Optimization of calcium-based bioclogging and biocementation of sand

Chu, Jian; Ivanov, Volodymyr; Naeimi, Maryam; Stabnikov, Viktor; Liu, Han-Long

2013

Chu, J., Ivanov, V., Naeimi, M., Stabnikov, V., & Liu, H.-L. (2014). Optimization of calcium-based bioclogging and biocementation of sand. Acta Geotechnica, 9(2), 277-285.

https://hdl.handle.net/10356/81878

https://doi.org/10.1007/s11440-013-0278-8

Optimization of calcium-based bioclogging and biocementation of sand

Jian Chu, Volodymyr Ivanov, Maryam Naeimi, Viktor Stabnikov, and Han-Long Liu

J. Chu (c**), V. Ivanov** Department of Civil, Construction & Environmental Engineering, Iowa State University, Ames, Iowa, 50011, USA. Tel.: +1-515-294-3157 Fax: +1-515-294-8216 Email: jchu@iastate.edu; Formerly School of Civil and Environmental Engineering, Nanyang Technological University, Singapore.

M. Naeimi School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Ave, Singapore, 639798.

V. Stabnikov Department of Biotechnology and Microbiology, National University of Food Technologies, 60 Volodymyrskaya Str., Kiev 01033, Ukraine

H.-L. Liu, College of Civil and Transportation Engineering, Hohai University, Nanjing, China

Abstract Bioclogging and biocementation can be used to improve the geotechnical properties of sand. These processes can be performed by adsorption of urease-producing bacterial cells on the sand grain surfaces, which is followed by crystallization of calcite produced from the calcium salt and urea solution due to bacterial hydrolysis of urea. In this paper, the effect of intact cell suspension of *Bacillus sp.* strain VS1, suspension of the washed bacterial cells, and culture liquid without bacterial cells on microbially-induced calcite precipitation in sand was studied. The test results showed that adsorption/retention of urease activity on sand treated with washed cells of *Bacillus sp.* strain VS1was 5 - 8 times higher than that treated with culture liquid. The unconfined compressive strength of sand treated with the suspension of washed cells was 1.7 times higher than that treated with culture liquid. This difference could be due to fast inactivation of urease by protease which was present in the culture liquid. The adsorption of bacterial cells on sand pre-treated with calcium, aluminum, or ferric salts was 29 to 37% higher as compared with that without pretreatment. The permeability of sand varied with the content of precipitated calcium. For

bioclogging of sand, the content of precipitated calcium had to be 1.3% (w/w) or higher. The shear strength of biotreated sand was also dependent on the content of precipitated calcium. To achieve an unconfined compressive strength of 1.5 MPa or higher, the content of precipitated calcium in the treated sand had to be 4.2% (w/w) or higher. These data can be used as the reference values for such geotechnical applications as bioclogging for reduction of permeability of sand and biocementation for increasing the shear strength of soil.

Keywords Bioclogging · Biocementation · Biogrouting · Sand

1 Introduction

Grouting with cement or chemical reagents is often used in geotechnical engineering to increase the strength or to reduce the permeability of soil [23]. For example, suspension of cement can be used to strengthen coarse sand for road or railways construction [1] or different grouting methods can adopted for ground improvement against liquefaction [9]. However, the viscosity of cement grout is too high to be used for fine sand or fine grained soil. Dissolved organic grouts can be used as alternatives. However, some of the organic grouts are expensive and toxic for human, animals, and plants [19].

A new grouting material, biogrout, has been developed in recent years [12, 19, 26]. Biogrout has low viscosity in solution and thus can penetrate better than cement or chemical grouts. The other advantages of biogrout over dissolved organic grouts are lower cost and lower toxicity [19].

Bioclogging is a process of filling the pores in soil with minerals and other substances that are generated microbially to reduce the soil permeability. Biocementation is a process to bind soil particles together with minerals and other substances to increase the compressive strength of soil. A common process for bioclogging and biocementation is microbially induced carbonate precipitation (MICP). As bioclogging and biocementation take place simultaneously most of the time, the two terms are used to refer to mainly the purposes of applications rather than the processes in practice.

