

# Effects of Biological Stabilization on the Water Retention Properties of Unsaturated Soils

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**Abstract:** The soil water retention curve (SWRC) is one of the most fundamental characteristics of unsaturated soils. Because unsaturated soils are subjected to a range of natural processes and engineered treatments, a thorough understanding is needed of how their retention properties change when exposed to each separate treatment. In recent years, several biological treatment methods, such as microbial-induced calcite precipitation (MICP), have been introduced as environmentally friendly techniques. This study investigates the effect of biological treatment by means of *Bacillus sphaericus* on the SWRC of both coarse-grained and fine-grained soil samples. Pore fluid pH, X-ray diffraction, and scanning electron microscopy tests are used to better delineate the mechanisms affecting biologically induced alterations of SWRCs. Results showed that higher bacterial concentrations produced higher air-entry values in coarse-grained soils. However, the fine-grained samples showed an initial increase followed by a decrease in the air-entry value when the bacterial concentration increased. Changes in the soil porous structure and double-layer thickness are thought to be main reasons causing the different behavior of fine-grained soils. DOI: 10.1061/(ASCE)GT.1943-5606.0002053. © 2019 American Society of Civil Engineers.

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## Introduction

Much of the Earth's surface is covered by soils that are partially saturated with water. The surface layers in arid and semiarid regions, which cover more than one-third of the Earth's surface, certainly fall into this category (Fredlund and Rahardjo 1993; Dijkema et al. 2017). Unsaturated soils are subject to far more complex hydromechanical processes than saturated soils. Because of the need to understand the behavior of unsaturated soils in engineering projects, the mechanics of unsaturated soils has received much attention in recent years (Fredlund and Rahardjo 1993; Lu and Likos 2004; among many others).

The mechanical behavior of unsaturated soil samples is known to be very much affected by the degree of fluid saturation. In fact, the basic hydraulic and mechanical properties of unsaturated soils are governed largely by the soil water retention curve (SWRC), which specifies the degree of fluid saturation as a function of the imposed suction (or capillary pressure). The soil water retention curve is a fundamental relationship in unsaturated soils for which different functions have been proposed over the years (e.g., van Genuchten 1980; van Genuchten and Nielsen 1985; Fredlund and

Xing 1994; Aubertin et al. 2003; Tarantino 2009), including their relationship with the mechanical and other properties of unsaturated media (Mualem 1976, Fredlund et al. 1996, Vanapalli et al. 1996; Khalili et al. 2008, Alonso et al. 2010).

Soil water retention is highly dependent on soil texture (notably particle-size distribution), soil structure, and various environmental conditions, including soil mineralogy, soil solution strength and composition, external loads, and temperature. Thus, for each engineering application, it is important to consider the individual effects of various environmental conditions, including changes in soil structure and chemical composition, that may alter the retention properties of soils.

Soil improvement projects often involve engineering applications for which the retention properties of soils are of considerable importance. In recent decades, new environmentally friendly techniques for soil improvement have been introduced. These techniques employ various biogeochemical processes, such as bioclogging and biocementation, to change the macroscale mechanical and hydraulic properties of a soil. Bioclogging, for example, has been used for sealing purposes in geotechnical engineering (Seki et al. 1998; Dennis and Turner 1998).

Various places on or near the Earth's surface contain naturally cemented sands, such as stromatolites in shallow high-salinity waters of Western Australia. Formations of this type have inspired the engineering use of microbial-induced calcite precipitation (MICP). They also point to the durability of this technique; as indicated by DeJong et al. (2013), MICP-treated sand is expected to be stable for more than 50 years as long as favorable conditions regarding pH and alkalinity persist. A 50-year durability is consistent with the lifetime of many earth structures. Because of lower energy consumption and lower CO<sub>2</sub> emissions, MICP is now increasingly used as an environmentally sustainable alternative to chemical and other soil improvement techniques.

MICP and resulting biocementation processes have been used to increase shear strength (DeJong et al. 2006; Al Qabany 2011;

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Burbank et al. 2013), reduce the liquefaction potential of soils (Montoya et al. 2013; He et al. 2013), and/or improve the mechanical properties of problematic soils such as swelling, collapsible, and dispersive soils (Sadjadi et al. 2014; Sisakht et al. 2015; Moravej et al. 2015, 2018; Saffari et al. 2017; among others). A comprehensive review of recent advancements in biological soil improvement techniques has been provided by DeJong et al. (2013).

The mechanical behavior of biologically improved soils (especially but not limited to their surface layers) that are exposed to various and often time-dependent hydrological conditions very much depends on how changes in prevalent biogeochemical processes lead to changes in the soil water retention properties. Biogeochemical processes can potentially have a broad range of effects, from changes in the soil microfabric (such as interlayer cations and the diffuse double-layer thickness), to changes in the soil macrofabric (such as the overall pore-size distribution and the presence of soil macropores), which all can alter the retention properties of unsaturated soils.

Several recent studies have addressed the effect of chemical soil-stabilizing materials, such as lime and fly ash, on the retention properties of artificially cemented soils (e.g., Russo 2005; Puppala et al. 2006; Thudi 2007; Aldaood et al. 2014; Wang et al. 2015; Suazo et al. 2016; Zhang et al. 2017). But only a very few works have focused on the effect of bacteria on the soil retention properties. Those studies did not investigate the effect of bacteria involved in MICP processes (e.g., Dello Sterpaio 2012), and the bacteria involved had different metabolic paths, metabolic products, and biochemical effects. Furthermore, most of the studies concerned the effects of microorganisms on the retention properties of coarse-grained soils. Therefore, to the best of the authors' knowledge, no comprehensive studies have been carried out specifically on the impact of biogeochemical effects induced by MICP processes on the water retention properties of unsaturated soils. Given the complex interplay of soil structure and biochemical reactions, especially for fine-grained soils, a need exists for well-designed experiments to address these shortcomings in current literature.

Following the aforementioned reasons, this study explores these different effects using a series of experimental tests on biologically stabilized soils using MICP technique at various bacterial densities. For this purpose, the effects of microbial calcite precipitation on basic soil properties (Atterberg limits) as well as soil water retention behavior were investigated. The soil water retention data were obtained by means of filter paper and pressure-plate techniques. Pore fluid chemistry and microfabric of fine-grained soils play an important role in their hydraulic behavior. Therefore, pore fluid pH measurements as well as X-ray diffraction (XRD) and scanning electron microscopy (SEM) tests were performed with the aim of examining the underlying mechanisms affecting biologically induced alterations of SWRCs.

The experiments were carried out using a low-plasticity clay. After a first set of experiments with the clay, another set of tests was performed on a coarse portion of the sample to distinguish the effect of biogeochemical processes on relatively fine-grained versus coarse-grained media, and at different (microscale and macroscale) levels.

The structure of the paper is as follows. First, the sample preparation and biological treatment techniques are described, and a brief explanation is provided of the adopted experimental procedures. Next, the results from different tests are presented and discussed. The paper ends with concluding remarks and suggestions for further studies. A brief discussion about the durability of MICP effects, as well as challenges in field applications of MICP, is presented in the Appendix.

## Materials and Methods

### Basic Soil Properties

First, the effects of biological treatment on the water retention properties of a fine-grained (swelling) soil sample were studied. The soil sample used was a synthetic mixture of three different media: a silty sand, a kaolinite, and a bentonite, with weight percentages of 70, 15, and 15, respectively. The same type of soil was used by Sadjadi et al. (2014) to investigate the effectiveness of biological stabilization of swelling soils.

