Factors Affecting Efficiency of Microbially Induced Calcite Precipitation

Ahmed Al Qabany[1]; Kenichi Soga, M.ASCE[2]; and Carlos Santamarina, A.M.ASCE[3]

Abstract: Microbially induced carbonate precipitation (MICP) using ureolytic bacteria shows promise in the field of geotechnical engineering for several different applications, such as ground improvement and groundwater control. This study examined optimal use and efficient control of *Sporosarcina pasteurii* to induce the precipitation of $CaCO_3$ in open environments. Laboratory tests were conducted to investigate the effect of changing treatment factors, such as chemical concentrations, retention times, and effective input rates (mol/L/h) on chemical efficiency. Chemical efficiency was measured based on weight measurements of $CaCO_3$ precipitation compared with the amount of chemical reactants injected to samples. Based on the experimental results, the optimal time required for the precipitation process to take place in porous media for a specific range of bacterial optical density was determined. Results show that, below a certain urea and $CaCl_2$ input rate (0.042 mol/L/h) and for a bacterial optical density (OD_{600}) between 0.8 and 1.2, the reaction efficiency remained high and the amount of precipitation was not affected by the liquid medium concentration (for input concentrations up to 1 M). However, the precipitation pattern at the pore scale was found to be affected by the injected concentration. Scanning electron microscopy images taken of different samples at different levels of cementation showed that, for the same amount of precipitation, the use of lower chemical concentrations in injections resulted in better distribution of calcite precipitation, especially at lower cementation levels. This variation in precipitation pattern is expected to affect the use of MICP for different applications. **DOI: 10.1061/(ASCE)GT.1943-5606.0000666.** © *2012 American Society of Civil Engineers.*

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Introduction

Interest in the use of biological technologies in geotechnical engineering has been rising over the past few years (Mitchell and Santamarina 2005). One technology that has shown some promise is the use of bioactivity in sand cementation via calcium carbonate precipitation, namely, microbially induced calcite precipitation (MICP). The most commonly used type of MICP is passive precipitation, where the pH of the system is changed as a result of bacterial activity (usually urea hydrolysis), carbonate ions are produced, and then chemical precipitation of $CaCO_3$ takes place inside the soil pore space in the presence of calcium ions.

Certain bacterial species produce urease enzyme, which is responsible for raising the pH of the system and the production of carbonate ion. The process starts by the hydrolysis of urea:

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ \quad (1)$$

When pH is above 8.3, calcium carbonate starts precipitating with an increasing rate up to pH = 9.0 (Stocks-Fischer et al. 1999; DeJong et al. 2006), where precipitation tends to lower pH back to neutral. However, the actual final pH of the solution depends on the reaction rates and substrate concentrations (van Paassen 2009).

$$HCO_3^- \rightarrow CO_3^{2-} + H^+ \quad (2)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow \quad (3)$$

The MICP process is defined as 100% efficient when all available urea is hydrolyzed by the bacteria and then the produced carbonate and present calcium precipitate as calcium carbonate at the targeted volume. In contrast, chemical efficiency is defined as the main injected chemicals (calcium and urea) precipitating as calcium carbonate. Thus, a 100% chemical efficiency does not ensure 100% overall process efficiency unless precipitation takes place in the target location.

Sporosarcina pasteurii (formerly known as *Bacillus pasteurii*) is a bacterial species that is well known for producing urease and hydrolyzing urea to form ammonia and bicarbonate ions, and was thus used in this study. *Bacillus* species (in which *S. pasteurii* was formerly classified) are known for their ubiquity in nature and their high resistance to chemical and physical agents, which enables their use in open environments in the field (Todar 2005). A study by Stocks-Fischer et al. (1999) is one of the earlier works that examined the kinetics of the microbial process as well as the effect of different factors, such as pH and bacterial growth, on the rate of precipitation. Other researchers investigated using a different type of bacterium, *Pseudomonas denitrificans*, for potential biological

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soil reinforcement via denitrification, which induces the precipitation of calcite (Karatas et al. 2008; van Paassen et al. 2009). However, despite that process being successful, some organic substrates used in the denitrification process are less soluble than those used for the urea hydrolysis process, which is a major challenge for using it in situ compared with ureolytic MICP (van Paassen et al. 2009).

Use of MICP has been proposed for several geotechnical engineering applications: (1) biogrout that increases the shear strength of the soil to enhance foundation bearing capacity and slope stability, and to facilitate excavation and tunneling (Whiffin et al. 2007; van Paassen 2009); (2) soil improvement against soil liquefaction during earthquakes (DeJong et al. 2006); and (3) foundation settlement reduction (Martinez and DeJong 2009). However, for successful use of MICP in engineering applications it is necessary to determine the most effective chemical treatment for conducting MICP, as well as to optimize the process to enable its use under different ground conditions.

Several factors must be considered to enable the use and control of the MICP process in field applications, including the concentrations of the chemical reactants, as well as methods to introduce the bacteria and these chemical reactants to the reaction medium. For example, clogging at locations close to the nutrient injection points needs to be prevented, especially at low injection rates; this is considered a major problem in terms of utilizing the process in different setups. Whiffin et al. (2007) conducted an experiment wherein bacteria and chemical reactants were applied to a 5-m-long tube and demonstrated that clogging could be prevented at the injection point using an injection rate as low as 350 mL/h. Harkes et al. (2010), who extended this work, suggested that injection of bacterial suspension followed by cementation fluid gave a homogeneous distribution of bacterial activity and calcite precipitation. It was also noted by Harkes et al. that the salinity of bacterial injection solutions or reactant solutions injected afterwards had a large impact on the retention of the bacteria in the soil. In that same study (Harkes et al. 2010), using high-salinity solutions such as a 0.05 M $CaCl_2$ solution, was found to stimulate adsorption and flocculation of the bacterial cells, whereas the use of a low-salinity solution, such as fresh surface water, resulted in stimulating transport and remobilization of these cells. Applying this optimized technique in large-scale experiments (treatment volume of up to 100 m^3), van Paassen et al. (2010) demonstrated that the use of biogrouting is technically feasible under conditions commonly found in practice.

Precipitation of $CaCO_3$ accumulates as more chemicals are injected to the soil, and the most efficient condition is when all of the chemical reactants (urea and $CaCl_2$) precipitate as calcium carbonate. This study aimed to find a chemical delivery technique that provides a high chemical efficiency. Laboratory tests were performed to examine the effect of different factors, such as chemical concentrations and retention times, on the chemical efficiency. Results were normalized in terms of the effective input rate of urea and $CaCl_2$ (i.e., mol/L/h), and the investigation also included whether the chemical efficiency was constant throughout the MICP process or variable at different degrees of cementation. Based on the findings, recommendations to achieve a high chemical efficiency are given.