MICP can be either a natural or engineered process that is controlled by different factors and through different mechanisms [6, 7, 27 - 29, 30, 31, 34]. One of them is the production of calcite in the porous soil by urease-producing bacteria (UPB) in the presence of urea, calcium ions, and either pure or enrichment

cultures [12, 14, 19, 20, 26, 33] or indigenous population of urease-producing bacteria (UPB) [4, 5]. This process is performed as follows:

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} + UPB \rightarrow CaCO_3 + 2NH_4^+ + UPB \quad (1)$$

The MICP process can be used for numerous applications in geotechnical and environmental engineering such as reduction of soil permeability through bioclogging or increase of shear strength of soil through biocementation [12, 19, 26; 27]. It has been demonstrated in the laboratory that the MICP process can significantly increase the strength [16, 17, 28, 37] or reduce the permeability of sand [15, 28, 33].

The MICP process in sand involves two major stages: 1) the adsorption of enzyme urease or cells of urease-producing bacteria on sand grains; and 2) the enzymatic hydrolysis of urea accompanying with the formation of calcium carbonate crystals. The adsorption of bacterial cells onto the sand particle surface and the movement of bacterial cells in the sand pores depends on the size, the surface charge (zeta-potential), and the surface hydrophobicity of the sand particles and bacterial cells, as well as the concentration of protons and other ions in environment [18, 21, 22, 35]. Coating the sand surface with calcium, ferric or aluminium cations enhances significantly the adhesion of bacterial cells to the sand grains [25, 35]. The objective of the research presented in this paper was to study ways to enhance the MICP-based biocementation of sand by treating it with different bioagents and cations.

2 Materials and methods

2.1 Materials

ASTM graded round sand was used in this study. The basic engineering properties of this sand are given in Table 1. Three different fractions of the sand were used to study the effect of grain size on the interaction of sand grains with bacteria and reagents. The three different fractions were prepared by sieving the sand through sieve sizes of 1.2, 0.6, and 0.2 mm so that the coarse fraction had grain sizes between 1.2 and 0.6 mm, the medium fraction between 0.6 and 0.2 mm, and the fine fraction below 0.2 mm. The sand specimens of the fractions of the coarse, medium, and fine sand were prepared with dry densities of 1540, 1510, and 1457 kg/m^3. The corresponding porosities were 42%, 43% and 45%, respectively.

The following reagents were used for bioclogging and biocementation of sand: 1) bioagents, which were bacterial suspension of halotolerant and alkalophilic strain of URB *Bacillus sp.* VS1 [8] or supernatant of culture liquid containing urease; 2) solution of the chemical reagents containing 82.5 g/L (0.75 M) calcium chloride and 90 g/L (1.5 M) of urea. The bacteria were grown in Tryptic Soya Broth medium as described earlier [33].

2.2 Treatment of sand with cations

Sand samples in 50 mL syringes with the sponge filter on the bottom were treated with water (as a control) or solutions of calcium, ferric and aluminum in experiments. The aliquots of 25 mL of 50 mM freshly prepared solutions of aluminum chloride, ferric chloride, or calcium chloride with the pH of 3.6, 1.8 and 5.8, respectively, were injected from the bottom of the sand sample to the top and incubated for 1 hour. The solution was then drained off by gravity and the sand was washed three times by the injection of 25 mL deionized water from the top to the bottom. The average of pH of water after the 3rd washing was 6.5, 6.9 and 7.2 for the samples pre-treated with Al^{3+}, Fe^{3+} and Ca^{2+} cations, respectively. In the control test, 25 mL deionized water was used instead of a salt solution. Adsorption of cells and urease was performed by addition of 25 mL of different biological agents to the treated sand for 2 h. Then, the optical density at 600 nm or urease activity of the drained liquid was measured. Some experiments were repeated to check the repeatability. The mean values and the standard deviations of the measurements were determined.