Based on the results of a sieve analysis [ASTM 2487 (ASTM 2011)] and following the Unified Soil Classification System, the prepared synthetic soil is classified as a clay with low plasticity. The results of a standard Proctor compaction test [ASTM D698 (ASTM 2012)] revealed that the optimum moisture content of the soil was 0.138, and the soil sample had a maximum dry unit weight of 18.2 kN/m<sup>3</sup>.

### Sample Preparation and Experimental Procedure

To investigate the effects of biological treatment on the water retention properties, soil samples were treated with solutions of *Bacillus sphaericus* bacteria. Lyophilized ampules of *Bacillus sphaericus* under the strain name of PTCC 1487 were obtained from the Iranian Organization of Scientific Research (IROST), Tehran, Iran. Ingredients of the media used to cultivate the bacteria are described in Table 1. Except for urea, the various ingredients of the culture media were poured into distilled water, after which the resulting solution was autoclaved for 20 min at 120 C. Urea was then added by means of filter paper having a pore size of 0.22  $\mu$ m.

A major focus of this study is the concentration of the bacterial solution and its effects on changes in the soil water retention properties. The concentration of a bacterial solution is often expressed in terms of its optical density (OD) as measured using a photospectrometer. The following procedure was used to determine the OD. The system was calibrated with the culture media without bacteria to obtain a reference line, followed by optical density measurements of the bacterial solution (determined based on the reference value). To determine the required time for the growth of bacteria, a growth rate curve was obtained first. The curve specifies the time required to reach a certain optical density. The growth rate curve obtained for the *Bacillus sphaericus* bacteria is presented in Fig. 1.

For the water retention measurements, soil samples having moisture contents of 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, and 0.24 by weight were prepared. The samples were allowed to equilibrate for 24 h in closed-lid containers, after which they were statically compacted. The compaction rate was set at 2 mm per minute. The compacted and treated samples were then kept in zipped plastic containers for 4 days, after which filter papers were placed on the samples for the retention measurements. Following ASTM D5298 (ASTM 2016), three filters (Whatman No. 42) were placed beneath the sample to measure the matric suction. The weights of the filters were determined after 10 days using a balance with

**Table 1.** Culture medium composition

Component of the liquid culture media	Amount (g/L)
Sodium bicarbonate	2.12
Nutrient broth	3.00
Ammonium chloride	10.00
Yeast extract	20.00
Urea	10.00

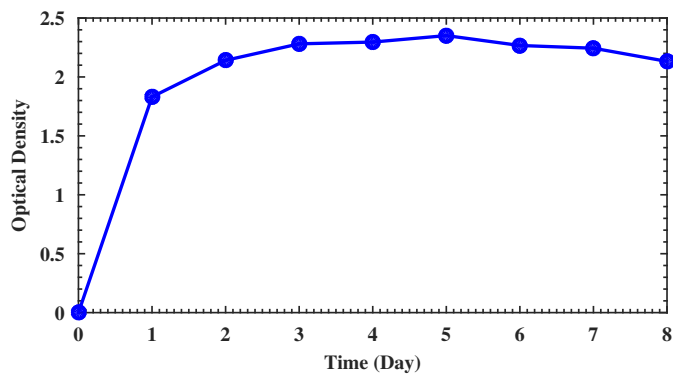


Fig. 1. Measured growth rate diagram of *Bacillus sphaericus*.

0.0001 g precision, thus providing measurements of their moisture contents. The matric suction was obtained based on the middle filter beneath the sample. The calibration graph of ASTM D5298 (ASTM 2016) was then employed to find the suction value corresponding to each moisture content, leading to plots of the soil water retention curve.

To better understand the effects of biological stabilization on the microfabric and macrofabric properties of the soil, additional tests were carried out on the coarse portion of the samples. For this purpose, the portion passing through a No. 40 sieve but retained by a No. 200 sieve were used. The coarse-grained portion of the sample drained quickly at relatively low values of the matric suction and hence did not need high-suction experiments. A pressure-plate apparatus was utilized to obtain the water retention data of the control and treated coarse-grained soil samples. The pressure-plate experiments used coarse-grained samples having unit dry weights of  $17 \text{ kN/m}^3$ , which were prepared using similar procedures as those used for the fine-grained samples.

Finally, to investigate expected mineralogical alterations induced by the *Bacillus sphaericus* bacteria, a series of complementary experiments were carried out, including SEM and XRD measurements, on the treated and untreated samples. For the XRD tests, a D8 Advance (Bruker AXS Corporation, Karlsruhe, Germany) was used, and the SEM tests used a Cambridge S360 (Leica Cambridge, Cambridge, UK). The pore fluid chemistry (notably pH) and Atterberg limits were monitored before and after treatment.

## Results and Discussion

Results of the various experiments are now provided. First, the results of the soil water retention tests are presented, followed by how *Bacillus sphaericus* bacteria affected the soil physical and chemical properties. Then, the effects of bacteria on the soil microfabric and related changes in the water retention properties of the soil are described.

### Results of the Soil Water Retention Experiments

Results of the effect of *Bacillus sphaericus* on the treated soil samples are presented in two parts. At first, measurements of the matric suction of the samples treated with a 12% biological solution (6% bacterial solution and 6% calcium chloride) are presented, followed by those for experiments using a 14% biological solution (7% bacterial solution and 7% calcium chloride). The concentrations of bacterial solution and calcium chloride are expressed in terms of weight percentages of the dry weight of the soil. The soil water retention measurements are presented in terms of gravimetric water contents.

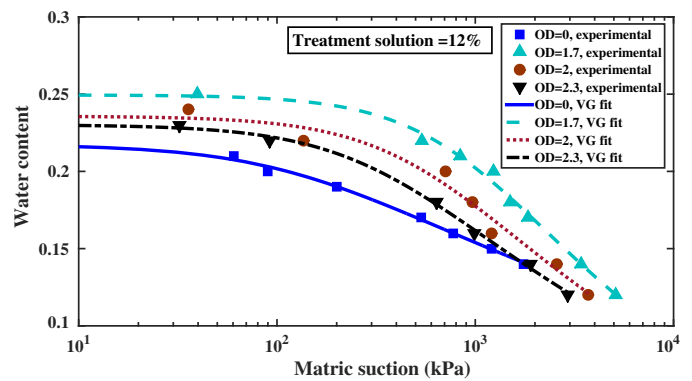


Fig. 2. Soil water retention curves of fine-grained samples mixed with 12% treatment solution, as obtained using the filter paper technique, at various bacterial densities. VG stands for the van Genuchten water retention equation.

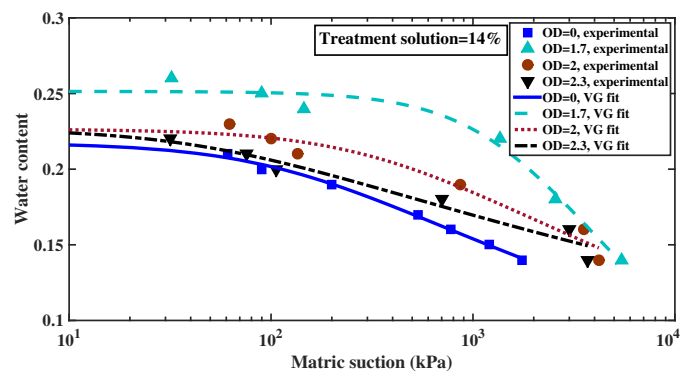


Fig. 3. Soil water retention curve of the fine-grained samples mixed with 14% treatment solution, as obtained using the filter paper technique, at various bacterial densities.