Materials and Methods

Bacterial Media and Growth

Sporosarcina pasteurii [American Type Culture Collection (ATCC) 11859] was used in all experiments due to its high ability to synthesize urea. The cells were obtained from ATCC biomaterials, grown on ATCC 1376 NH_4-YE media plates, and incubated at 30°C. All ingredients [20 g of yeast extract, 10 g of $(NH_4)_2SO_4$, and 20 g of agar in 0.13 M Tris buffer in pH 9.0] were laboratory-grade chemicals obtained from Fisher Scientific and sterilized separately at 121°C for 25 min before mixing. After plate growth, bacteria were harvested and inoculated in NH_4-YE liquid media (same components but no agar), where they were grown for 24–28 h with an aeration ratio of 1:5 (i.e., 200 mL of media in a 1-L flask) to an optical density of 600 nm (OD_{600}) of 0.8–1.2 (10^7 cells/mL). Abiotic (without bacteria) bottles of media were always prepared and incubated under the same conditions as control samples to check for contamination and to ensure that the growth obtained in the remaining inoculated liquid media was indeed only *S. pasteurii*.

This optical density was used because it ensures high urease activity, given that it was reported by Stocks-Fischer et al. (1999) that the specific rate of ammonium production (urea hydrolysis) decreased with increased cell concentration. In this study, the urea hydrolysis rate was measured by means of electrical conductivity at different stages before bacteria were introduced to the samples, and was found to be in the range of 5–20 mM urea/h.

Preparation of the Initial Inoculate

Urea-$CaCl_2$ liquid medium was used in injections in all experiments. Cells grown in stock NH4-YE liquid media were washed with saline solution, harvested, and resuspended in urea-$CaCl_2$ media. This was made by adding PBS (phosphate buffered saline) of 3–5 mL in 50-mL solution, centrifuging at 3,000 rpm for 20 min for two or three times to remove the metabolic waste and any metabolism by-products, then disposing of the supernatant while keeping the bacterial cells pellet-concentrated at the bottom of the test tube. The washed solution containing bacteria (liquid stock medium) was then mixed with the work injection solution (urea and $CaCl_2$ solution) and injected into the soil samples. For larger (1-L) samples, where pumps were used for injections and injection took a longer time, bacteria were mixed with a work solution containing nutrient broth, ammonium chloride, and sodium bicarbonate in the absence of urea and $CaCl_2$ for the initial injection.

Urea [$CO(NH_2)_2$] was used as an energy and ammonium source for the hydrolysis process (DeJong et al. 2010; Mitchell and Santamarina 2005), and $CaCl_2$ was used as a calcium source. The test liquid media also contained 3 g of nutrient broth as a nutrient source for the bacteria, in addition to 10 g of NH_4Cl and 2.12 g of $NaHCO_3$ per liter of deionized water for stabilization of the pH of the solution before the injections. The total amount that was introduced to any specimen in different experiments was based on chemical stoichiometric calculations and the amount of precipitation targeted in each experiment. Different concentrations of $CaCl_2$ and urea in the work solution (0.1–1.1 M) were used to examine the effect of chemical concentration on the precipitation pattern.

Soil Type and Size

To ensure optimal results, sand of particle sizes ranging from 90 to 300 μm was used for all experiments. Rebata-Landa (2007) found that the most optimal range of grain size for the biocementation process is between 50 and 400 μm because bacterial activity cannot take place in very fine soils and larger amounts of nutrients are needed to increase the stiffness and strength in coarser soils. Silica sands of two different grain sizes (British standard grades D and E sand) were used (Grade D: $d_{50} = 165$ μm and $d_{90} = 250$ μm; Grade E: $d_{50} = 140$ μm and $d_{90} = 150$ μm).

Materials and Setup

Plastic syringes with 100-mL volume were used as test soil columns, as shown in Fig. 1. The syringes were filled with sand

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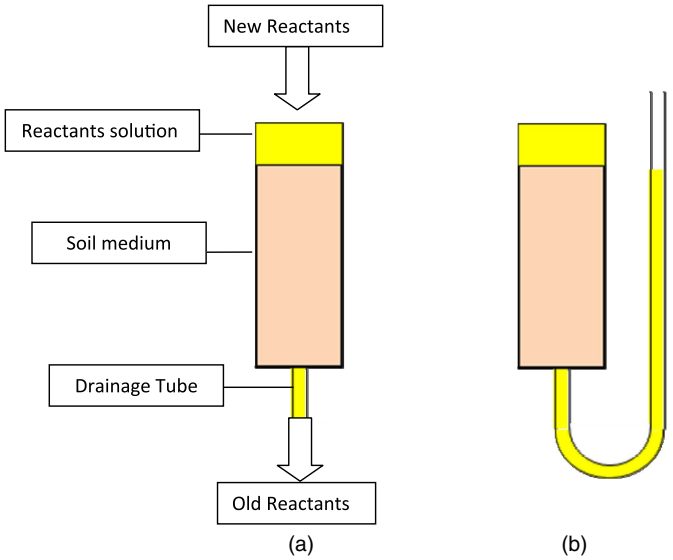

Fig. 1. Advection experiments setup

and connected to plastic tubing at the bottom for drainage of nutrients, where sand was packed in the columns in the presence of the bacterial liquid medium (1 PV with $OD_{(600)}$ of 0.8–1). The porosity was 0.37 (relative density = 98%) for Fraction D sand ($e_{max} = 0.9875$; $e_{min} = 0.585$) and 0.44 (relative density = 57%) for Fraction E sand ($e_{max} = 1.014$, $e_{min} = 0.613$).

The effective input rate (or the retention time) was varied by injecting urea-$CaCl_2$ liquid media of a given molar concentration and leaving it for different time durations to react. The injection was made by adding new liquid media at the top soil boundary under gravity [Fig. 1(a)]. During the retention stage, the sand was always kept slightly overtopped with a liquid [Fig. 1(b)] to ensure that it remained saturated at all times; evaporation losses and leakages were regularly checked. After a predetermined retention time, the old liquid medium in the specimen was replaced with a new one. This process of injection-retention was performed several times to provide a certain mass input of liquid media into the specimens.