2.3 Treatment of sand with bioagents

Sand can be treated in a number of ways: the cycles of batch treatment, the batch or continuous surface spray or the surface percolation [8, 33], the continuous injection, and the applications with the different sequence of supply of bacterial suspension and cementation reagents [32**].** The cycles of batch treatment were used in this research. After 48 hours of batch cultivation, as described earlier [8], 500 mL of intact bacterial suspension (culture liquid) with a biomass concentration of 8 g dry biomass/L was divided into two fractions using centrifugation with 1000 x g for 20 minutes. One fraction was the bacterial biomass

collected by centrifugation. It was re-suspended in 500 mL of 0.9% solution of NaCl. Another fraction was 500 mL of liquid supernatant with urease activity. The aliquotes of 25 mL of three different bioagents such as the intact bacterial suspension, the suspension of washed bacterial cells, or the supernatant, were injected from the bottom of the sand sample to the top and the sand samples then were incubated for 2 hours. The suspension/solution was then drained off by the gravity. The enzymatic hydrolysis of urea in sand typically increased pH up to 8.9 - 9.3. Some experiments were repeated to check the repeatability. Mean values and standard deviations of the measurements were determined.

2.4. Precipitation of calcium by different bioagents

Aliquotes of 0.5 L of biocementing solution containing 82.5 g/L (0.75 M) calcium chloride and 90 g/L (1.5 M) of urea were mixed with 50 mL of either the intact bacterial suspension, or the washed suspension of bacterial cells, or the supernatant and incubated for 30, 60 min, 120 min, and 180 min at room temperature on the shaker at 100 rpm. Quantity of calcium carbonate was measured by the standard method APHA 2540 D for total suspended solids [3] by filtration through the glass-fiber filter following with drying at 103^{o}C.

2.5. Bioclogging,and biocementation of sand

After coarse sand was treated with bioagents, 25 mL of biocementing solution (it is approximately one pore volume of sand) containing 82.5 g/L (0.75 M) calcium chloride, 90 g/L (1.5 M) of urea was injected from the bottom of the sand sample to the top and the sand sample was incubated for 24 hours. Then the solution was drained off by gravity.

Permeability tests were conducted on the biotreated sand in the syringe using a falling head method. This measurement was used to estimate the reduction in the permeability of sand due to MICP.

When the treatment was finished, the bottom of the plastic syringe was cut out and the specimen was removed carefully from the syringe. The specimens were dried at 60^{o}C until the weight became constant and then used for unconfined compression tests.

For the study on the precipitated calcium dosage on bioclogging and biocementation, the treatments of sand were repeated several times. The content of $CaCO_3$ in the treated sand was determined through the mixing of 10 g of the crushed sample with 100 mL of 10N HCl for 12 h, following with filtration of the solution and the measurement of calcium concentration using a standard method APHA 2340C with ethylene diaminetetraacetate (EDTA) titration [3]. Some experiments were repeated to check the repeatability. Mean values and standard deviations of the measurements were determined.

2.6. Measurements and microscopy

Scanning electron microscopy (SEM) and light microscopy were conducted using the Leica Stereoscan 420 and the Olympus SZx9 stereomicroscope, respectively. Urease activity of bacterial suspension was measured by changes in the conductivity as described earlier [8, 33]. Urease activity was also measured by the production of ammonia determined by the Nessler method [3]. Concentrations of metals in the solution before and after adsorption test with sand were determined using Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) Perkin Elmer [3].

The protease activity was measured using the Sigma's non-specific protease activity assay [10]. To inhibit the protease activity, 0.5 mL of the Protease Inhibitor Cocktail (Sigma-Aldrich, St Louis, USA) was added to per 50 mL of the supernatant. According to the manual of the manufacturer, the cocktail included such proteases inhibitors as AEBSF – [4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride], Aprotinin, bestatin hydrochloride, E-64-[N-(trans-epoxysuccinyl)-L-leucine-4-guanidinobutylamide], Leupeptin hemisulfate salt, and Pepstatin A.

3 Results

3.1. Precipitation of calcium from the solution by different bioagents

Initial rate of the calcium carbonate precipitation from the solution of calcium chloride and urea using either the intact bacterial suspension, or the washed suspension of bacterial cells, or the supernatant was

0.38, 027, 0.17 g/L/min (mean values) , respectively (Fig.1). Therefore, as far as the microbial precipitation of calcium is concerned, the best option is the use of the intact bacterial suspension. However, all rates dropped to 0.03- 0.05 g/L/min in 3 hrs as observed from this study (Fig.1).

