the full specimen length using staged injection alone. Significant cohesion was gained over the portions of the specimens between the injection points and the specimen mid-heights (i.e. a linear distance of ~70 mm). However, no significant strength improvement was achieved for greater distances, with the unconfined sand in the distant halves of the treated specimens easily crumbling by hand. Similar behaviour was observed by Whiffin *et al.* (2007) and Rong *et al.* (2012), with the former reporting mobilised UCS values in the range 0–500 kPa over the length of sand columns treated by MICP in this manner. This behaviour occurs on account of the accumulation of bacterial cells near the injection points, which hampers their transportation over greater distances along the specimen length.

The protocol adopted for the main testing programme of the present investigation was staged injection with retention periods. Given the limited distances through which the bacterial cells had been transported during the preliminary tests, it was decided to inject the bacterial cell solution under relatively high pressure via the tops of the sand specimens. After a 12 h retention period, the urea–$CaCl_2$ cementation solution was injected, but without applying a pressure head (i.e. slow flow rate) in order to mitigate against possible leaching of adsorbed bacterial cells.

3.3 One-cycle staged injection with retention periods

For the main testing programme, the MICP treatment was applied to loose specimens of sands A and B using staged injection (downward flow), including retention periods, and with a pressure head applied during injection of the bacterial cell solution, as follows.

- The sand specimens were de-aired by permeating water of volume ~$2V_v$ down through the specimens, where V_v is the volume of the specimen pore voids, which was 130·2 ml for the 140 mm-long specimens of sands A and B prepared in the manner described earlier.
- A bacterial cell solution of volume $1{\cdot}5V_v$ was injected into each specimen under a pressure head (initially 1·2 m but falling slightly over the course of the injection (Figure 3(a)), with the outflow rate of ~10 ml/min from the specimen base controlled by a peristaltic pump. The purpose of the pressure head was to promote transportation of more bacterial cells and over greater distances from the injection points; in other words to achieve a more uniform distribution over the specimen length.
- After this volume of bacterial cell solution had been introduced into each specimen (now fully saturated with bacterial solution), the flow was stopped for a 12 h period, allowing the bacterial cells to be adsorbed by the sand grains. At the end of this retention period, the peristaltic pump was disconnected from the hydraulic line, and the bacterial cell solution was allowed to drain under gravity from the specimen base.
- Reservoirs containing the urea–$CaCl_2$ solutions were placed directly above the specimens (i.e. applying a negligible pressure head). The cementation solutions, which had slightly lower densities compared with the bacterial cell solutions, were allowed to permeate down through the specimens, with the outflow rate of 3·0 ml/h from the base of each specimen controlled by a peristaltic pump (Figure 3(b)).
- After the urea–$CaCl_2$ cementation solution of volume V_v had entered each specimen, the flow was stopped for a 24 h period to allow the bacteria to react with the cementation solution. At the end of this 24 h retention period, the peristaltic pump was disconnected from the hydraulic line, and each sand specimen was allowed to drain under gravity from its base.

The effect of MICP treatment using different concentrations of bacterial cell and urea–$CaCl_2$ cementation solutions was assessed in terms of measured strength, stiffness and permeability coefficient values. SEM images were also used to evaluate the success of the staged MICP injection protocol adopted for the main testing programme as well as the formation and distribution of calcite crystals in the two treated sands.