Gravimetric SWRCs of the samples treated with the 12% and 14% biological solutions are depicted in Figs. 2 and 3, respectively. In addition to the amount of treatment solution, the OD of the solution (as a measure of the bacterial concentration) was considered as an additional variable. Soil water retention curves were obtained at OD values of 0, 1.7, 2, and 2.3.

Observed water contents ( $w$ ) as a function of the suction head ( $h$ ) were analyzed in terms of the standard van Genuchten (1980) equation given by

$$w(h) = w_r + \frac{w_s - w_r}{[1 + (\alpha h)^n]^{1-1/n}} \quad h \geq 0 \quad (1)$$

where  $w_r$  and  $w_s$  = residual and saturated gravimetric water contents, respectively; and  $\alpha$  and  $n$  (with  $n > 1$ ) = quasi-empirical shape factors. The value of  $\alpha$  is approximately the inverse of the air-entry value of a soil. The water retention curves as fitted to the data using the RETention Curve (RETC) code of van Genuchten et al. (1991) are shown in Figs. 2 and 3. Values of the van Genuchten (VG) parameters obtained for the samples mixed with the 12% treatment solution are presented in Table 2, and those for the 14% treatment solution are listed in Table 3.

As shown in Figs. 2 and 3, by treating the soil with the culture media and bacterial solutions, the suction value required to drain the soil to a certain water content increases first as the SWRC moves upward, but then the curves move back and downward with

**Table 2.** van Genuchten hydraulic parameters obtained for biologically treated fine-grained soil sample mixed with 12% treatment solution

VG parameter	Optical density			
	0	1.7	2	2.3
$w_s$	0.217	0.249	0.236	0.230
$w_r$	0	0	0	0
$n$	1.17	1.43	1.36	1.30
$\alpha$ (kPa <sup>-1</sup> )	0.00722	0.00101	0.00162	0.00264

**Table 3.** van Genuchten hydraulic parameters obtained for biologically treated fine-grained soil sample mixed with 14% treatment solution

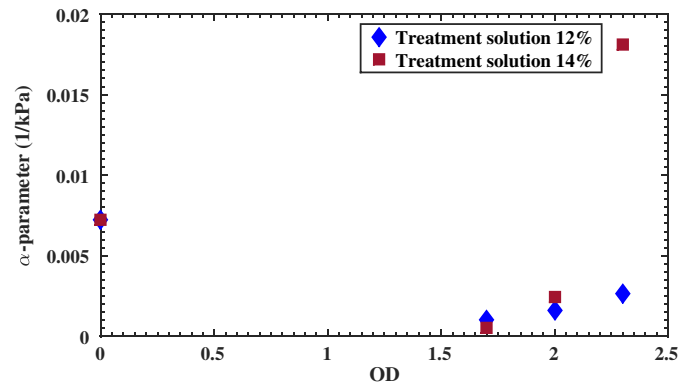
VG parameter	Optical density			
	0	1.7	2	2.3
$w_s$	0.217	0.251	0.226	0.227
$w_r$	0	0	0	0
$n$	1.17	1.52	1.18	1.10
$\alpha$ (kPa <sup>-1</sup> )	0.00722	0.00051	0.00242	0.0181

further increases in the bacterial concentration. The authors hypothesize that bacterial soil treatment affects the water retention properties of unsaturated soil through different biogeochemical processes. By increasing the bacterial concentration (from OD = 0 to 1.7), processes such as bioclogging and biocementation lead to smaller pore radii. Therefore, the number of pores with smaller radii that contribute to the same water content increases; consequently, the matric suction required to drain the soil to that water content rises. Furthermore, it is expected that the air-entry value (which is closely related to the pore-size distribution) will be altered.

Bioclogging and biocementation are accompanied by other biochemical processes, most notably by the effect of bacterial treatment on soil pH. As bacterial cell concentrations further increase and as the soil pH changes, another mechanism comes into play: the effect of biological treatment on the double diffuse layer of the soil. As the cell concentration increases, the ammonium and calcite production rates are affected. For example, Stocks-Fischer et al. (1999) demonstrated that at higher cell numbers of calcite-precipitating bacteria, the ammonium production rate decreases considerably, which means that at higher cell concentrations, the final pH will be less than that at lower cell concentrations. The lower the pH, the lower the diffuse double-layer thickness (Mitchell 1993; van Olphen 1991; Gratchev and Towhata 2013). The lower the double-layer thickness, the lower the water retention tendency (François and Ettahiri 2012).

However, care should be taken because the results of Stocks-Fischer et al. (1999) described the metabolism of *Bacillus pasteurii*, whereas the present study used *Bacillus sphaericus* (another calcite-precipitating bacteria). Nonetheless, one can at least speculate that the changes in the water retention curves, first upward and then downward, can be attributed to the twofold influence of the cell concentration on soil pH and precipitation-induced pore clogging, including their competition. Additional evidence for the change in soil pH of the treated soil are presented separately in subsequent sections where the biochemical reactions involved are also summarized.

The presence of bacteria and their active involvement in the biogeochemical processes operative in a complex clay-water environment involves the two processes causing the forward and then backward (nonmonotonic) movement of the SWRCs. Variations in the  $\alpha$ -parameter for the biologically treated clay mixed with



**Fig. 4.** Effect of different treatment solutions and bacterial concentration on the  $\alpha$ -parameter of fine-grained soil (as obtained using the van Genuchten retention model).

12% and 14% treatment solutions are depicted in Fig. 4. The  $\alpha$ -parameter, which is closely related to the inverse of the soil air-entry value, is of importance when considering the hydromechanical behavior of unsaturated soils. The two treatments (12% and 14%) show different trends in the value of  $\alpha$  with increasing OD values (bacterial concentrations), which are attributed to the competition between different biochemical processes and their effect on soil fabric and chemistry.

For fine-grained soils, it is important to investigate how both capillary water and adsorbed water are influenced by microbial calcite precipitation. Although van Genuchten's classical model provides a general understanding of how retention properties are affected, another model is needed to account for adsorbed water. For this purpose, the model proposed by Lu (2016) was used to investigate the effect of *Bacillus sphaericus* on the adsorbed water content of fine-grained soil samples. The model of Lu (2016) is expressed as follows:

$$w(h) = w_{ac} \left\{ 1 - \left[ \exp\left(\frac{h - h_{\max}}{h}\right) \right]^m \right\} + 0.5 \left[ 1 - \operatorname{erf}\left(\sqrt{2} \frac{h - h_c}{h_c}\right) \right] \times \left[ w_s - w_{ac} \left\{ 1 - \left[ \exp\left(\frac{h - h_{\max}}{h}\right) \right]^m \right\} \right] [1 + (\alpha h)^n]^{\frac{1}{n}-1} \quad (2)$$

where  $w_s$  = saturated water content, as before;  $1/\alpha$  = air-entry suction;  $n$  = pore-size distribution coefficient;  $h_c$  = cavitation suction;  $h_{\max}$  = highest suction;  $w_{ac}$  = adsorption capacity; and  $m$  = adsorption strength. Whereas the original formulation of Lu (2016) is expressed based on volumetric water content, here, the same formula in terms of gravimetric water content will be used. When all parameters of Lu's (2016) equation are obtained, one can determine the adsorbed water content using

$$w_a(h) = w_{ac} \left\{ 1 - \left[ \exp\left(\frac{h - h_{\max}}{h}\right) \right]^m \right\} \quad (3)$$