3.2 Treatment of sand with different bioagents

The intact bacterial suspension was centrifuged to produce two bioagents: the saline-washed suspension of bacterial cells and the culture liquid, i.e. the supernatant containing urease. The adsorption/retention of these bioagents on sand was quite different. After incubation of washed suspension of the bacterial cells with the coarse or the fine sand for 1 hr, the adsorption of urease activity was 89% and 100% of the initial activity, respectively. Meanwhile, after incubation of the supernatant with the coarse or fine sand for 1 hr, the adsorption of urease activity was 11% and 20% of the initial activity, respectively. So, the adsorption/retention of UPB cells on sand was 5 - 8 times more effective than that of urease. It could be due to the difference in adsorption of cells or enzyme on sand surface or just due to aggregation of *Bacillus sp*. cells [8] and retention of these aggregates in the sand pores.

3.3 Instability of biocementation activity of different bioagents

The higher biocementation efficiency of the washed bacterial cells in comparison with the supernatant and the non-centrifuged cultural suspension may be hypothetically explained by the presence of protease activity in the supernatant and the non-centrifuged cultural liquid and the absence of this activity in the suspension of the washed bacterial cells. The protease activity in the supernatant and the intact bacterial suspension was within the range 0.1 - 0.2 μmoles of tyrosine/mL·min and can cause hydrolysis of urease protein. Simultaneous measurements of the urease and the protease activities during cultivation of *Bacillus* sp. strain VS1 showed that the high urease activity was at time when the protease activity was low (Fig. 2). The protease activity was not detected in the suspension of the washed bacterial cells. This may be one of the reasons for the higher biocementation activity of the washed bacterial cells in comparison with the supernatant.

As shown in Fig. 2, the urease activity could decrease to a very low value in 3 hrs in all three solutions (Fig. 3). The presence of protease explained why the urease activity was low and unstable in the supernatant. It was dropped in the supernatant from 0.50 to 0.17 mM NH_4^+/min for 60 min incubation at 30^{o}C. Meanwhile, the reduction of the urease activity for the washed bacterial suspension for the same time and temperature was from 1.30 to 0.75 mM NH_4^+/min. An addition of the proteases inhibitors cocktail to bioagents blocked the reduction of the urease activity in the supernatant but not that in the suspension of bacterial cells. Therefore, not only the protease but also some other non-identified factors of the urease instability are present in the cell suspension.

3.4 Modification of the cell adsorption on sand by cations

The uptake of bacterial cells from the suspension to the sand particles with the sizes between 0.2 and 0.6 mm was determined by measuring the depletion of cells (optical density at 600 nm, OD_{600}) and the urease activity from the bacterial suspension (Fig. 4). Adsorption of cells on sand was completed in 60 min (Fig. 4), therefore the duration of all experiments on the effects of cations on adsorption of cells was 2 hours. The efficiencies of the bacterial cells adsorption on the sand grain surface after pre-treatment of sand with the different cations are given in Table 2. Pre-treatment of sand with Ca^{2+}, Fe^{3+}, or Al^{3+} solution increased adsorption of bacterial cells by 31 ± 6% (mean ± standard deviation for triplicates) compared with the control which was the treatment of sand with water.

Table 2 also indicates that the efficiency of the bacterial cells adsorption was not significantly affected by the grain size of sand within the range of 0.2 to 2 mm. This can be hypothetically explained as that the efficiency of the bacterial cells adsorption does not depend on the specific surface of sand but on the number of positively charged sites created by cations adsorbed on the sand grains surface.

3.5 Effect of precipitated calcium content on the properties of treated sand

The SEM images of sand after the MICP process are shown in Fig. 5. The MICP process resulted in either adhesion of bacterial cells and formation of crystals on sand surface (Fig. 5a), clogging of the channels between the sand grains (Fig. 5b), or filling of the pores with carbonate crystals (Fig. 5c).

Almost all supplied calcium ions, typically from 95 to 98%, were precipitated as calcium carbonate. The distribution of calcium content along the length of the specimen was uniform, probably, due to the small size of the specimen.

