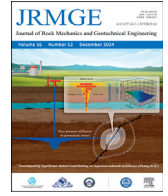




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Full Length Article

A new bacterial concentration method for large-scale applications of biomineralization

Hanjiang Lai^{a, b}, Xingzhi Ding^{a, b}, Mingjuan Cui^{c, *}, Junjie Zheng^d, Jian Chu^e,
Zhibo Chen^{a, b}, Jianwei Zhang^f

^a Zijin School of Geology and Mining, Fuzhou University, Fuzhou, 350108, China^b Fujian-Taiwan Science and Technology Cooperation Base of Fujian Province on Intelligent Geo-Environmental Engineering, Fuzhou, 350116, China^c College of Civil Engineering, Fuzhou University, Fuzhou, 350108, China^d School of Civil Engineering, Wuhan University, Wuhan, 430072, China^e School of Civil and Environmental Engineering, Nanyang Technological University, Singapore^f School of Civil Engineering and Architecture, Henan University, Kaifeng, 475004, China

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ABSTRACT

Bacterial suspension is an essential component of microbially induced carbonate precipitation (MICP)-based biocement and a large-scale production is required for field applications. In this study, a new bacterial concentration method is proposed to enable high concentration bacterial suspension to be produced to facilitate field work. By adding low concentration calcium to bacterial suspension, flocs are formed and bacterial cells are adsorbed on the flocs to achieve bacterial concentration. Compared to the traditional bacterial concentration method using centrifugation and freezing-drying method, the proposed method can concentrate a large volume of bacterial suspension without using special equipment. The feasibility of this method is verified by bacterial concentration tests, solution tests and sand column treatment tests. The results of both the solution test and the sand column treatment test show that the bacterial suspension concentrated by the proposed method can be effectively used for soil biocementation. There is a threshold calcium concentration that allows a complete bacterial concentration for the proposed method, and this threshold calcium concentration tends to increase linearly with the optical density of the cell suspension at a wavelength of 600 nm (OD_{600}).

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1. Introduction

Biomineralization based on microbially induced carbonate precipitation (MICP) is an emerging environmentally friendly technology in geotechnical and geological engineering that has attracted much attention worldwide in the last two decades. This technology has the potential to be a promising alternative in many fields, such as soil improvement (Ivanov and Chu, 2008; Van Paassen et al., 2010; DeJong et al., 2010; Jiang and Soga, 2019; Lai et al., 2021a; Wang et al., 2023a), heavy metal remediation (Qiao et al., 2021; Niu et al., 2022; Song et al., 2022; Dong et al., 2023;

Naeimi et al., 2023), concrete and rock crack remediation (Tiano et al., 2006; Van Tittelboom et al., 2010; Abo-El-Enein et al., 2012; Intarasoontron et al., 2021; Sohail et al., 2022; Turner et al., 2023; Yu and Zhang, 2023a), historical building restoration (Yang et al., 2011, 2024; Yang and Cheng, 2013; Liu et al., 2020), rainfall erosion mitigation (Jiang et al., 2019; Liu et al., 2021), seepage erosion control (Jiang et al., 2017; Clarà Saracho et al., 2021; Pan et al., 2023), tidal erosion mitigation (Salifu et al., 2016; Zhang et al., 2023a), wind erosion control (Maleki et al., 2016; Chae et al., 2021; Dagliya et al., 2022; He et al., 2023), dust suppression (Naeimi and Chu, 2017; Zhu et al., 2021; Wang et al., 2023b; Zhou et al., 2023), and CO₂ geological sequestration (Okyay and Rodrigues, 2015; Nathan and Ammini, 2019; Yu and Zhang, 2023b). In terms of soil improvement, a large number of studies have been conducted in the past two decades to investigate the engineering properties (Xiao et al., 2021; Konstantinou et al., 2021; Lai et al., 2021b; Sharma et al., 2021; Ahenkorah et al., 2023; Chen

* Corresponding author.

E-mail address: cuijm@fzu.edu.cn (M. Cui).

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et al., 2023; Tang et al., 2023; Wang et al., 2023c; Zhang et al., 2023b), possible influencing factors (Ma et al., 2022; Lv et al., 2022; Lai et al., 2023; Su et al., 2023; Cui et al., 2024), and intrinsic micro-mechanisms (Cui et al., 2021a; Xiao et al., 2022, 2024; Zhao et al., 2023) of the MICP-based biocemented soils. Meanwhile, several upscaled model tests (Van Paassen et al., 2010; DeJong et al., 2014; Li, 2015; Salifu et al., 2016; Zhu et al., 2016; Gomez et al., 2017; Sharma et al., 2022; Sang et al., 2023) and field trials (Saneiyani et al., 2019; Kirkland et al., 2020; Li et al., 2023) have been carried out to investigate the feasibility of MICP-based biomineralization technology in practical engineering. However, only a few studies have been conducted on the pilot application of this technology (Van Paassen, 2011; Gomez et al., 2014; Kirkland et al., 2020).