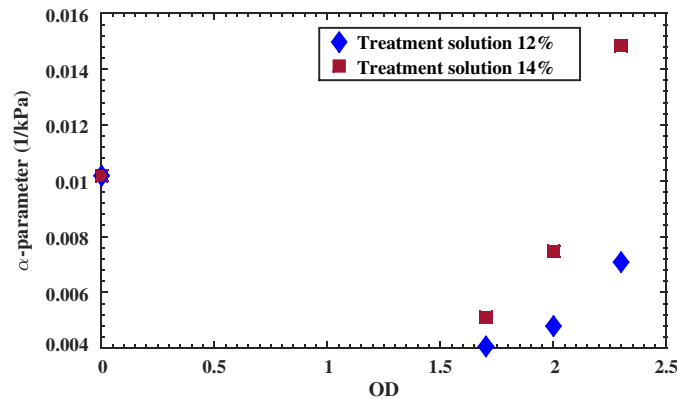
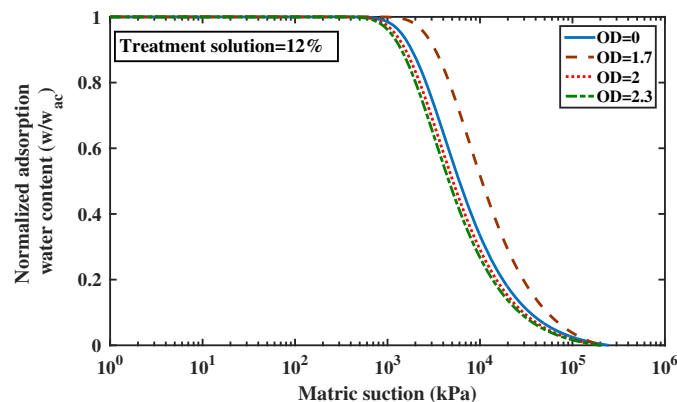
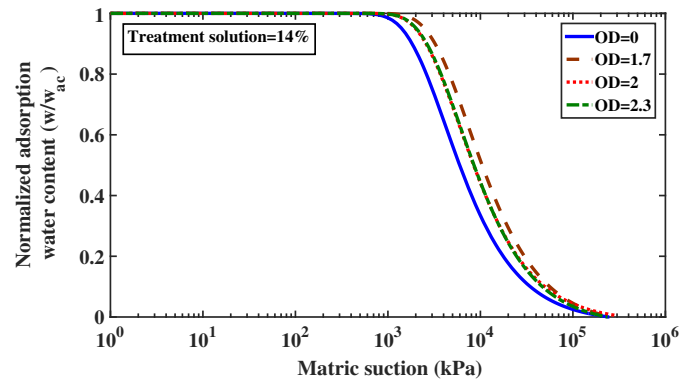
Values of the Lu (2016) parameters obtained for the samples mixed with the 12% treatment solution are presented in Table 4, and those for the 14% treatment solution are listed in Table 5. Fig. 5 presents the variation in the  $\alpha$ -parameter as obtained using Lu's (2016) method versus optical density. The  $\alpha$ -parameter values obtained with the Lu (2016) method followed the same pattern as

**Table 4.** Values of the Lu (2016) model parameters obtained for biologically treated fine-grained soil sample mixed with 12% treatment solution

Lu parameters	Optical density			
	0	1.7	2	2.3
$h_{\max}$ (kPa)	$2.469 \times 10^5$	$2.011 \times 10^5$	$2.016 \times 10^5$	$2.013 \times 10^5$
$h_c$ (kPa)	645.7	1,422	192.8	186.3
$w_{ac}$	0.154	0.1565	0.1867	0.174
$m$	0.01718	0.03812	0.01797	0.01626
$n$	1.544	1.223	5.408	7.239
$\alpha$ (kPa $^{-1}$ )	0.01017	0.004077	0.004782	0.007089

**Table 5.** Values of the Lu (2016) model parameters obtained for biologically treated fine-grained soil sample mixed with 14% treatment solution

Lu parameters	Optical density			
	0	1.7	2	2.3
$h_{\max}$ (kPa)	$2.464 \times 10^5$	$2.589 \times 10^5$	$2.009 \times 10^5$	$2.358 \times 10^5$
$h_c$ (kPa)	645.9	1,592	532.4	4,312
$w_{ac}$	0.154	0.1868	0.191	0.1786
$m$	0.01721	0.02913	0.0302	0.026
$n$	1.544	1.125	4.937	2.262
$\alpha$ (kPa $^{-1}$ )	0.01017	0.005094	0.007485	0.01483

**Fig. 5.** Effect of different treatment solutions and bacterial concentration on the  $\alpha$ -parameter of fine-grained soil [as obtained using the Lu (2016) retention model].**Fig. 6.** Adsorbed retention curve for the fine-grained soil samples mixed with 12% treatment solution.**Fig. 7.** Adsorbed retention curve for the fine-grained soil samples mixed with 14% treatment solution.

those determined using van Genuchten's equation. Figs. 6 and 7 illustrate the adsorbed water content curves. As indicated in these figures, although there is an increase in adsorbed water until an optical density of 1.7, the values tend to decrease by increasing the bacterial concentration. As mentioned previously, such a pattern is attributed to the competition and effect of different mechanisms. The involved mechanisms are listed in Table 6.

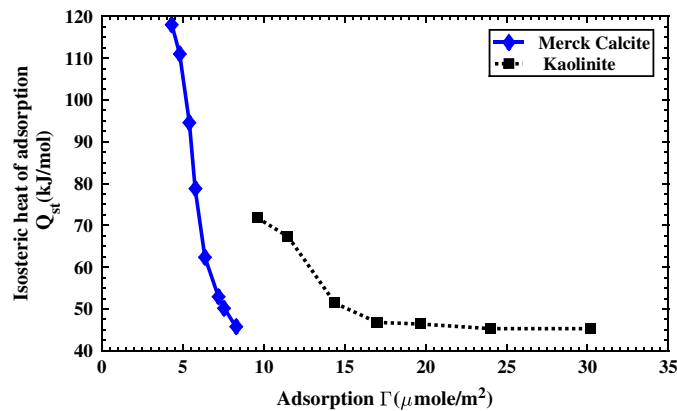
At lower bacterial concentrations, the effect of precipitated calcite crystals is dominant relative to the other mechanisms. The effects of precipitated calcite crystals are twofold. Calcite crystals precipitated on grains and in pores reduce the size of pores and increase the suction required for the air phase to invade the partially clogged pores. The precipitated calcite on the grains can, in fact, increase their surface water adsorption. A comparison between the adsorption energy of calcite and kaolinite presented by Ohkrimenko et al. (2013) can shed light onto this effect (Fig. 8). Furthermore, as stated by Ercole et al. (2007) *Bacillus sphaericus* produces extracellular polymeric substances (EPS) in addition to calcite. Having a spongelike microstructure, EPS has been shown to have a considerable water-holding capacity, as much as 10 or more times of its dry weight (e.g., Zheng et al. 2018). The high affinity of precipitated calcite and the water-holding capacity of EPS can lead to the observed increase in the adsorbed water content.