The unconfined compressive strengths obtained are plotted against the content of precipitated calcium for five biotreated sand specimens in Fig. 6. Accuracy (ratio of standard deviation to mean) in all geotechnical experiments was lower 20%. Within the tested range, a linear relationship can be established as follow:

$$S = 366\ C, \quad \text{kPa} \quad (R^2 = 0.98) \qquad (2)$$

where: S = unconfined compressive strength (in kPa), C = content of precipitated calcium (% w/w of dry treated sand).

The permeability of treated sand, k, is also affected by the content of precipitated calcium, C. The higher the C value, the higher the permeability. A correlation between permeability and content of precipitated calcium is also shown in Fig. 7. Within the range of experiments, the linear equation can also be established as:

$$k = 507 - 403C, \quad 10^{-7}\ \text{m/s}, \quad (R^2 = 0.98) \qquad (3)$$

The permeability of the clean sand was 5×10^{-5} m/s. The permeability at C = 1.24% was 1.6×10^{-7} m/s.

4 Discussion

4.1 Biocementation activity of different bioagents

The study presented in this paper has revealed that bioclogging and biocementation of sand with the suspension of bacterial cells was more effective than the treatment of sand with the supernatant containing urease. Effect of increased urease activity of bacterial cells after dilution of bacterial suspension with saline solution was known and has been hypothetically explained by an increased release of urease from the cells under the presence of NaCl by Harkes et al. [17]. The increased urease activity in sand treated with the

washed cells suspension as compared with that with the supernatant was explained in this paper by the presence of urease-inactivating protease in the supernatant. This is based on the following observations: 1) the inactivating effect of proteases is well known for urease and many other enzymes; 2) protease activity was detected in the supernatant but not in the washed cells suspension; 3) the inactivation of urease in the supernatant was faster, whereas the inactivation of urease in the suspension of the washed bacterial cells was slower; and 4) the use of the proteases inhibitors cocktail blocked the reduction of urease activity in the supernatant during the first 18 hours of incubation, whereas it did not affect the change of the urease activity in the suspension of bacterial cells.

The difference in the reduction rate of the urease activity (Fig. 2) and in the rate of $CaCO_3$ production due to the urease activity (Fig. 3) could be due to the removal of protease adsorbed on the fresh precipitate of calcium carbonate as well as the buffering of pH in the solution of urea and calcium chloride during the formation of calcium carbonate. The above findings have important practical implication. It means that bioclogging and biocementation of soil can be better performed using a concentrated bacterial suspension or an intact bacterial suspension rather than the supernatant or filtrate containing only dissolved enzymes.

The urease activities in our experiments were lower than those reported in some other papers [16, 17, 27, 34]. However, this could be better for biocementation because a low urea hydrolysis rate leads to stronger calcium carbonate aggregates [28, 30]. The biocementation activity of the washed cells can be also different. For example, the washed cells of urease-producing bacteria from exponential phase of the batch culture were significantly more active in sand cementation than cells from the stationary phase [7] but the reason of this difference is not clear.

The depth of the bacterial cells penetration in soil depends on the rate of the cells retention (adsorption) by the soil particles and the permeability of soil. For saturated sand with a permeability of $1x10^{-4}$ m/s with a time of 1 hour to complete the adsorption of bacterial cells on sand, as in the case of our study, the calculated depth of the bacterial cells penetration for one cycle of the sand treatment was about 36 cm. Therefore, to increase the depth of bioclogging and biocementation, the sand must be treated with bacterial suspension either continuously for a defined period of time or using injection of bacterial suspension at different depths from the surface.

4.2 Effect of cations on bacterial cells adsorption

It is known that coating of sand surface with cations significantly enhances the adhesion of bacterial cells to the sand grains [25, 35]. It could be due to the increased density of the positively charged sites at the sand grain surface that attracts the negatively charged sites of the bacterial cell surface. In our study, pre-treatments of sand with trivalent cations, Fe^{3+} and Al^{3+}, or divalent cation Ca^{2+} enhanced the adsorption of bacterial cells for almost the same value, 31 ± 6%, notwithstanding that trivalent cations bridges between sand and bacterial cell could have 1.5 times stronger net bonds comparing to the salt bridges created by divalent cations. It means that the cations-enhanced bacterial cells adsorption is due to the increase in the number of the positively charged sites, but not due to the strength of the bonds between cations and the surfaces.