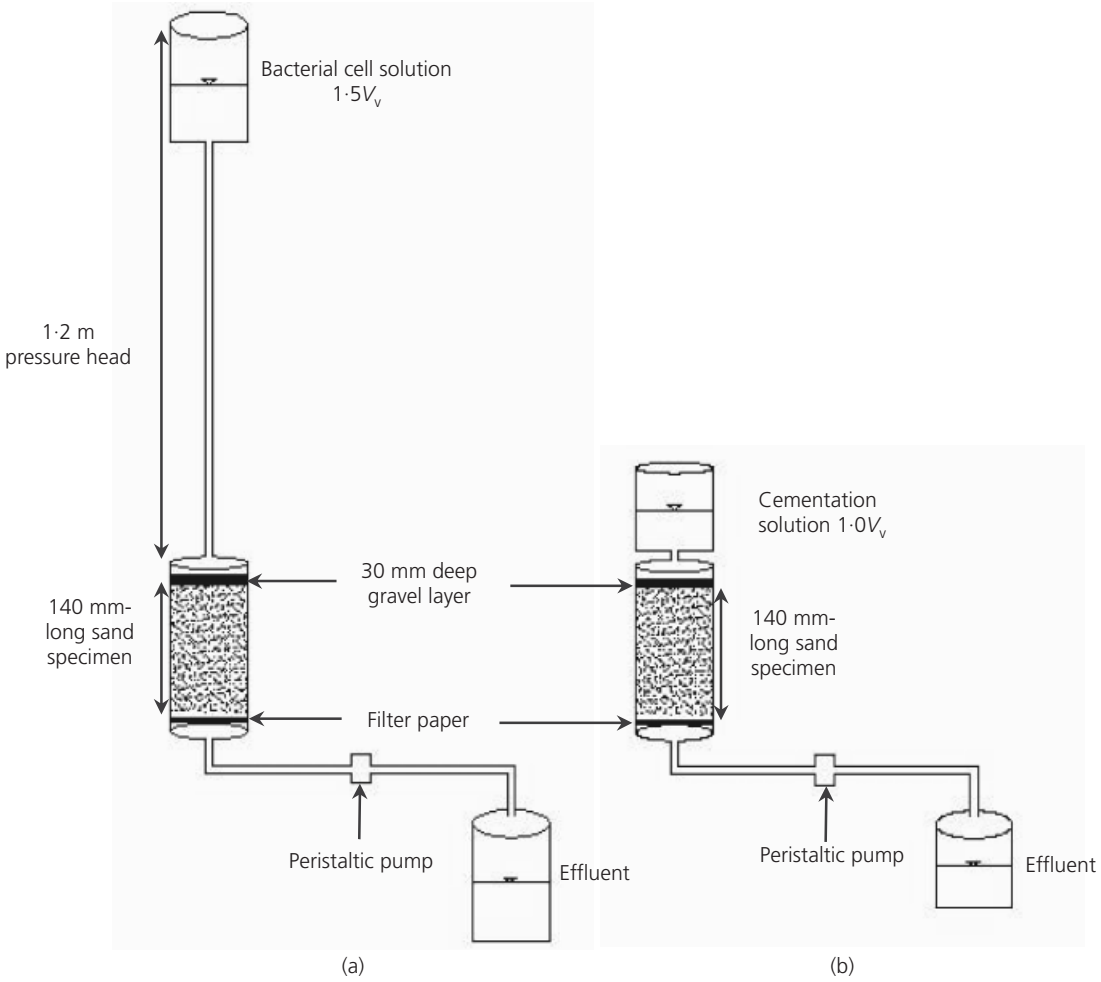


Figure 3. The MICP staged injection protocol adopted: (a) injection of the bacterial cell solution; (b) injection of the cementation solution

3.4 Strength and permeability tests

After completing the MICP treatment, the drained specimens were allowed to cure over a 21 d period at an ambient laboratory temperature of 20 ± 2°C. The specimens for strength testing were then released from their split moulds, and their ends trimmed as necessary to produce right cylinders, thereby ensuring uniform contact with the specimen loading platens. The stress–strain–strength characteristics of these specimens (50 mm in diameter by ~140 mm long) were determined in unconfined compression at an axial strain rate of 0·36%/min using an Instron apparatus. The permeability coefficient values of the cured specimens (still contained in their moulds) were determined using the falling-head method.

3.5 SEM tests

SEM images were obtained from horizontal sections through the top, middle and bottom portions of specimens treated with 1×10^8 cells/ml and 1·85 M urea–1·0 M $CaCl_2$ solutions in order to investigate the formation and spatial distribution of calcite crystals over the specimen length. These specimens were sectioned using a sharp knife. SEM images were also obtained from specimens treated with the same bacterial cell solution concentration but using 0·25 M urea–0·25 M $CaCl_2$ cementation solution, in order to investigate the effect of different cementation solution concentrations on calcite formation. Finally, SEM images were obtained from specimens treated with the same 1·0 M urea–0·5 M $CaCl_2$ cementation solution but using 6×10^7 or 4×10^8 cells/ml bacterial solution concentration, in order to investigate the effect of different bacterial cell concentrations on calcite formation.