Retention Times and Chemical Concentrations

All injections were based on chemical stoichiometric calculations and were predetermined before the tests started. The amount of chemicals injected into each sample depended on the level of cementation required. For example, to produce 1 M of $CaCO_3$ (100 g/M), 1 M of $CaCl_2$ (111 g/M), and 1 M of urea (60.06 g/M) are required [refer to Eq. (1)].

A number of treatment combinations were examined to determine the effect of chemical concentration and retention time of the reactants on the reaction efficiency of MICP. Based on initial tests, it was found that samples with a precipitation of 120 kg $CaCO_3/m^3$ of sand were highly cemented for a fine sand (d_{50} = 110 μm) (Rebata-Landa 2007). Accordingly, this value was set as an approximate upper limit and different values below this limit were selected as intermediate targets.

Different combinations of injection frequency (retention time) and chemical concentrations of the liquid media were applied to (1) estimate the optimal time required for the reactants to precipitate in a porous medium for this optical density and (2) assess the effect of different pumping rates (i. e., retention times) on the efficiency of the process for field application. In the first test series, four different retention times of 6 h, 12 h, 24 h, and 2 days were applied with 0.25 M (urea and $CaCl_2$) solution, which provided different reactant input rates. All experiments were conducted at 20°C; it has been reported that an increase in temperature will result in an increase in urease activity up to a temperature of 60°C (Whiffin 2004; van Paassen 2009).

The total reactant mass input was also varied to produce samples with different amounts of precipitation; the largest one would be reached by a total of 14 injections. Therefore, the highest amount of mass input was 3.5 M × sample liquid volume for fraction D samples ($n = 0.37$) and 3 M × sample liquid volume (for fraction E samples ($n = 0.44$). This was equivalent to a precipitation value of about 130 kg $CaCO_3/m^3$ if all the reactants precipitated as $CaCO_3$.

In the second test series, the input chemical concentration was increased to 0.5 M to examine the effect of chemical concentration on chemical efficiency. Two different retention times (6 and 24 h) were tested to examine the effect of the reactant input rate on chemical efficiency. The injection sequences of the two test series are shown in Fig. 2. The slopes of the lines (mol/sample liquid volume/h) are defined here as reactant input rates.

A third series of tests was conducted by using a urea-$CaCl_2$ input concentration of 0.1 M with a retention time of 3 h. In total, more than 80 samples were tested and two or more data points were obtained for each combination to assure the repeatability of the results.

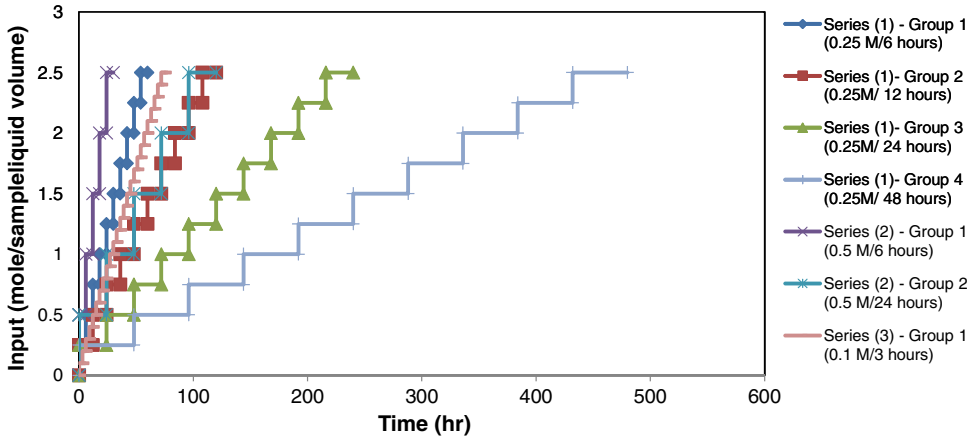

Fig. 2. Examined input patterns

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Fig. 3. Cemented sample after 3 days (100 kg/m^3)

Experiment Termination Procedure

The experiments were terminated in the following manner. The liquid medium was first drained and the soil was washed with deionized water to remove excess materials in the remaining nutrient. Then the specimens were oven-dried to stop metabolism and the test columns were cut with a mechanical saw to extract the hardened soil specimens. Samples with high calcite precipitation were found to be fully cemented, as shown in Fig. 3. Cementation was uniformly distributed all over the samples, even at lower precipitation values. This was visually observed in almost all of the samples and was confirmed by measuring the weights of $CaCO_3$ for two sections in some samples, where equal precipitation values were obtained. For most of the samples it was difficult to break the cementation to facilitate dissolution using HCl.

The weight of the dry specimens was recorded before the soil was washed with a 0.5 M HCl solution to dissolve the precipitated carbonates. Heavily cemented samples (above 80 kg $CaCO_3/m^3$ sand) required a large amount of acid for complete dissolution of the precipitated $CaCO_3$ (approximately 200 mL of a 0.5 M HCl solution for the 100-mL specimens).

Finally, the soil was rinsed with deionized water, drained, and oven-dried, and the weight of the dry specimens was recorded. Although it is also possible to measure the amount of CO_2 released upon the addition of acid, in this study the difference between the two weights was considered to be the weight of the carbonates that were present in the original specimen. The efficiency of MICP was then computed as the ratio of the actual precipitated mass to the mass based obtained from chemical calculations, taking into account the total amount of chemical inputs until each specimen was terminated.

Two control samples were run alongside the test. For the first control sample, chemical reactants were injected into the specimens without any bacteria, and insignificant $CaCO_3$ precipitation was obtained in these samples at the end of the experiment (approximately 0.5% of the sample weight). For the second control sample, deionized water was also injected simultaneously. Upon termination and addition of HCl, no change in weight was found.

Scanning Electron Microscopy

A JEOL JSM-5800LV scanning electron microscope (SEM) was used where backscattered imaging was applied on these samples. Some samples were also sputter-coated with platinum or carbon using an Emitech K550 sputter coater to determine the most suitable imaging method for detection of precipitation pattern. It was found that the backscatter detection technique was the most appropriate for the purpose of the imaging.

Quantitative analysis of the chemical composition of samples was also conducted on several specimens using an energy dispersive *X*-ray analyzer (EDX) at an accelerating voltage of 15 kV. This was done to confirm that the crystals observed in the images were actually $CaCO_3$ crystals precipitated on the silica sand and to detect whether there were any other elements in the samples after the precipitation process. The most abundant materials were indeed found to be SiO_2 (quartz) and $CaCO_3$.