4.3 Effect of the precipitated calcium dosage

The binding of soil particles can improve the soil structure and the agricultural properties of sandy soils [11, 13]. Microorganisms can also naturally induce the clogging of the soil pores and reduce the permeability of soil [19]. Different dosages of calcium deposited during engineered MICP lead to different levels of bioclogging or biocementation of sand.

Content of precipitated calcium required to reduce the permeability of sand to a certain value can be estimated using Eq. (3). It can be worked out from Eq. (3) that to reduce the permeability to a value smaller than 10^{-7} m/s, the content of precipitated calcium is only 1.24%. However, at this content of precipitated calcium, the compressive strength of the treated sand is only 454 kPa according to Eq. (2). To achieve a compressive strength of 1000 kPa, the content of precipitated calcium required is estimated to be 2.73% using Eq. (2). Therefore, the permeability of sand can become quite low before the required shear strength can be achieved through biocementation. The low permeability will certainly make it harder for subsequent biocementation to be implemented.

However, it should be pointed out that Eqs. (2) and (3) may not be applicable to other types of soil as different quantities of precipitated calcium carbonate may be required to produce the same value of strength for different soils. More experimental data are required to verify this point.

The duration of the bioclogging can be calculated from the urease activity *in situ* and the content of precipitated calcium. If the urease activity is a rate-limiting step of the bioclogging and its value *in situ* = 1 mM/min ≈ 4 mmol precipitated $CaCO_3$/L of sand min (for porosity about 50%) and the content of precipitated calcium is 1.26% (≈ 43 mmol/L sand), the calculated duration of the bioclogging is about 11 h. The duration of biocementation can be calculated similarly. If the urease activity is a rate-limiting step of the biocementtion and its value *in situ* is 1 mM/min ≈ 4 mmol of precipitated $CaCO_3$/L of sand/min (for sand porosity of 50%), the content of precipitated calcium is about 4.2 % (≈ 143 mmol/L sand), the calculated duration of the biocementation to reach the compressive strength of 1.5 MPa is about 36 h.

It is well known that solubility of calcite in water-gas phase depends on pH, temperature, ionic strength, and partial pressure of CO_2 in gas. For all studied systems pH was above 9.2, temperature about 25^{o}C, and partial pressure of CO_2 in the pores of sand was close to atmospheric one. It could be calculated using [24] that the solubility of calcite in water in contact with atmospheric content of CO_2 gives the concentration of calcium about 0.5 mM (19 mg Ca^{2+} /L), while under CO_2 partial pressure 0.1 atm the concentration of calcium will be about 3 mM (128 mg Ca^{2+} /L). Therefore, bioclogging and biocementation decreasing the permeability of treated sand for several orders magnitude will ensure stability of precipitated calcite.

Bacteria attached to the sand granules are supposed to provide the nucleation sites where calcite is precipitated due to the hydrolysis of urea, an increase of pH [34], and, probably, transformation of bicarbonate to carbonate. Our SEM images showed that calcite crystals precipitated on the sand surface (Fig. 5a). An accumulation of precipitates finally clogged the pores in sand. The shear strength enhancement of biocemented sand is caused by the point-to-point contacts of $CaCO_3$ crystals that bridge the sand granules together [2].

For future research and practical applications of bioclogging and biocementation in geotechnical engineering the following research tasks could be essential:

1) Strains of halophilic and alkaliphilic urease-producing bacteria with low protease activity have to be selected to ensure stability of biocementation rate;
2) The mechanism of the involvement of intracellular urease in the biocementation process has to be studied;
3) The technology of low cost dry biocement as a stable and an efficient geotechnical material has to be developed.

5 Conclusions

The objective of the research presented in this paper was to study ways to enhance the MICP-based biocementation of sand by treating it with different bioagents and cations. The compressive strength of sand treated with suspension of the washed cells was 1.7 times higher than that treated with the cultural liquid. Pre-treatment of sand with calcium, aluminum or ferric salts increased the adsorption of bacterial cells on the sand surface by 29 to 37% in comparison with the control. Permeability of sand (k) varied with the content of precipitated calcium (C, % w/w) by the equation $k = (507 – 403C)\ 10^{-7}$ m/s. The unconfined compressive strength of dry biotreated sand (S) increased with the content of precipitated calcium (C, % w/w) by the equation S = 366 C kPa.