4. Experimental results and analyses

4.1 Strength and stiffness in unconfined compression

Measures of the absolute improvements in strength and stiffness under confined compression achieved by the MICP treatment

could not be determined since the untreated sand specimens (controls) mobilised no shear resistance. Hence, the effects of different bacterial cell and urea–$CaCl_2$ solution concentrations were assessed by comparing the treated specimens' UCS and stiffness values. The effect of the bacterial cell solution concentration was considered first (Table 4 and Figure 4) followed by the cementation solution concentration (Table 3 and Figure 5). The UCS values were determined as the peak deviatoric stresses mobilised, which generally occurred for 0·5–2% axial strain. The general mode of failure for the treated specimens was nearest to longitudinal splitting.

The stiffness values were determined as the gradients of the steepest, approximately linear portions of the deviatoric stress–strain plots. Each UCS and stiffness value reported in Tables 3 and 4 is the mean of the respective values measured for the three specimens tested for each experimental condition, with good reproducibility achieved; that is, similar deviatoric stress–strain plots were obtained for each set of three identically prepared and treated specimens.

All of the data in Table 4 and Figure 4 indicate that for the same cementation solution concentration but different bacterial concentrations of 6×10^7, 1×10^8 and 4×10^8 cells/ml investigated, mobilised stiffness and UCS values decreased with increasing bacterial cell concentration. The finer of the two test sands (i.e. sand A, see Table 2 and Figure 1) consistently mobilised higher stiffness and UCS values, apart from the UCS of specimens treated with the 4×10^8 cells/ml solution. Similar findings were reported in previous studies by Ismail *et al.* (2002). However, it is recognised that the two test sands had slightly different grading, particle shape characteristics and densification levels (see Table 2 and Figures 1 and 2), and hence presumably different friction angles, which would also influence the shear resistances mobilised by the two sands.

Table 3 indicates that for the same bacterial concentration of 1×10^8 cells/ml, the treated specimens generally mobilised greater stiffness and UCS values for higher urea–$CaCl_2$ solution concentrations. Similar findings have been reported by Yasuhara *et al.* (2011). In the present study, the lowest and highest UCS

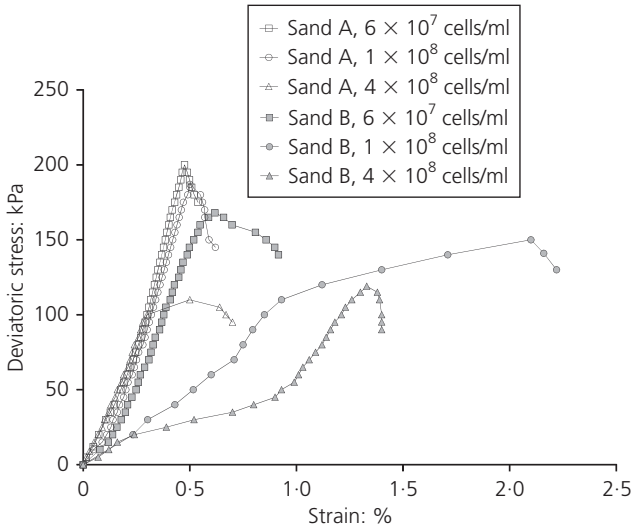


Figure 4. Deviatoric stress–strain responses of MICP-treated sand specimens using the 1·0 M urea–0·5 M $CaCl_2$ cementation solution

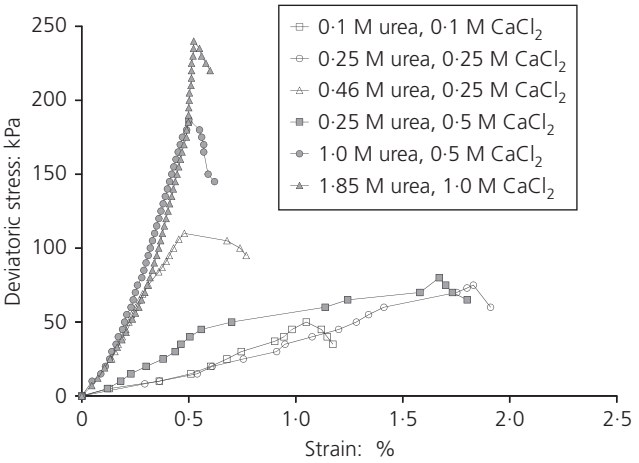


Figure 5. Deviatoric stress–strain responses of MICP-treated sand A using the 1×10^8 cells/ml bacterial solution