Precipitation of calcite and other biological byproducts (such as EPS) on the surface of grains additionally can increase the grain surface roughness, thereby increasing the specific surface area as well as the water film thickness on the grains. There is much evidence in the literature that an increase in surface roughness will increase the water film thickness (Kibbey 2013; Zheng et al. 2015). Given a relationship proposed by Tuller and Or (2005) connecting the soil capacity for adsorbing water ( $\theta$ ) and the soil specific surface area (i.e.,  $\theta = h \times SA \times \rho_w$ , where  $h$  is water film thickness,  $SA$  stands for specific surface area, and  $\rho_w$  is water density), one would expect the adsorbed water content to increase. All of these mechanisms are in favor of increasing the adsorbed water content. As stated previously, the pore-size reduction as a result of the production of EPS and calcite leads to an increase in capillary water retention, and hence the water retention capacity of a soil.

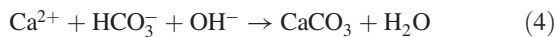
However, by increasing calcite precipitation (because of the increase in bacterial concentration), a lower pH is attained in the soil-water-bacteria system, which for the case of fine-grained soils affects the double layer in two ways. The lower pH directly results in a lower thickness of the double layer and consequently lower water retention. In addition, the dynamics of calcite precipitation can be affected by pH. Because pH is known to affect the precipitation-solution

**Table 6.** Processes affecting the water retention properties of fine-grained soils in MICP technique

Process	Cause	Effect
Reduction in soil pore and throat radii	Bioclogging due to precipitation of calcite and EPS on and among grains	Increase in water retention
Increase in grain roughness	Precipitation of calcite and EPS on the surface of grains	Increase in water film thickness and adsorbed water retention
Changing grain surface adsorption properties	Precipitation of calcite and EPS (i.e., high water-holding capacity of EPS and adsorption affinity of calcite)	Increase in adsorbed water retention
Change in surface wettability	Precipitation of calcite and EPS on the surface of grains	Depending on the amount of EPS and calcite, different contact angles can be achieved
Change in double-layer thickness	Change in pH induced by bacterial metabolism and calcite precipitation	Reduction in adsorbed water retention due to pH decrease
Precipitation-solution dynamics of calcite in favor of the presence of free $\text{Ca}^{2+}$ cations, and their substitution in clay structure	Change in pH induced by bacterial metabolism and calcite precipitation	Reduction in adsorbed water retention due to pH decrease

**Fig. 8.** Comparison of the adsorption energies of calcite and kaolinite. (Data from Ohkrimenko et al. 2013.)

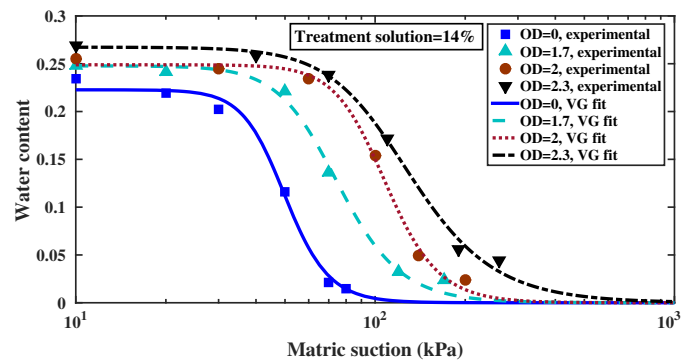
dynamics of calcite [Eq. (4)], at lower pH values, the rate of calcium precipitation is reduced (Krauskopf and Bird 1995)



For these reasons, not all calcium cations would be consumed and precipitated. Although the resulting free  $\text{Ca}^{2+}$  cations will not influence a coarse-grained soil, for fine-grained soils, they will substitute cations present in the double layer and, consequently, decrease its thickness. The cation substitution mechanism has also been proposed to influence the retention properties of lime-treated and cement-treated soils (Thudi 2007).

Several other processes, generally having less influence, may also be operative. They include an increase in viscosity of adsorbed water due to EPS (Roberson and Firestone 1992; Zheng et al. 2018) and changes in the contact angle of the grain surface due to precipitation of EPS and calcite, which seem to generally lead to higher contact angles [e.g., Ethington (1990) reported values of the contact angle on pure calcite substrate]. Of course, the exact contact angle would depend on the amount of precipitated EPS and calcite on the grains, which requires additional experiments (e.g., by means of environmental scanning electron microscopy or X-ray microtomography), possibly in future studies.

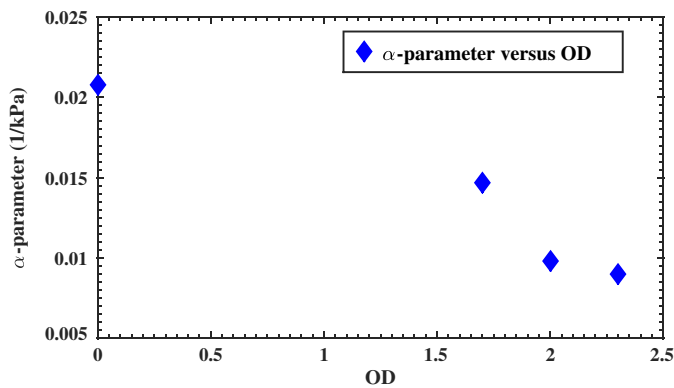
Still, the competition of the various processes discussed in preceding paragraphs appear to explain the patterns observed in the adsorbed water content curves as obtained with the Lu (2016) model for the fine-grained soil samples.

**Fig. 9.** Soil water retention curves of the coarse-grained samples mixed with 14% treatment solution obtained with pressure-plate tests at various bacterial densities.

In order to further distinguish the effects of the aforementioned mechanisms (i.e., bioclogging-induced changes in the soil pore network and pH-induced changes in the double-layer thickness, among others), the SWRCs of the coarse portion of the soil samples (before and after treatment with the 14% biological solution) were obtained. For the coarse-grained soil, the dominant retention mechanism is capillarity, and the pH-induced changes in the soil double layer should be absent, which would lead to a monotonic change in the air-entry value and consequently the  $\alpha$ -parameter. The SWRCs of the coarse portion of the soil sample (passing a No. 40 sieve but retained by a No. 200 sieve, which is designated as sand) are shown in Fig. 9. The parameters of the van Genuchten equation for this soil sample are presented in Table 7. The variation in  $\alpha$  versus bacterial concentration is illustrated in Fig. 10. As expected, in the absence of double-layer effects, the first hypothesized mechanism (bioclogging and biocementation) is the sole

**Table 7.** van Genuchten parameters obtained for coarse-grained soil samples treated with 14% biological solution

VG parameter	Optical density			
	0	1.7	2.0	2.3
$w_s$	0.223	0.248	0.249	0.267
$w_r$	0	0	0	0
$n$	6.32	4.56	5.31	3.50
$\alpha$ ( $\text{kPa}^{-1}$ )	0.0208	0.0144	0.00973	0.00880



**Fig. 10.** Variation in the van Genuchten  $\alpha$ -parameter as a function of OD (sand sample and 14% treatment solution).

mechanism causing an increase in air-entry value (a decrease in  $\alpha$ -parameter), meaning a forward movement of the curves.