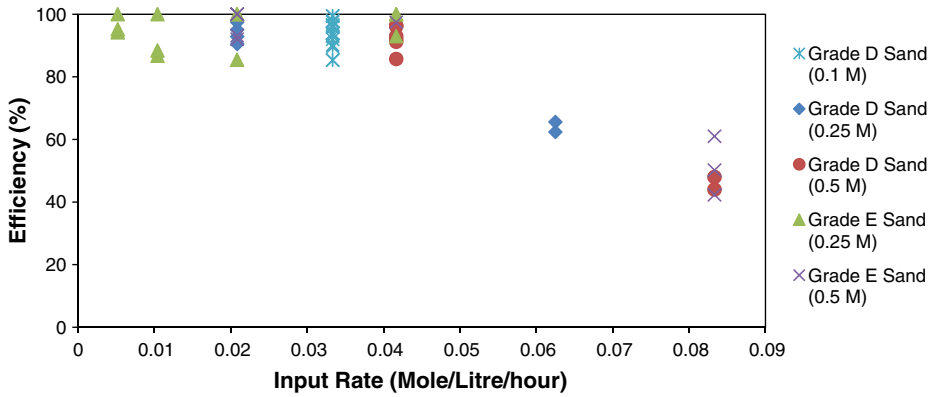

Fig. 4. Reactants efficiency for different input rates

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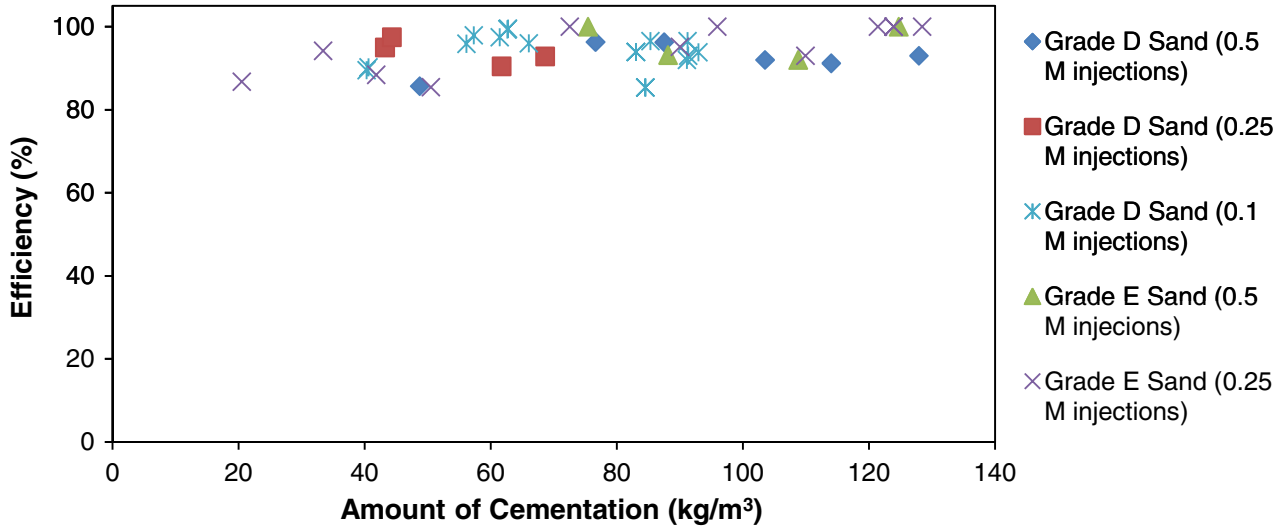

Fig. 5. Reactants efficiency at different stages of cementation

Results and Discussion

Reactants Efficiency and Input Fluxes

Fig. 4 shows the effect of the reactants input rate on the chemical efficiency of the process. For the tested chemical concentrations, the highest urea-$CaCl_2$ input rate reached was 0.042 mol/L/h while maintaining the chemical efficiency at $> 90\%$. This efficiency decreased to an average of 50% for an input rate of 0.084 mol/L/h under the same conditions. The differences in efficiency between specimens for a given input rate could be a result of the variations that could occur in any bacteria-related process, or due to experimental inconsistencies that might have occurred, such as variations in sand packing. The variation in sand type between fraction D and E did not have any significant effect on the results.

As long as the reactants per injection were given enough time to react (i.e., the reactants input rate was < 0.042 mol/L/h), samples that terminated at different stages of cementation showed that the chemical efficiency remained constant throughout the entire process, as shown in Fig. 5. Also, varying chemical concentration (0.1, 0.25, or 0.5 M) did not make a significant difference in the overall efficiency. However, it was found that for a given amount of precipitation, the precipitation pattern in the soil pores varied depending on the input urea-$CaCl_2$ concentrations, which will be discussed in "Effect of Liquid Media Concentration."

The input rates of some experiments published in the literature are shown in Table 1. The values are below the upper limit of 0.042 mol/L/h evaluated in this study and all had high chemical efficiencies. The case with the lowest concentration of 0.1 M (DeJong et al. 2006) was reported to produce high levels of cementation, whereas the case with the highest concentration of 1.1 M (Whiffin et al. 2007) gave a chemical efficiency similar to the one measured in this study.

Table 1. Effective Input Rates Reported in the Literature

Study	DeJong et al. (2006)	Rebata-Landa (2007)	Whiffin et al. (2007)
Input rate (mol/L/h)	0.025	0.042	0.0088
Concentration (M)	0.1	0.25	1.1
Efficiency (%)	92 (Jason DeJong, personal communication, 2009)	95	88

The data produced in this study, as well as those shown in Table 1, give the upper limit of the input rate to achieve high reaction efficiency. The lower limit will be governed by the bacterial activity. In this study, a bacterial optical density (OD_{600}) of 0.8–1.2 was used in all experiments, and bacterial solutions were used within 48 h of preparation. The use of constant optical density ensured relatively consistent bacterial activity and precipitation results in different tests regardless of the initial bacterial activity, and was found to guarantee high process efficiency in porous media within the input rates determined here. Despite the variation noticed in the measured urea hydrolysis rate before introduction to samples (5–20 mM urea/h), bacterial activity was not found to affect the results obtained.

Experiments conducted by Rebata-Landa (2007) suggested that precipitation usually becomes insignificant after a certain period of time. In early tests conducted in this study, it was found that the bacterial activity started to drop to a level that affects efficiency after 16 days when the urea-$CaCl_2$ input rate was 0.042 mol/L/h. Rebata-Landa (2007) reported the bacterial activity drop to start between 16 and 32 days, as shown in Fig. 6.