Bacterial concentration: cells/ml	Stiffness: MPa		UCS: kPa		Permeability coefficient: m/s	
	Sand A	Sand B	Sand A	Sand B	Sand A	Sand B
6×10^7	59	37	200	168	$5{\cdot}9 \times 10^{-5}$	$5{\cdot}6 \times 10^{-4}$
1×10^8	51	23	180	150	$8{\cdot}1 \times 10^{-5}$	$6{\cdot}3 \times 10^{-4}$
4×10^8	38	20	110	119	$1{\cdot}2 \times 10^{-4}$	$7{\cdot}2 \times 10^{-4}$
0 (control)	—	—	—	—	$4{\cdot}2 \times 10^{-4}$	$3{\cdot}1 \times 10^{-3}$

Table 4. Some properties of MICP-treated sands using 1·0 M urea–0·5 M $CaCl_2$ cementation solution

values of 50 and 240 kPa were mobilised for the lowest and highest solution concentrations investigated of 0·1 M urea–0·1 M $CaCl_2$ and 1·85 M urea–1·0 M $CaCl_2$, respectively. This range of UCS values is approximately in line with that reported by Whiffin *et al.* (2007) and Palmén (2012) for MICP studies on sands using a single injection cycle (refer to Table 1). Although generally stiffer, higher-solution concentrations typically produced more brittle responses (Figure 5). More specifically, increasing urea molarity correlated with increasing stiffness and UCS values: for example, compare cementation combination 2 with 3, and 4 with 5, in Table 3. For sand A, the axial strain at failure (ε_f) reduced from ~1·8% strain (0·25 M urea–0·25 M $CaCl_2$) to 0·5% strain (1·85 M urea–1·0 M $CaCl_2$). From Figure 4, treated sand B had consistently lower stiffness and a larger ε_f in the range 0·6–2·2%, compared with $\varepsilon_f \approx 0{\cdot}5\%$ for sand A. It is not clear why the sand B specimen treated with a bacterial solution of 1×10^8 cells/ml and 1·0 M urea–0·5 M $CaCl_2$ cementation solution was more ductile under compression, with $\varepsilon_f \approx 2{\cdot}2\%$ (Figure 4). The limited test data suggest that increasing the $CaCl_2$ molarity, with the urea molarity remaining unchanged, does not appear to produce significant improvements in UCS: for example, compare cementation combination 2 with 4 in Table 3.

4.2 Coefficient of permeability

The measured permeability coefficient (k) values for the MICP-treated and -untreated (control) specimens are reported in Tables 3 and 4, with each value determined as the mean of the values measured for the three specimens tested for each experimental condition. Approximately similar k value reductions have been reported in previous MICP studies on sand: for example, see data from Soon *et al.* (2013), Whiffin *et al.* (2007) and Yasuhara *et al.* (2011) summarised in Table 1. Compared with the controls, the k values of specimens treated using the same 1·0 M urea–0·5 M $CaCl_2$ solution, but different bacterial concentrations in the range 6×10^7 to 40×10^8 cells/ml, were between approximately 71% and 86% lower considering both test sands (Table 4). A 90% reduction corresponds to one order of magnitude. The maximum and minimum permeability reductions were achieved for the lowest and highest bacterial cell concentrations investigated respectively. Compared with the controls, k value reductions of between 50% and 94% were achieved for sand A treated using the same bacterial solution of 1×10^8 cells/ml but different urea and $CaCl_2$ solution concentrations in the ranges 0·1–1·85 and 0·1–1·0 M, respectively (Table 3). Greater reductions in the permeability coefficient were generally achieved for higher cementation solution concentrations: for example, k value reductions were approximately one order of magnitude greater for 1·85 M urea–1·0 M $CaCl_2$ compared with 0·1 M urea–0·1 M $CaCl_2$. These findings are consistent with Yasuhara *et al.* (2011). More specifically, from the data in Table 3, it would appear that, for non-equimolar cementation solutions, higher urea molarity produced greater reductions in permeability.