Acknowledgements Part of the study presented in this paper was supported by the SERC Grant No. 0921420043 from the Agency for Science, Technology and Research, Singapore. Part of it also forms part of a collaborative program with Hohai University, China, under the University Characteristics Project of the Ministry of Education, People Republic of China, Grant No. Ts2012HHDX029.

References

1. Ajorloo AM, Mroueh H, Lancelot L (2012) Experimental investigation of cement treated sand behavior under triaxial test. Geotech Geol Eng 30:129-143
2. Al-Thawadi SM (2011) Ureolytic bacteria and calcium carbonate formation as a mechanism of strength enhancement of sand. J Adv Sci Eng Res 1:98-114

3. American Public Health Association (APHA) (1999) Standard Methods for the Analysis of Water and Wastewater, 20th ed. American Public Health Association, Washington DC
4. Burbank MB, Weaver TJ, Green T, Williams BC, Crawford RL (2011) Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soil. Geomicrobiol J 28:301-312
5. Burbank MB, Weaver TJ, Williams BC, Crawford RL (2012) Urease activity of ureolytic bacteria isolated from six soils in which calcite was precipitated by indigenous bacteria. Geomicrobiol J 29:389 - 395
6. Cacchio P, Ercole C, Cappuccio G, Lepidi A (2003) Calcium carbonate precipitation by bacterial strains isolated from a limestone cave and from a loamy soil. Geomicrobiol J 20:85-98
7. Chou CW, Seagren EA, Aydilek AH, Lai M (2011) Biocalcification of sand through ureolysis. J Geotechn Geoenviron Eng 137:1179-1189
8. Chu J, Ivanov V, Stabnikov V (2012) Microbially induced calcium carbonate precipitation on surface or in the bulk of soil. Geomicrobiol J 29:544-549
9. Chu J, Varaksin, S, Klotz, U, Menge, P (2009) State of the art report: construction processes. Proceed. 17th Int. Conf. on Soil Mechanics and Geotechnical Engineering, M. Hamza et al. (Eds.), 4:3006-3135.
10. Cupp-Enyard C (2008) Sigma's non-specific protease activity assay-casein as a substrate. J Visual Exper 19:899-910
11. Degens BP (1997) Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Austral J Soil Res 35:431-459
12. DeJong JT, Mortensen BM, Martinez BC, Nelson DC (2010) Bio-mediated soil improvement. Ecol Eng Res 36:197-210
13. Forster SM (1990) The role of microorganisms in aggregate formation and soil stabilization: types of aggregation. Arid Soil Res Rehab 4:85-98
14. Frankel RB, Bazylinski DA (2003) Biologically induced mineralization by bacteria. Rev Mineral Geochem 54:95-114
15. Gollapudi UK, Knutson CL, Bang SS, Islam MR (1995) A new method for controlling leaching through permeable channels. Chemosphere 30:695-705