### Effect of Bacteria on Soil Properties and Microfabric

#### Atterberg Limit Test

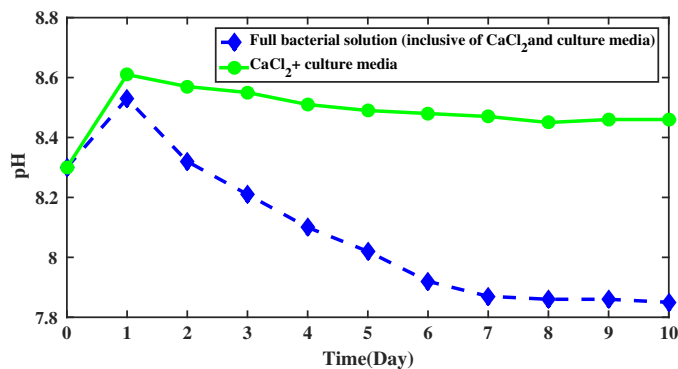
Table 8 presents the effects of biological treatment (with 14% biological solution, OD = 2.3) on the plasticity properties of the soil in terms of the Atterberg limits as obtained with ASTM D4318 (ASTM 2017) standard tests. The results reveal that the liquid limit reduced considerably as a result of biological stabilization, but that the plasticity limit was not noticeably affected. The change in the plasticity index is induced mainly by changes in the liquid limit. This finding confirms the proposed mechanisms that biological treatment of the fine-grained soil not only causes changes in the pore-size distribution and soil porous structure, but also induces biochemical processes affecting the clay mineral-water system. These biogeochemical effects (pH changes, double-layer alterations, and pore fluid chemistry alterations) contribute to changes in the water adsorption potential. They eventually alter both the Atterberg limits as well as the soil water retention curves, which both are sensitive to changes in the soil microfabric. To further elaborate the effects of biological treatment on the soil microfabric and to determine possible biogeochemical interactions, pH measurements and XRD and SEM analyses were performed. Results are discussed in subsequent sections.

#### Soil Pore Fluid Chemistry: Measurement of Soil pH

In order to monitor soil pore fluid geochemical changes, variations in the pH of two separate soil samples were investigated. The tests involved one soil sample treated with the culture media and CaCl<sub>2</sub> only, and another sample treated with the 14% biological solution (OD = 2.3) in addition to the culture media and CaCl<sub>2</sub> (precipitating agent). Results are presented in Fig. 11. The initial pH of the solution containing culture media and CaCl<sub>2</sub> was 8.0, and the untreated soil sample had a pH of 8.3.

**Table 8.** Atterberg limits of fine-grained samples treated with 14% biological solution (OD = 2.3) and untreated samples

Soil property	Untreated sample	Treated sample
Liquid limit (%)	41	30
Plastic limit (%)	17	16
Plasticity index (%)	24	14



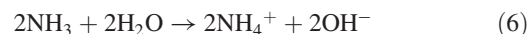
**Fig. 11.** Variations in the pore fluid pH with time following treatment.

Consistent with previous studies (e.g., van Paassen 2009; Al Qabany et al. 2012), the results in Fig. 11 indicate that the urease enzyme is responsible for the initial increase in soil pH, whereas calcite precipitation later tends to lower the pH back to neutral, with the actual final pH of the soil sample depending upon the reaction rates and substrate concentrations. As Fig. 11 indicates, for the mixture of the full bacterial solution and soil, a noticeable decrease in pH occurred, which can be attributed to the formation of calcite crystals. The decreasing pattern in pH with time is not that pronounced when the soil is treated with a solution containing only the culture media and CaCl<sub>2</sub>.

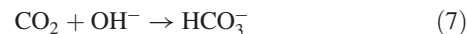
To better understand the effects of biochemical processes on the observed soil pH changes, the MICP reactions are summarized as follows. The hydrolysis of urea is



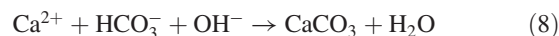
Decomposition of NH<sub>3</sub> and creation of hydroxyls is



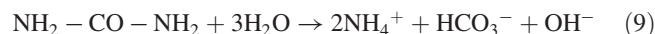
Creation of carbonic acid as a result of CO<sub>2</sub> production by bacteria is



CaCO<sub>3</sub> precipitation is

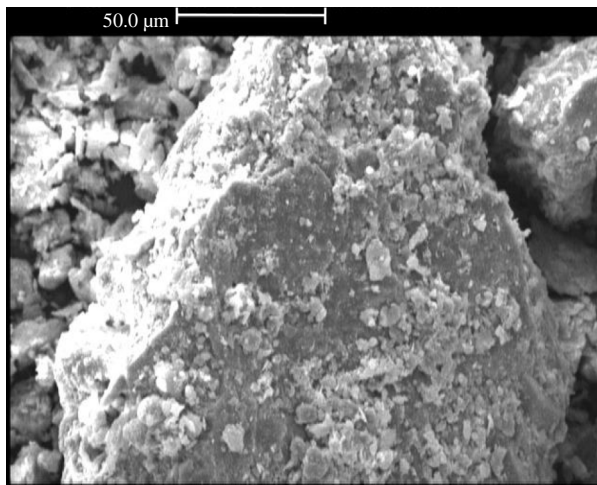


Net urea hydrolysis reaction is



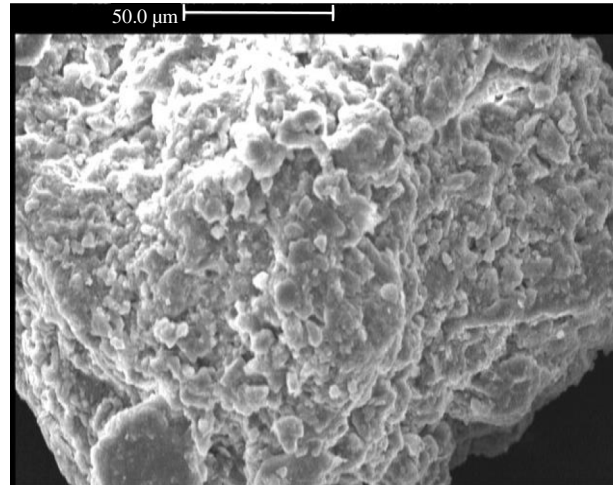
As Fig. 11 shows for the soil treated with the full bacterial solution, the pH first rises to 8.5, which can be attributed to decomposition of urea and the production of OH<sup>-</sup>. But after this, a decrease in the trend occurs due to consumption of OH<sup>-</sup> during the precipitation process [Eqs. (5)–(9)], leading to a pH of 7.8 after 10 days. For the sample mixed only with the culture media, the decrease in pH is considerably less than that of the fully treated sample. The presence of calcium cations in the solution containing the culture media and CaCl<sub>2</sub> (treatment solution without bacteria) causes a consumption of hydroxyl ions. The formation of calcite crystals, with the accompanying consumption of hydroxyl ions and decreasing pH values, is intensified by the presence of *Bacillus sphaericus*, which is indicative of the catalyzing role of bacteria and their potential impact on clay-water interactions.

L-SE1 EHT=20.0 KV WD=11 mm MAG = X 600. PHOTO=0



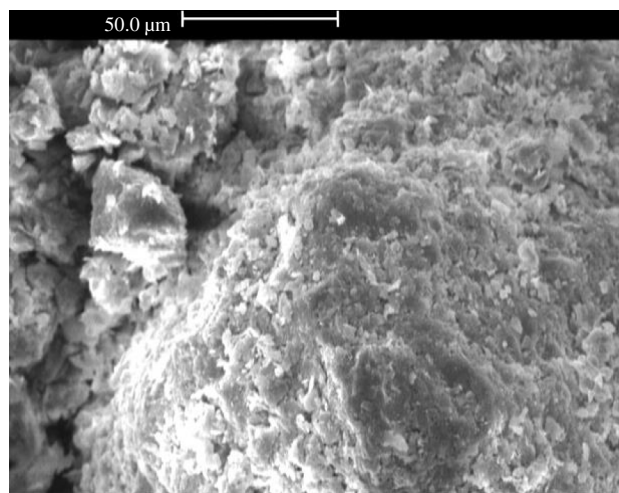
(a)

L-SE1 EHT=20.0 KV WD=12 mm MAG = X 600. PHOTO=0



(b)

L-SE1 EHT=20.0 KV WD=10 mm MAG = X 600. PHOTO=0



(c)

**Fig. 12.** SEM micrographs of (a) untreated sample; (b) treated sample after 4 days (treated with 14% biological solution, OD = 2.3); and (c) treated sample after 60 days (treated with 14% biological solution, OD = 2.3).