4.3 SEM analyses

Figure 6 shows SEM images obtained from the top, middle and bottom portions of a test specimen of sand A that had been treated with bacterial cell and cementation solutions of 1×10^8 cells/ml and 1·85 M urea–1·0 M $CaCl_2$ (the latter being the highest concentration investigated). Calcite was observed to have deposited at all three locations, indicating that the reagents had distributed/permeated over the full specimen length of 140 mm. Hence, from this point of view, the staged injection (with retention periods) protocol adopted for the main testing programme in the present study was judged a success. However, the distribution of calcite deposition was not entirely uniform over the specimen length. Compared with the specimen mid-height, significantly greater amounts of precipitated calcite had formed within the top and bottom portions, appearing to cause significant clogging of the pore voids/throats at these locations (Figures 6(a) and 6(c)). At the specimen mid-height, individual calcite crystals appeared smaller but approximately similar in size (Figure 6(b)). Similar calcite deposition patterns have been observed by van Paassen (2009) over the length of MICP-treated sand columns. Possible reasons for the significantly lower levels of calcite deposition occurring near the specimen mid-height may include a lack of oxygen and (or) reagents present at this location. Oxygen may have an influence on the precipitation rate and characteristics of the calcite crystals formed in the pore voids. Palmén (2012) reported that exposure of MICP-treated sand to air (oxygen) produced further strength improvement. On the other hand, Whiffin (2004) and Mortensen *et al.* (2011) consider that the urea hydrolysis rate is not adversely affected by lack of oxygen. The investigation of this issue was outside the scope of the present study. However, it was not likely that a lack of reagents was responsible for the significantly lower levels of calcite deposition occurring near the specimen mid-height. This is justified, since, compared with the mid-height, significantly greater amounts of calcite had been deposited in the bottom portion of the specimen (i.e. furthest from the injection point). Hence, it can be postulated that a lack of oxygen may have been responsible for the formation of fewer and smaller calcite crystals near the specimen mid-height; further research is necessary in this regard.

Figure 7 shows SEM images obtained from the top portions of test specimens of sand A (i.e. nearest the injection points) that had been treated with different bacterial cell and cementation solution concentrations. For the same 1·0 M urea–0·5 M $CaCl_2$ solution (Figures 7(a) and 7(b)), the coverage depth of calcite deposited on the sand grains appeared thinner but more uniform for the lower bacterial concentration of 6×10^7 cells/ml (Figure 7(b)). For the higher bacterial concentration of 4×10^8 cells/ml, concentrated areas of calcite precipitate were observed to have formed on the grain suraces and in the pore voids (Figure 7(a)). These concentrated areas of calcite precipitate only formed at the top portions of these specimens, with significantly less precipitation occurring on sand grains at greater distances from the injection point. This was not the case for the 6×10^7 cells/ml bacterial solution. For lower concentrations, the bacteria are less likely to accumulate near injection points (Johnson and Logan, 1996) and more likely to permeate through the specimen, thereby