Van Paassen (2009) reported that bacterial activity dropped below 5 mM urea/h after 20 days. Then, after the injection of another batch of bacteria, the bacterial activity remained at 15 mM urea/h for another 20 days. The explanation for this decline in bacterial activity could be hydraulic constraints, such as restraint of the bacteria (i. e., encapsulation of bacteria as a result of precipitation or being trapped inside pores) and interruption of chemical transport in the pore space after precipitation (which would prevent nutrients required for growth and chemicals required for further precipitation from reaching the bacteria). Both reasons (cells encapsulation and starvation) were also reported by van Paassen (2009), along with bacterial flushing out as reasons for decline in bacterial activity. Another reason could also be space limitation in the case of saturation of the pore fluid (1-μm-size bacteria such as *S. pasteurii* could reach approximately 10^8 bacteria/mL (Mitchell and Santamarina 2005). Although Stocks-Fisher et al. (1999) reported that urease is still active in degrading urea even during the stationary phase of cell growth, these reasons—in addition to possible accumulation of metabolic waste—are suspected to result in the decrease of bacterial urease activity, which in turn reduces its affinity to perform its role in the precipitation process.

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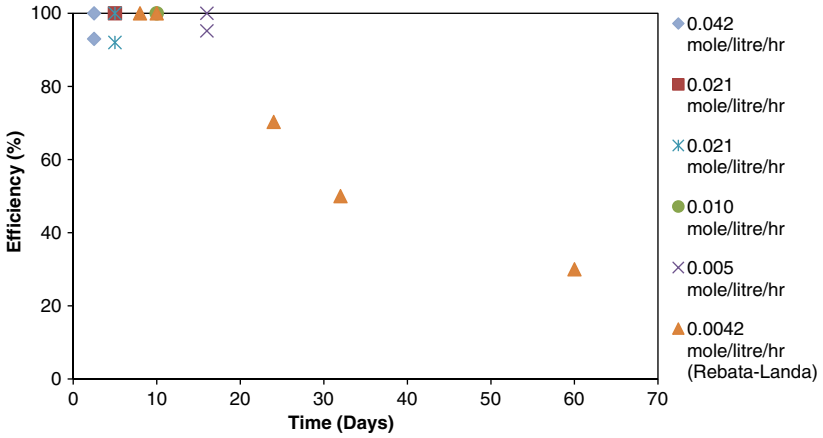

Fig. 6. Decline in bacterial activity with time for different effective input rates

Upscaling

To confirm that the boundaries obtained from the previous tests could be applied on a larger scale, additional tests were conducted in 1-L rigid cells where the same experimental setup was used and a peristaltic pump was used for injection of the chemicals. A confining pressure of 20 kPa was applied on the cells and a 0.5 M $CaCl_2$-ureasolution was used. Injections were made at a rate of 10 mL/ min and reactants were allowed to react for 12 h (an effective input rate of 0.042 mol/L/h). Chemical injections were made from the bottom of the samples after sand was compacted in the bacterial solution at the beginning of the test. The average efficiency of more than 20 samples was found to be 90%. In addition, the same input rate of 0.042 mol/L/h was applied on a 10-L sample tested at the Public Works Research Institute (PWRI) in Japan, and a high efficiency of about 80% was also found (Inagaki et al. 2011).

When MICP is used in a larger field scale, additional factors must be taken into account. including bacterial injection and attachment to the soil grains as well as its distribution over the treatment volume, which was studied previously by Harkes et al. (2010). In addition, the treatment time in such a scale should be divided into two parts. The first part would be delivery time of the reactants to the target area, and the second part would be the retention time required for precipitation of these reactants (mainly urea and $CaCl_2$) within that target area. The delivery time would depend on site-specific conditions such as well location (with respect to target area), groundwater flow, or even pumping costs. The retention time of reactants in the target area can also be deduced from the maximum effective input rate that can be determined from the laboratory tests for a given soil and bacterial activity conditions (0.042 mol/L/h in this study). Because the total treatment time should not be more than 16 days so that bacterial activity does not decrease to below the desired range, the use of high-concentration liquid media will result in a shorter treatment time to achieve a given target calcite precipitation. This is because the retention time will be the same but the total delivery time will increase with an increasing the number of injections (i. e., the use of lower concentrations). However, the effect of liquid concentration on the calcite precipitation pattern needs to be examined, which is discussed in the next section.

In some cases, bacterial activity and the hydrolysis rate may be much higher than the ranges used in this study (5–20 mM urea/h). Nevertheless, this does not necessarily mean higher process efficiency for higher input rates. Very high urease activities may result in the initial precipitation of vaterite, as reported by van Paassen (2009) and Al Qabany (2011). This vaterite could actually be flushed away if not given enough time to redissolve and precipitate as calcite, which would also result in reduced process efficiency. In this study, the determined maximum effective input rate ensured that the injected urea was hydrolyzed by bacteria, and that the precipitated $CaCO_3$ remained in the tested samples (i. e., remained in the target volume).

Effect of Liquid Media Concentration

These findings are based on the test results using 0.25 and 0.5 M liquid media. During the termination of the experiment, samples that were treated with a higher chemical concentration and a smaller number of injections seemed to produce more hardened specimens in which it was harder to dissociate the calcite by acid, and they had a greater tendency to clog. This indicates that the input chemical concentration potentially has an effect on the precipitation pattern. The authors investigated this by conducting SEM imaging on samples that had undergone treatments of different chemical concentrations.

Scanning electron microscopy was conducted on two series of samples; the first series was of samples injected with a 0.5 M solution every 12 h, whereas the second series was of samples injected with a 0.25 M solution every 6 h. The total mass injections were varied. Figs. 7 and 8 show the development of $CaCO_3$ crystals in the two series. To ensure that images taken were representative of the entire specimen, for each specimen, more than one sample was taken and several images were taken for each of these samples. In Fig. 9 an image of a sample treated with a 1 M urea-$CaCl_2$ solution is shown at a precipitation value of 70 kg/m^3.

Despite the apparent exterior resemblance between samples treated with different chemical concentrations, it was clear from the images that the cementation distributions were different at the microscale. A low-concentration treatment (0.25 M) was found to generally result in a uniform distribution of calcite precipitation at different levels of cementation, as shown in Fig. 7. At low precipitation values [Figs. 7(a) and 7(b)], crystals were distributed all over the sand grains where no areas of concentrated precipitation could be found, because precipitation seemed to take place over the surface of the sand grains rather than accumulating over the crystals. This was even clearer as precipitation increased [Figs. 7(d) and 7(e)], where a larger number of crystals covered the sand grains uniformly and the crystals size did not seem to be any larger as precipitation increased.