16. Hammes F, Boon N, De Villiers J, Verstraete W, Siciliano SD (2003) Strain-specific ureolytic microbial calcium carbonate precipitation. Appl Environ Microbiol 69:4901-4909
17. Harkes MP, van Paassen LA, Booster JL, Whiffin VS, van Loosdrecht MCM (2010) Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. Ecol Eng 36:112–117
18. Hendricks DW, Post FJ, Khairnar DR (1979) Adsorption of bacteria on soils: Experiments, thermodynamic rationale, and application. Water Air Soil Poll 12:219-232
19. Ivanov V, Chu J (2008) Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil *in situ.* Rev Environ Sci Biotechnol 7:39-153
20. Ivanov V (2010) Environmental Microbiology for Engineers. CRC Press, Boca Raton.
21. Jacobs A, Lafolie F, Herry JM, Debroux M (2007) Kinetic adhesion of bacterial cells to sand: Cell surface properties and adhesion rate. Colloids Surfaces B 59:35-45
22. Jiang G, Noonan MJ, Buchan GD, Smith N (2007) Transport of *Escherichia coli* through variably saturated sand columns and modeling approaches. J Contam Hydrol 93:2-20
23. Karol RH (2003) Chemical Grouting and Soil Stabilization, 3rd ed. M Dekker, New York
24. Krauskopf KB, Bird DK (1995) Introduction to Geochemistry (3rd ed.), McGraw-Hill, Inc., New York, 647 pp.
25. Lukasik J, Cheng YF, Lu F, Tamplin M, Farrah SR (1999) Removal of microorganisms from water by columns containing sand coated with ferric and aluminum hydroxides. Water Res 33:769-777
26. Mitchell JK, Santamarina JC (2005) Biological considerations in geotechnical engineering. ASCE J Geotechn Geoenviron Eng 131:1222-1233
27. De Muynck, W., N. De Belie and W. Verstraete (2010). "Microbial carbonate precipitation in construction materials: A review." Ecological Engineering 36(2): 118-136.
28. Nemati M, Voordouw G (2003) Modification of porous media permeability, using calcium carbonate produced enzymatically in situ. Enzyme Microb Technol 33:635-642
29. van Paassen LA, Ghose R, van der Linden TJM, van der Star WRL, van Loosdrecht MCM (2010) Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. J Geotechn Geoenviron Eng 36:1721-1728

30. Qian C, Wang J, Wang RW, Cheng L (2009) Corrosion protection of cement-based building materials by surface deposition of $CaCO_3$ by *Bacillus pasteurii*. Materials Sci Eng 29:1273-1280

31. Rivadeneyra MA, Perez-Garcia I, Salmeron V, Ramos-Cormenzana A (1985) Bacterial precipitation of calcium carbonate in presence of phosphate. Soil Biol Biochem 17:171-172

32. Rong H, Qian CX, Li LZ (2012) Influence of molding process on mechanical properties of sandstone cemented by microbe cement. Construction and Building Materials 28:238-243.

33. Stabnikov V, Naemi M, Ivanov V, Chu J (2011) Formation of water-impermeable crust on sand surface using biocement. Cement Concrete Res 41:1143–1149

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

35. Tan Y, Bond W, Rovira AD, Brisbane PG, Griffin DM (1991) Movement through soil of a biological control agent, *Pseudomonas fluorescens*. Soil Biol Biochem 23:821-825

36. Vasarhelyi B, Van P (2006) Influence of water content on the strength of rock. Eng Geol 84:70-74

37. Whiffin VS, van Paassen LA, Harkes MP (2007) Microbial carbonate precipitation as a soil improvement technique. Geomicrobiol J 24**:**417-423

Legends to Figures

Fig. 1. Correlation between the urease and the protease activities during growth of *Bacillus sp*. strain VS1.

Fig. 2. Changes of the urease activity in the supernatant (Curve 1), the intact cultural liquid (Curve 2), and in the washed bacterial suspension (Curve 3) during their incubation at 30^oC.

Fig. 3. Changes of calcium carbonate precipitation rate, g/L· min in the supernatant (Curve 1),the intact cultural liquid (Curve 2), and in the washed bacterial suspension (Curve 3) during their incubation at 30^oC. The calcium precipitation rates were shown for time intervals 0-30 min, 30-60 min, 60-120 min, and 120-180 min, so each point on the graph is corresponding to the middle of these time intervals.

Fig. 4. Adsorption of bacterial cells on sand: Curve 1, adsorption measured by OD_{600}; Curve 2, adsorption measured by urease activity of cells.

Fig. 5. The SEM images of microbially-induced calcium carbonate crystallization on sand (I) and the schematics of the process stages (II): a) adsorption of cells and formation of crystals on sand surface; b): clogging of the channels between sand grains; c) filling of the pores with calcium carbonate crystals.

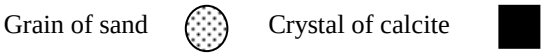

Fig. 6. Effect of precipitated calcium on permeability of the samples of biocemented sand.

Fig. 7. Effect of precipitated calcium on unconfined compressed strength for dry samples of biocemented sand.