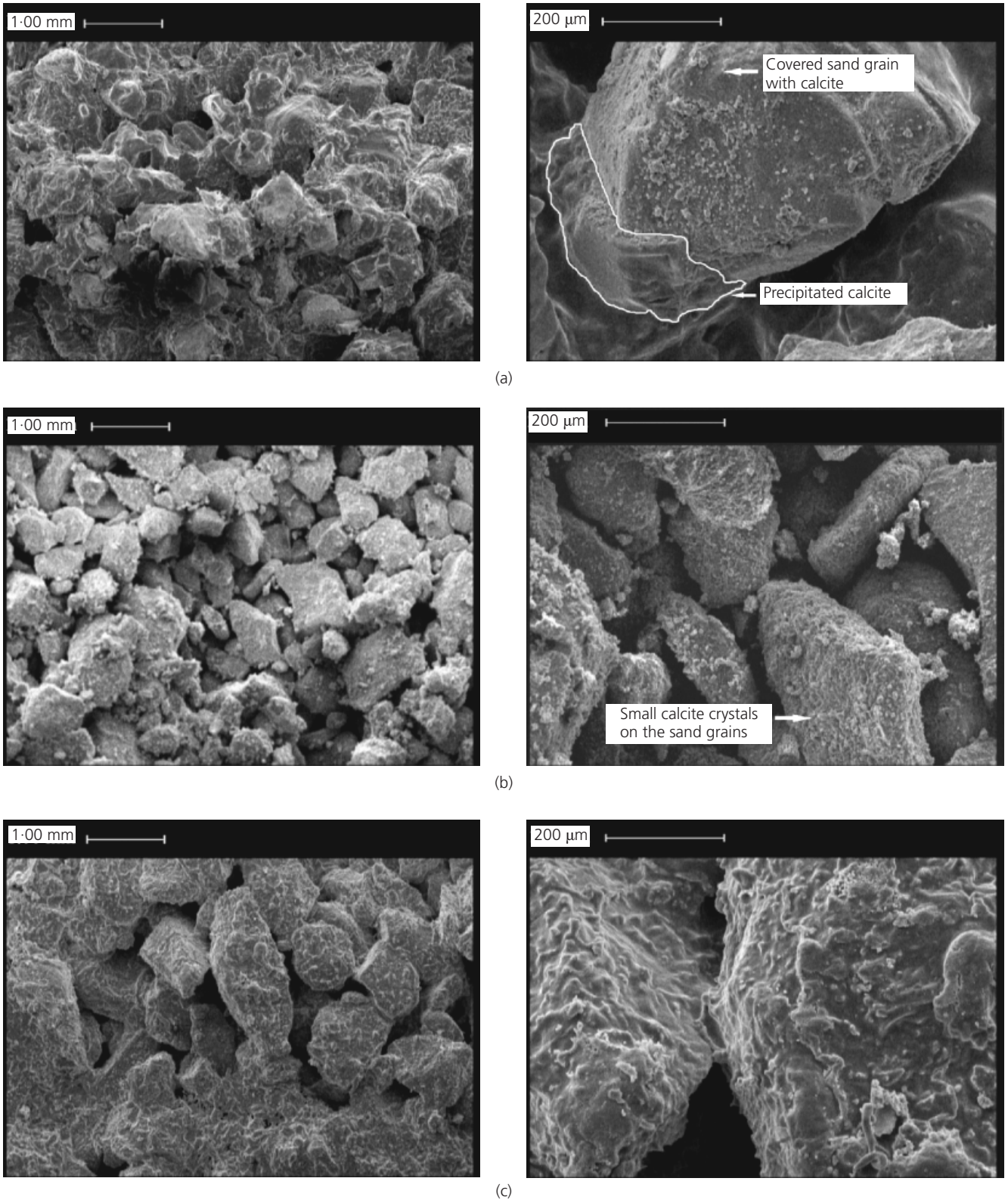


Figure 6. SEM images of sand A treated with 1×10^8 cells/ml bacterial solution and 1·85 M urea–1·0 M $CaCl_2$ cementation solution: (a) top (nearest the injection point); (b) mid-height; (c) bottom