### Imaging with Scanning Electron Microscopy

SEM images were used to investigate the presence of  $\text{CaCO}_3$  crystals. SEM monographs of, respectively, the untreated sample and samples after treatments of 4 and 60 days (using the 14% biological solution, OD = 2.3) are presented in Figs. 12(a–c). The plots show that the amount of produced calcite crystals increases as time progresses. Calcite precipitation on the soil grains (biocementation) and into the pore space (bioclogging) causes a decrease in the pore-size distribution, the primary mechanism for the observed changes in the soil water retention curves. The resulting change in water retention of the fine-grained soils is mainly a function of the competition between the two proposed mechanisms of biocementation as well as changes in a double-layer thickness of the fine-grained soils.

### X-Ray Diffraction

XRD analyses were further carried out to identify the composition of the precipitates. Both treated and untreated samples were investigated for this purpose to explore the relative changes in soil

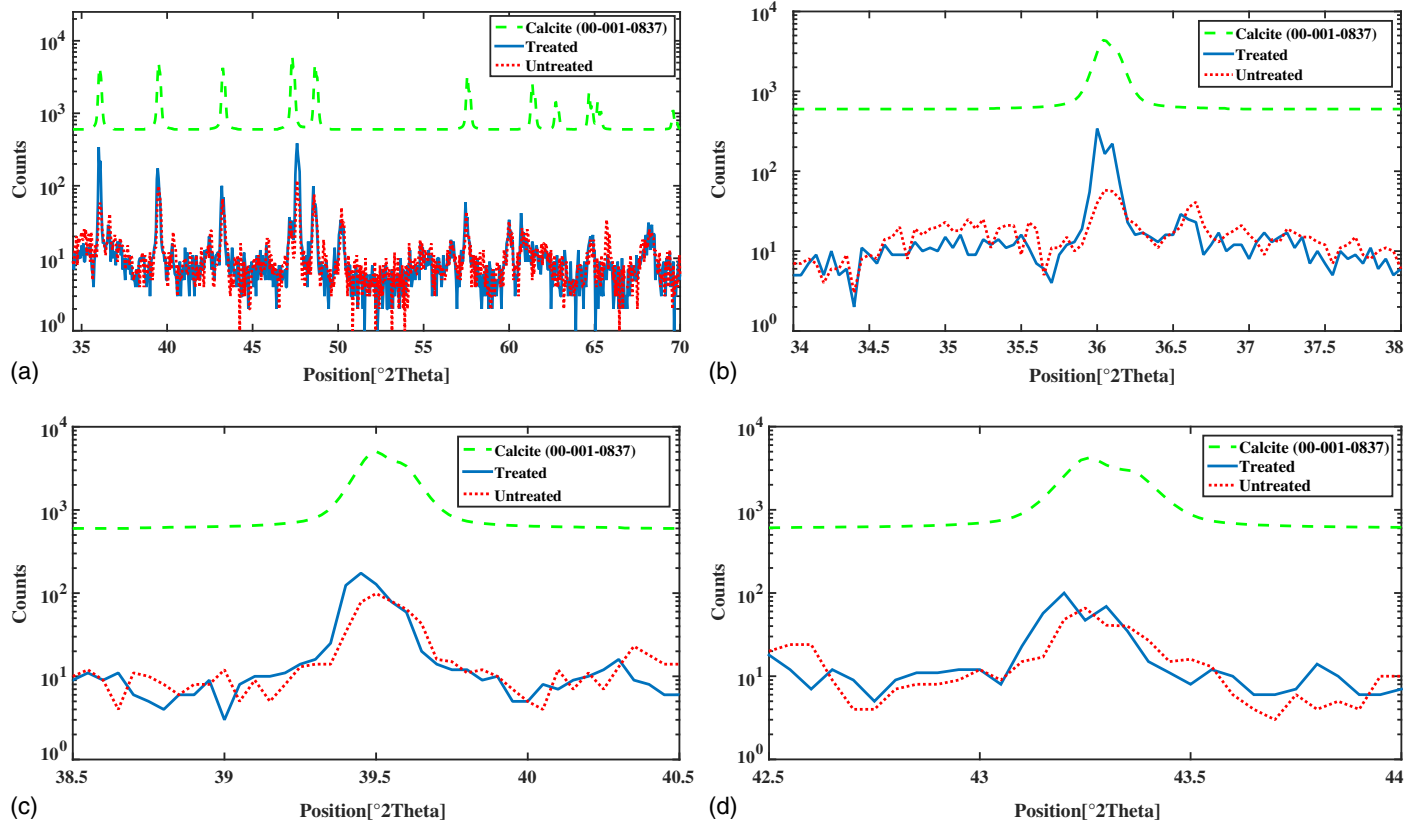
composition. Particular attention was paid to identifying calcite, which presumably is the main material produced by biological treatment.

To determine whether or not a certain compound is present in a sample, three main peaks of that compound in the standard XRD patterns should be compared with the peaks found in the XRD pattern of that sample (Sanches et al. 2003; Sun et al. 2015). If they are present, the soil contains that compound. Increasing and decreasing peak values then demonstrate an increase or decrease in the amount of that compound in the soil sample, respectively. Comparing the XRD monographs of the sample treated with the biological solution (14%, OD = 2.3) and the untreated sample (Fig. 13) indicates that calcite crystals are present in both samples, but that the amount had increased remarkably in the treated sample.

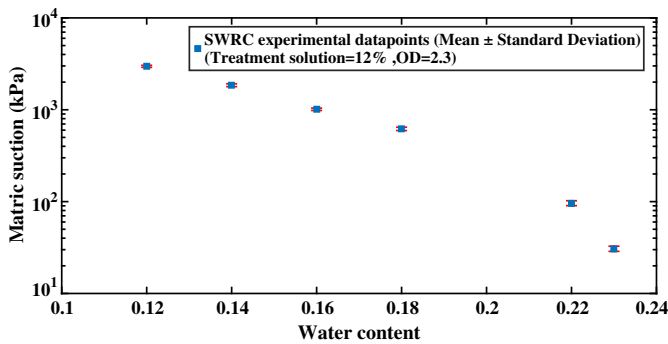
### Possible Experimental Errors and Their Importance

Performing soil water retention tests, especially on fine-grained soils is, per se, a time-consuming task. In addition, investigating

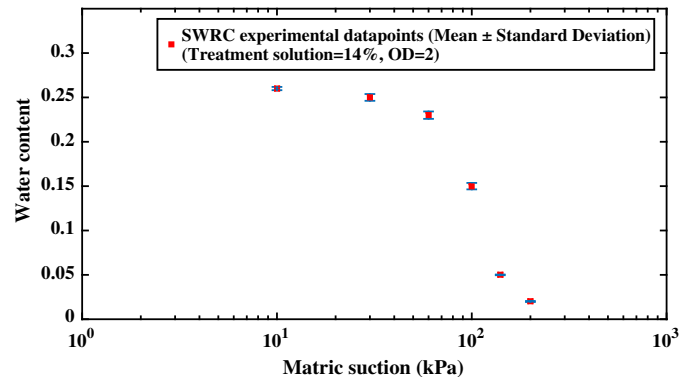




**Fig. 13.** (a) XRD patterns of the untreated samples and samples treated with 14% biological solution, OD = 2.3, and calcite; (b) XRD patterns of the first main peak; (c) XRD patterns of the second main peak; and (d) XRD patterns of the third main peak.