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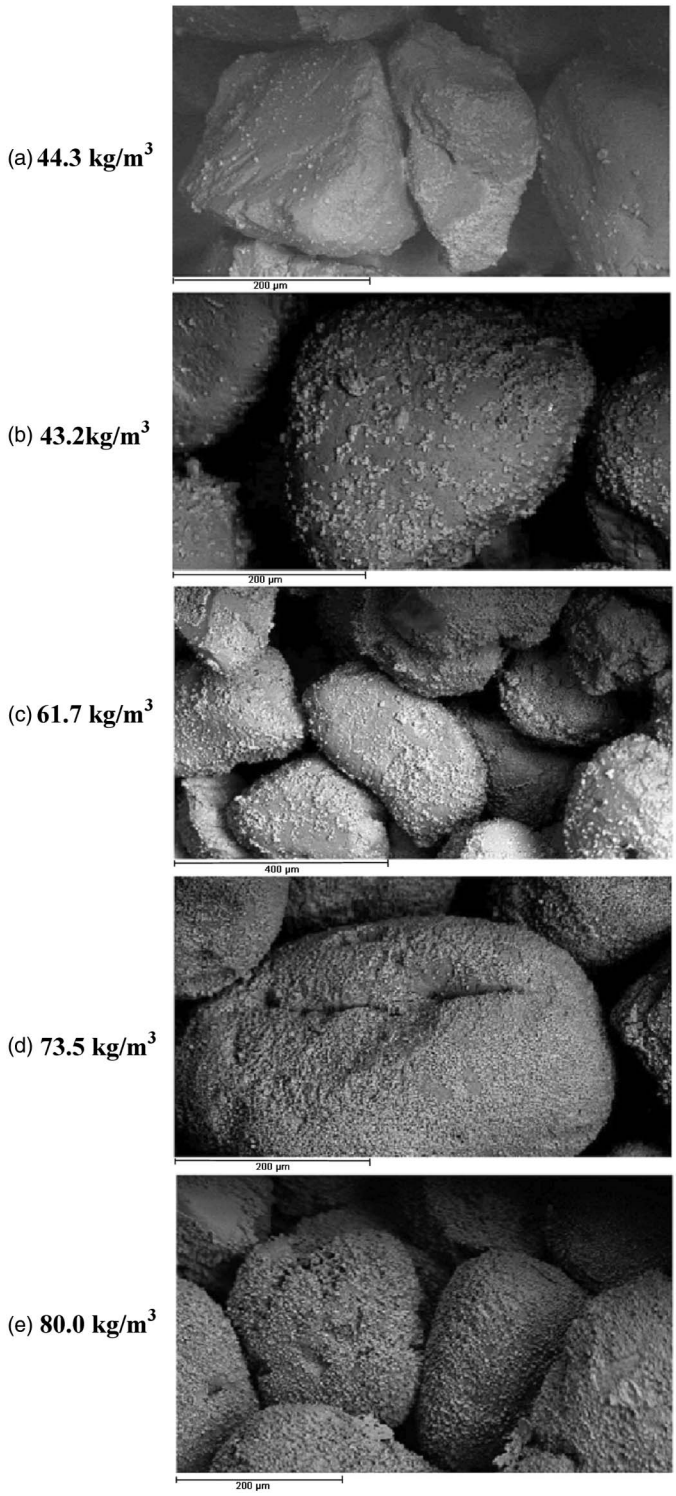

Fig. 7. SEM images for series 1: 0.25 M/6 h

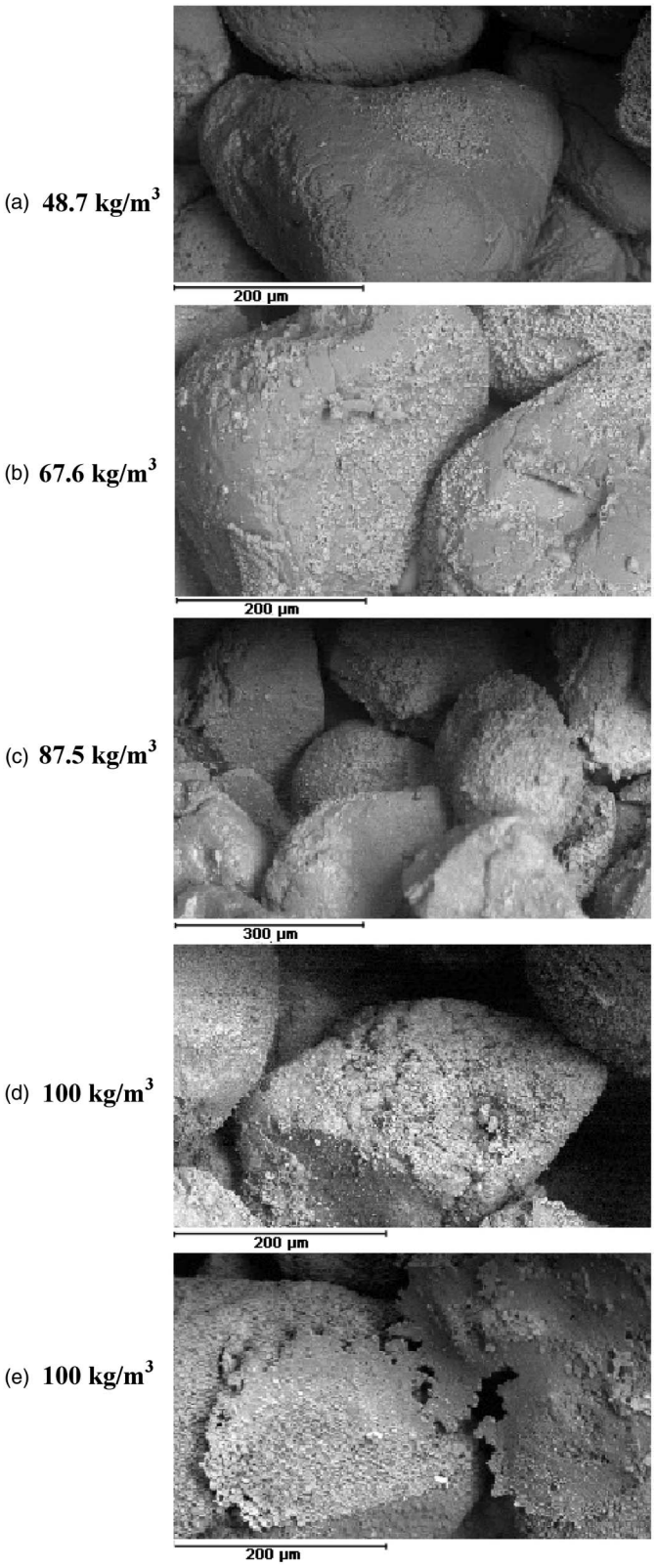

Fig. 8. SEM images for series 2: 0.5 M/12 h

When an intermediate concentration treatment (0.5 M) was performed, a more random distribution of cementation was observed, as shown in Fig. 8. This nonuniformity was clearer for lower precipitation values [up to 67 kg/m^3, as shown in Figs. 8(a) and 8(b)]. As more calcite accumulated, the precipitation pattern was more random. At a given amount of precipitation, some samples showed a nonuniform pattern [Fig. 8(d)], whereas others showed relatively more even spreading of $CaCO_3$ crystals all over the samples [Fig. 8(e)].