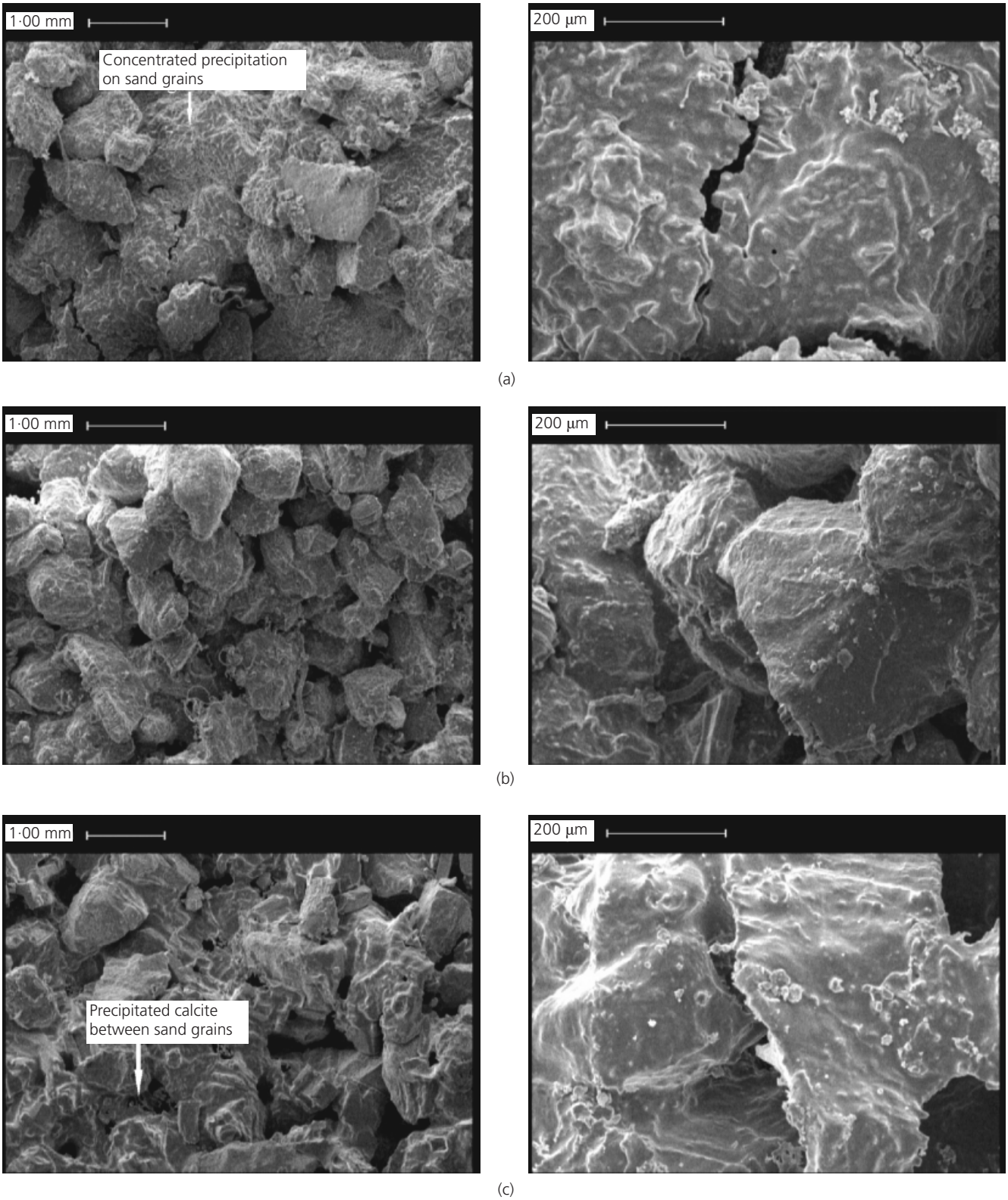


Figure 7. SEM images taken from the top portions of sand A specimens treated with different concentrations of bacterial cell and cementation solutions: (a) 4×10^8 cells/ml and 1·0 M urea–0·5 M $CaCl_2$; (b) 6×10^7 cells/ml and 1·0 M urea–0·5 M $CaCl_2$; (c) 1×10^8 cells/ml and 0·25 M urea–0·25 M $CaCl_2$

allowing a more even attachment of bacteria to the sand grain surfaces over the specimen's length.

5. Discussion

According to Stocks-Fischer *et al.* (1999), the level of calcite precipitation reduces for increasing bacterial concentrations above $\sim 10^8$ cells/ml. For the three bacterial solutions of 6×10^7, 1×10^8 and 4×10^8 cells/ml investigated in the present study, higher UCS and stiffness values along with greater reductions in permeability coefficient values were achieved for the (lower) 6×10^7 cells/ml solution concentration (see Table 4). The distinctly different calcite deposition patterns observed for the different bacterial cell solution concentrations can be explained as follows. For the 4×10^8 cells/ml concentration, clogging of the pore voids with bacterial cells occurred close to the injection points (i.e. the tops of the specimens), hampering permeation of the bacterial cell solution along the specimen length under the applied pressure head. On subsequent injection of the cementation solution, calcite deposited as concentrated areas on the grain surfaces and in the pore voids near the tops of the specimens, with the coverage depth on the sand grain surfaces reducing along the specimen length. For the 6×10^7 cells/ml concentration, the bacterial solution permeated more easily, and, on injecting the cementation solution, almost uniform calcite precipitation occurred on the grain surfaces (contributing to bond formation) over the full specimen length. This hypothesis is substantiated by the SEM images in Figures 7(a) and 7(b). Hence, in these element tests (i.e. for the specimen as a whole and not just its top portion), higher UCS and lower permeability coefficient values were measured for reducing bacterial cell solution concentrations for both sands that were tested (see Table 4).