**Fig. 14.** Extent of errors in the soil water retention curve of the fine-grained samples mixed with 12% treatment solution as obtained from filter paper tests at OD = 2.3.



**Fig. 15.** Extent of errors in the soil water retention curve of the coarse-grained samples mixed with 14% treatment solution as obtained from pressure-plate tests at OD = 2.

various factors considered in this study requires a multitude of experiments, which added to the complexity of the experimental design. Therefore, given the available laboratory and time resources, several control experiments were performed to inspect the extent to which experimental errors could influence the obtained parameters and, consequently, this study's conclusions. For this purpose, extra filter paper tests were performed on the fine-grained soil sample treated with 12% biological solution having an OD value of 2.3. Furthermore, pressure-plate tests were performed on the sand sample treated with 14% biological solution having an OD value of 2.

Because the moisture content of the sample can be controlled and the suction is measured for filter paper tests, whereas for

the pressure-plate test, the intended suction value is imposed and the water content is measured, the error bars are considered for suction and water content for these two tests, respectively (Figs. 14 and 15). Furthermore, the relative errors between the obtained retention parameters of the original and control experiments (replicates) were obtained and are presented in Tables 9 and 10. As can be seen, the relative errors in the retention model parameters are fairly low (maximum of 3.4%), which indicates a good level of repeatability (reliability) of the obtained retention data and parameters. However, given the complex interplay of soil mineral

**Table 9.** Soil water retention parameters obtained for biologically treated fine-grained soil sample mixed with 12% treatment solution (OD = 2.3)

VG parameter	Original experiment	Repeated test case	Relative error (%)
$w_s$	0.2302	0.2308	0.3
$w_r$	0	0	0
$n$	1.30	1.29	0.8
$\alpha$ (kPa <sup>-1</sup> )	0.00264	0.00273	3.4

**Table 10.** van Genuchten parameters obtained for biologically treated coarse-grained samples mixed with 14% treatment solution (OD = 2)

VG parameter	Original experiment	Repeated test case	Relative error (%)
$w_s$	0.249	0.252	1.2
$w_r$	0	0	0
$n$	5.31	5.14	3.2
$\alpha$ (kPa <sup>-1</sup> )	0.00973	0.01002	2.98

characteristics, the soil-water-bacteria environment, and the biochemical factors involved in MICP, further studies with various clay mineralogical properties and soil types are recommended so as to broaden the scope and applicability of the results and to provide a more in-depth analysis of the observed patterns and mechanisms involved.

## Conclusions

This study investigated the effects of biological treatment using *Bacillus sphaericus* on the water retention properties of a fine-grained soil (low-plasticity clay) and a coarse-grained soil (sand). Results demonstrated that biological treatment alters the soil water retention curves of the fine-grained and coarse-grained samples in different ways. By increasing the bacterial concentration, the air-entry value of the SWRCs (or van Genuchten  $\alpha$ -parameter) of the coarse-grained soil monotonically increased (decreased), whereas for the fine-grained soil, the air-entry value first increased and then decreased. For the latter case, at a certain saturation, the value of suction required to drain the soil at that saturation hence first increased and then decreased.

It was hypothesized that two mechanisms govern the observed behavior in fine-grained soils: biocementation, which clogs pores of smaller radii and thus causes SWRCs to shift forward and up, and biogeochemical processes caused by the additional presence of bacteria, which lead to a decrease in the diffuse double-layer thickness and lower matric suctions at the same fluid saturation. Atterberg limit tests further confirmed the effect of bacteria on the water adsorption tendency of soil, and XRD and SEM tests indicated calcite precipitation and changes in the microfabric of the soil samples.

The conducted tests were limited to one relatively fine-textured and one relatively coarse-textured sample. More extensive tests are required to investigate the effects of microbial-induced calcite precipitation on soils with different grain-size distributions and different mineralogy and to investigate in depth other treatment scenarios. Finally, the use of environmental scanning electron microscopy (ESEM) and X-ray microtomography on the treated samples is recommended to further explore the different processes discussed in this study.

## Appendix. Durability of MICP Effects: Limitations, Scope of Application, and Future Perspectives

One may ask the extent to which the effects induced by MICP are durable. Although the primary focus of this study was not the durability of MICP, this aspect has practical significance and is worthy of some comments. The use of MICP as a soil improvement technique aims for changes in soil fabric and structure in order to improve soil engineering properties (e.g., shear strength, collapse potential, and unconfined compressive strength). For such applications, the main concern is not necessarily the longevity of the bacteria but the durability of the calcite precipitated in the sample (and the structure formed therein) against severe environmental conditions. Cheng et al. (2016) examined biologically treated sand columns where only 0.7 g weight loss was observed when the samples were exposed to 12 L of acid rain, corresponding to 5 years of rainfall (1,000 mm/year). Salifu et al. (2016) investigated the erosion mitigation of sandy soil foreshore slopes against tidal waves. MICP treatment of the slopes resulted in negligible erosion and no change in the slopes, whereas the untreated slopes collapsed to the angle of repose at the first tidal event, followed by the erosion of 0.2° of the slope angle per tidal event during subsequent tidal events. Moravej et al. (2018) further investigated the potential of using MICP to improve dispersive soils. They found that MICP was capable of decreasing the level of dispersivity of the soils involved. These studies clearly demonstrate the durability of MICP effects on the soil structure for different environmental conditions. The use of MICP as a protective layer in the exterior of historical monuments adds further evidence of its potential level of durability (Perito et al. 2014).

There are of course optimal environmental conditions for MICP process, which include appropriate temperature and pH ranges. Kim et al. (2018) showed that different calcite precipitation-inducing microorganisms express different sensitivity levels to environmental conditions (notably pH and temperature). They found that the optimal temperature for MICP processes was 30°C. However, calcite precipitation did not stop at higher temperatures. Moreover, once calcite precipitates are formed, they are durable under extreme environmental conditions (rainfall and temperature, among others).

MICP has also shown considerable success in field trials aimed at ground reinforcement (van Paassen et al. 2009, 2010; DeJong et al. 2014; Gomez et al. 2015; Esnault-Filet et al. 2016; Terzis and Laloui 2017). However, applications of MICP face a great challenge in terms of the nonheterogeneous distribution or penetration of treatment across and into soil volumes. New procedures have to be formulated and further studies are required when improving soil mechanical and hydraulic properties for relatively large soil volumes is concerned. Furthermore, in order to treat large volumes of soil, more economic methods for producing the ingredients of the culture media are needed. A possible alternative to deal with complex on-site bacteria cultivation is to use MICP utilizing lyophilized cells instead of bacterial cells in their vegetative state (Terzis and Laloui 2018). A thorough review of MICP, and limitations encountered when upscaling to field conditions has been given by DeJong et al. (2013) and Haouzi and Courcelles (2018).

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