Figs. 9 and 10 highlight the differences obtained between the 0.25 and the 0.5 M treatments at the particle contact points. For the 0.25 M treatment (Fig. 9), the calcite crystals all had a similar size, were very well distributed spatially, and covered the contact area uniformly. For the 0.5 M treatment (Fig. 10), the crystals were

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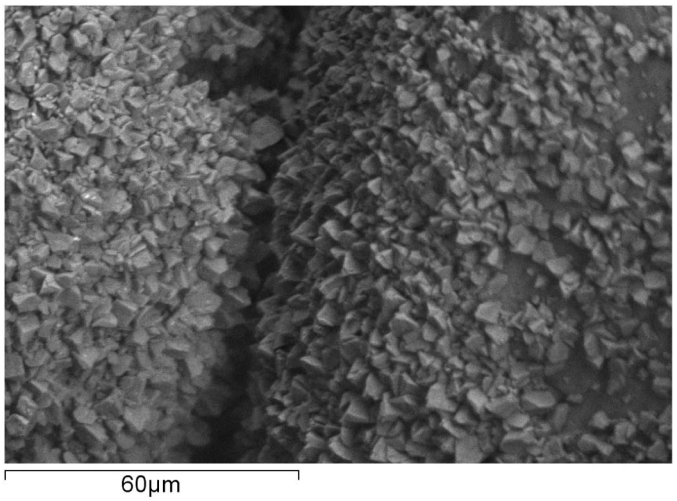

Fig. 9. Particle contact point for 0.25 M treatment (80 kg/m^3)

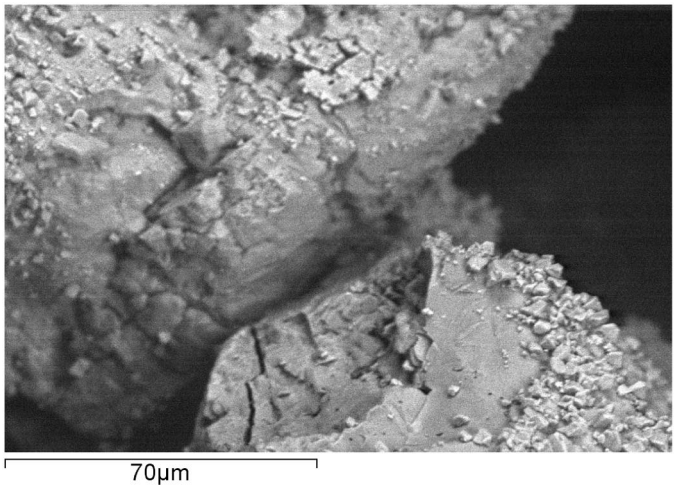

Fig. 10. Particle contact point for 0.5 M treatment (100 kg/m^3)

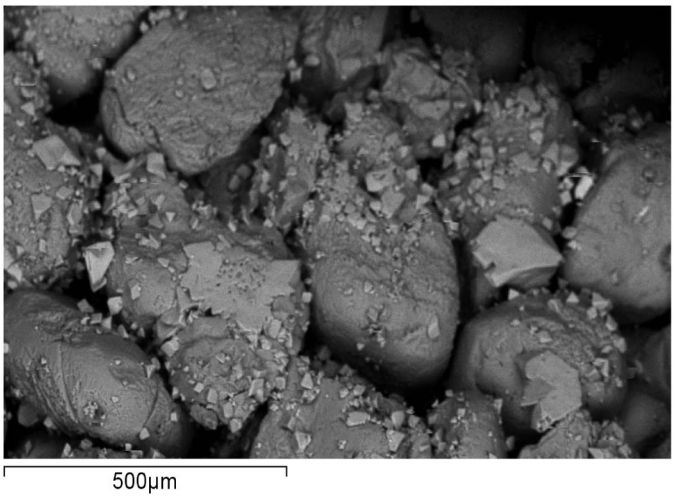

Fig. 11. SEM image for sample: 1 M/24 h (65 kg/m^3)

not very well distributed spatially and had different sizes (where at some locations precipitation accumulated over the calcite crystals, resulting in larger crystals rather than being uniformly distributed over the surface of the sand grains).

When a high-concentration treatment (1 M) was performed, the precipitation pattern was less uniform with larger crystal sizes, as shown in Fig. 11. These observed patterns indicate that—in samples tested in this study—the use of lower chemical concentrations over a larger number of injections resulted in a more homogeneous cementation.

Two mechanisms are typically reported for precipitation in MICP (Stocks-Fischer et al. 1999; DeJong et al. 2006; Rebata-Landa 2007). The first mechanism is that bacterial cells act as nucleation sites for $CaCO_3$ precipitation (Ca^{2+} is bound to the cell, which is active precipitation). The second mechanism is the urea hydrolysis, which raises the pH around the cells and produces the conditions favoring precipitation. In this study, bacterial distribution was not expected to have an effect on precipitation patterns for different concentrations because bacteria were applied in the same way to all samples.

An explanation of the noted variation in precipitation distribution could be the distribution of the urea molecules with respect to the bacterial cells. In the case of higher urea concentrations, a higher and more localized rise in pH takes place around some bacterial cells as more urea molecules are available, and thus a thick layer of precipitation takes place (i. e., the second mechanism is much more dominant than the first). In contrast, in lower concentrations, smaller amounts of calcite precipitate at every injection and the higher number of injections could allow the urea molecules to be distributed over more bacterial cells, resulting in an overall better distribution of precipitation.