For the same bacterial concentration of 1×10^8 cells/ml, higher UCS and stiffness values along with greater permeability coefficient reductions were achieved for higher cementation solution concentrations (see Table 3). Similar qualitative findings have been reported by Yasuhara *et al.* (2011) from the MICP treatment of sand using 0·5 and 1·0 M (equimolar) urea–$CaCl_2$ solutions. This may be attributed to the amount and larger size of individual calcite crystals deposited (Al-Thawadi, 2008), which were confirmed by SEM observations in the present study. Comparing the images in Figures 6(a) and 7(c), the greater levels of calcite precipitation for higher-concentration cementation solutions manifested as larger crystal formation on the sand grains and as concentrated deposits in the pore voids. Referring to Table 3, the effect was even greater for non-equimolar urea–$CaCl_2$ solutions comprising a higher molarity urea component. This may be attributable to high specific urease activity (Whiffin, 2004). It is postulated that higher-concentration solutions comprising greater urea molarity produce better (stronger) bonding between sand grains on account of greater levels of calcite precipitation.

Another key factor in modifying the geomechanical and (or) hydraulic properties of sand by MICP is the number of injection cycles. For sand, more injection cycles using high urea–$CaCl_2$ solution concentrations produce greater improvements in geomechanical properties and permeability coefficient reductions (Yasuhara *et al.*, 2011). Al Qabany and Soga (2013) reported that for four treatment cycles, higher cementation solution concentrations over the range 0·1–1·0 M examined were found to produce more rapid and greater permeability coefficient reductions. This is consistent with the findings of the present study, which investigated a single treatment cycle. However, Al Qabany and Soga (2013) found that, for a greater number of injections, low-concentration cementation solutions produced stronger specimens for the same amount of calcite precipitation. This apparent contradiction with the present study (i.e. higher UCS and stiffness values were achieved for higher urea–$CaCl_2$ concentrations) may be largely explained by differences between the injection protocols adopted for the two studies. A high number of treatment cycles necessitates the use of low cementation solution concentrations, otherwise UCS and stiffness values are reduced overall on account of non-uniform calcite deposition patterns occurring over the specimen length arising from localised clogging of pore voids close to the injection points. In contrast, for the single-cycle staged injection (with retention periods) protocol adopted for the main testing programme in the present study, greater improvements in stiffness and strength were achieved using higher cementation solution concentrations.

6. Summary and conclusions

In this study, different injection strategies for the MICP treatment of sand were investigated at bench scale with varying success. The concentrations of the reagents, especially of the bacterial cell solution (in the range 6×10^7 to 40×10^8 cells/ml investigated), were particularly important. Rapid clogging of the pore voids next to the injection points occurred when the reagents were mixing together beforehand and also for single-phase (simultaneous) injection. Clogging of the pore voids was not a particular problem for two-phase injection using lower bacterial cell solution concentrations, but the toughness of the treated sand specimens did not significantly increase away from the injection points, possibly due to leaching/flushing of bacterial cells out of these specimens during subsequent injection of the cementation solution. Staged injection including retention periods and with a pressure head applied during downward injection of the bacterial cell solution proved most effective. For lower bacterial cell solution concentrations, this approach was found to produce a more uniform distribution of calcite crystal formation over greater permeation distances. Single-cycle staged injection along these lines produced significant improvements in the UCS and stiffness values of 140 mm-long test specimens of the two loose medium quartz sands investigated. The permeability coefficient values of the treated sands reduced by less than approximately one order of magnitude, so their drainage capacities were not significantly affected. Greater stiffness/strength improvements and, to a lesser degree, permeability coefficient reductions were achieved for bacterial solution at 6×10^7 cells/ml concentration. This was the lower concentration value investigated, and is within the reported range for higher urease activity. Higher cementation solution

concentrations were also found to produce greater improvements in stiffness/strength, particularly for non-equimolar solutions having a higher molarity of urea. These experimental findings were supported by SEM observations of the treated sands. Stiffer, higher cementation solution concentrations generally produced a more brittle specimen response under unconfined compression. Another factor was grading (particle size), with the finer of the two treated sands investigated mobilising higher UCS and stiffness values.

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