Gandhi et al. (1995) reported that nucleation of new crystals would compete with the process of crystal growth for the available supersaturation, such that the formation of fine particles depends on circumstances in which nucleation of new particles prevails over the growth of those that exist. Taking this finding into account, Somani et al. (2006) reported that the higher the carbonate concentration is, the larger the average particle size of precipitates becomes. At lower carbonate concentration, on the other hand, the carbonate ions may be consumed mainly by the nucleation of calcite rather than the growth of calcite crystals. Such results suggest that, at higher supersaturation resulting from bacterial activity (i. e., when enough urea for hydrolysis is available), there could be a greater tendency for precipitation over existing crystals (i. e., growth of crystals) rather than nucleation in new sites. This is also further supported by Snoeyink and Jenkins (1980), who stated that the necessary degree of supersaturation for precipitation tends to be larger for homogeneous nucleation (i. e., growth of calcite crystals) than for heterogeneous nucleation (i. e., nucleation over sand grains). Such high supersaturation may also be a result of organics produced by bacteria [such as extracellular polymeric substances (EPS)] acting as crystallization inhibitors (Rodriguez-Navarro et al. 2007).

The observations made by Somani et al. (2006) could also defend that at high calcium concentrations precipitation will start at a relatively low carbonate concentration, which could result in smaller crystals precipitating. However, this could be the case only initially until carbonate levels increase as a result of high urea concentration (despite ongoing precipitation). Thus, increasing supersaturation in the solution would result in precipitation accumulating over these initial small crystals and formation of larger crystal sizes, or possibly a mixture of different sizes, as shown in the 0.5 M input cases (Figs. 8 and 10). For a low-input $CaCl_2$-urea concentration (0.25 M), the level of supersaturation would not be expected to

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increase to such a condition, which results in continuous heterogeneous precipitation over the sand grains.

On the other hand, some studies such as Sondi and Salopek-Sondi (2005) showed that, in addition to general precipitation rules, the presence of organic macromolecules, such as enzymes, directly affect the precipitation process either through nucleation, crystal growth, or even morphology obtained. Furthermore, Stocks-Fischer et al. (1999) reported that the availability of microbial cells and the extracellular urease enzyme produced around these cells have a significant impact on the rate of ammonia production and, consequently, precipitation. Van Paassen (2009) reported how reaction (hydrolysis) and diffusion rates could also have a large impact on crystal properties at different stages of precipitation and should be taken into account when discussing distribution of solutes (urea molecules) with respect to crystals, because they could affect the supply of carbonate ions toward the crystal surface.

These different findings and observations may make it difficult to predict a precipitation pattern for different chemical concentrations in the presence of bacteria and organic substances. However, the precipitation patterns observed in this study suggest that the use of higher concentrations not only results in thicker calcite matrices, but possibly also gives a faster decline in the bacterial activity, because the urea becomes less available to the encapsulated microbial cells to hydrolyze. A more detailed discussion of these differences in precipitation pattern and crystal size, along with the quantification of these differences, could be the subject of future study.

The $CaCO_3$ precipitation pattern could have a significant impact on applications targeted for biocementation. It will influence the amount of contact between soil grains by forming bridges between these grains, and thus the way in which load is transferred between them and the strength and stiffness of the material (Harkes et al. 2010). It also influences the pore space shape/structure through local accumulation of crystals, which could have an effect in the flow properties of porous media. Further work is needed to examine the effect of precipitation pattern on the change in engineering properties, such as permeability and stiffness/strength.

Conclusions

Identification of different process limitations, such as bacterial activity and reaction rates, enables the control of MICP for its use in geotechnical engineering. Understanding how different treatment methods could affect different applications is an important aspect of using MICP in practice. Although no quantitative measurement of engineering properties was made here on samples after treatment, this study highlights the significance of treatment method to ensure that the treatment used not only suits field conditions, but is also optimal for the application it was designed for and achieves the best possible results from the process.

The precipitation process is controlled chemically by the availability of calcium and carbon sources for precipitation, and biologically by the ability of the bacteria to hydrolyze the urea and produce alkalinity and carbonate. The rate at which bacteria hydrolyze urea (bacterial activity) could be a good indication of this. However, it does not necessarily determine the rate of precipitation in the field, because when it comes to reaction in porous media, several other factors are involved and could influence the process. This study examined the effect of two liquid media input variables (i. e., retention time and liquid media concentration) on the efficiency of MICP.

Laboratory investigation with different treatment combinations was performed on sands using bacteria with an optical density (OD_{600}) of 0.8–1.2 (bacterial concentration of 10^7 cells/mL). Results show that, when the input rate of the liquid medium (0.1, 0.25, or 0.5 M urea-$CaCl_2$ concentration) was less than 0.042 mol/L/h, high chemical efficiency (up to 100%) of MICP was achieved under the closed-system conditions (where the solution becomes saturated and no additional space for bacterial growth is available). Within this rate, the concentration of urea and $CaCl_2$ in the input could be as high as 1 M and as low as needed, depending on the number of injections and amount of injection solution desired for operating conditions. The efficiency was found to be constant through different stages of cementation until a precipitation value of 130 kg $CaCO_3/m^3$ of sand was reached. At higher input rates, the efficiency decreased because the rate of bacterial urea hydrolysis was slower than the input rate. At lower input rates, the ability of the bacteria to hydrolyze 0.042 mol/L/h urea and precipitate $CaCO_3$ started declining after a certain period of time (more than 16 days in this study), even when nutrients were supplied for bacterial growth.

The data presented in this paper could improve the design of future experiments and field applications because any higher input rate (for the same optical density) would probably be a waste of reactants. Also, although the values in this study were obtained from pulse flow tests to ensure a more controlled retention time of reactants over the entire sample, it is expected that they would still be applicable in the case of continuous injection. However, the pulse flow may provide a better distribution of cementation and more control of the retention time for bacteria and chemicals. This is because the injection rate in pulse flow tests would be expected to be too high compared with the hydrolysis rate, such that most precipitation takes place during the residence time in which chemicals are distributed uniformly over the entire length of the sample.

Within the range of optimal input rates, the amount of precipitation was not affected by the liquid media concentrations (up to 1 M). However, the precipitation pattern of $CaCO_3$ at the pore scale level was affected by the liquid media concentration. At the same level of cementation, an input with low liquid medium concentration resulted in a more homogeneous distribution of precipitation over the sand grains at the microscale. Hence, cemented soil properties may be different depending on the retention time of the reactants and the liquid medium concentration because of the difference in precipitation pattern. Further study is needed to examine the effects of precipitation patterns on engineering properties.

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