Large-scale field application of MICP treatment requires large volumes of bacterial suspension. Whiffin et al. (2007) found that the strength of biocemented sand can only be increased when the calcium carbonate content is greater than 60 kg/m³. Li (2015) used a total of 120 L of bacterial suspension to achieve a calcium carbonate content of 65 kg/m³ in a 1 m³ upscaled model test. Furthermore, DeJong et al. (2014) injected 30 L of bacterial suspension into a treatment zone with dimensions of 0.5 m × 0.5 m × 0.15 m, while Sharma et al. (2022) used 200 L of bacterial suspension for a 1.35 m × 1.13 m × 0.65 m model test. It is worth noting that the storage and transportation of such a large volume of bacterial suspension will present significant challenges for the field application of MICP-based ground treatment. Therefore, further measures are necessary to address this issue. One potential solution is to reduce the volume of the bacterial suspension through concentration techniques, such as centrifugation (Cuthbert et al., 2013; Nayanthara et al., 2019; Mu et al., 2021) or freezing-drying (Pungrasmi et al., 2019; Leeprasert et al., 2022; Nuaklong et al., 2023). However, these methods require special equipment and repetitive operations, given that the size of the equipment typically available in the laboratory is usually limited.

This paper proposes a new method of bacterial concentration that facilitates the transportation of large quantities of bacteria and is suitable for field applications. To verify the validity of the bacterial concentration method, bacterial concentration tests, solution tests and sand column treatment tests were carried out. The data obtained from this study can provide a reference for the site construction of MICP treatment.

2. New bacterial concentration method

A new method of bacterial concentration is proposed, in which a solution containing a low concentration of Ca²⁺ is added to the bacterial suspension. Once the Ca²⁺ is added to the bacterial suspension, large amounts of flocs are formed in the solution, which serve as carriers for the bacterial cells. In this way, bacterial cells can be concentrated through the settling of the flocs. After the flocs have settled, the volume of the bacterial suspension can be greatly reduced by draining the supernatant. Compared to the conventional centrifugation-based bacterial concentration method, the proposed method can be used to concentrate large quantities of bacterial suspension at once without the necessity of special equipment. As shown in Fig. 1a, the solution containing Ca²⁺ is simply added to the bacterial suspension and stirred thoroughly. The mixture is then allowed to stand for 24 h, after which the concentrated bacterial suspension (i.e. floc solution) can be obtained by draining the supernatant. The concentration of bacterial cells in the floc solution will be higher than that of the original bacterial suspension. For field applications, as shown in Fig. 1b, the concentrated bacterial suspension can be diluted by simply adding tap water on site.

3. Verification tests

3.1. Materials

In this study, *Sporosarcina pasteurii*, a type of urease-producing bacteria (UPB), was used. The sterile NH₄-YE medium consisting of 20 g/L yeast extract, 15 g/L NH₄Cl, and 0.1 mmol/L NiCl₂, at pH = 9.25, was employed to cultivate the bacteria. The original bacterial suspension with an OD₆₀₀ of about 3.36 was used in solution tests and sand column treatment tests, where OD₆₀₀ is the optical density of a cell suspension at a wavelength of 600 nm. Silica sand with a grain size of 0.6–0.85 mm ($G_s = 2.65$, $e_{max} = 1.15$, and $e_{min} = 0.7$) was utilized to construct the sand column. The cementation solution (2 mol/L) containing equal mole of calcium chloride and urea was used as the stock solution for the preparation of the biotreatment solution.

3.2. Experimental program

3.2.1. Bacterial suspension concentration test

The proposed method of bacterial concentration is achieved by the addition of a solution containing Ca²⁺ to the bacterial suspension. Both the bacterial cell concentration and Ca²⁺ concentration can influence the concentration effect. In this context, different concentrations of bacterial cells and Ca²⁺ were employed as the parameters for bacterial concentration test. In this paper, OD₆₀₀ is used to represent the concentration of bacterial cells in the solution. A bacterial suspension of different OD₆₀₀ values (i.e. 0.56, 1.12, 1.68, 2.24, 2.8 and 3.36) was prepared for this test. The test program is summarized in Table 1. The test procedure is outlined as follows:

- (1) The bacterial suspension was mixed with a calcium chloride solution of 0.5 mol/L at varying ratios (Table 2) to achieve a final calcium concentration (0.005–0.2 mol/L) in the mixed solution.
- (2) The mixture was allowed to settle for 24 h, after which the deposition of flocs was observed.
- (3) The supernatant was extracted to measure its OD₆₀₀, and the supernatant was removed to obtain the concentrated bacterial suspension.
- (4) The concentrated bacterial suspension was diluted to the same concentration as the original bacterial suspension using sterilized medium or tap water, and its urease activity was measured.

All the aforementioned steps were conducted at room temperature (25 °C ± 1 °C).

3.2.2. Solution test

A solution test was performed to monitor the MICP process and to determine if the concentration method would affect the biomineralization of UPB. The solution test program is summarized in Table 3, which is briefly described as follows:

- (1) The concentrated bacterial suspension was diluted to the same concentration as the original bacterial suspension using sterilized medium or tap water.
- (2) The bacterial suspension was mixed with a cementation solution of 2 mol/L (i.e. urea and calcium chloride) at a volume ratio of 1:1 to prepare the biotreatment solution.
- (3) The biotreatment solution was incubated at room temperature (about 25 °C ± 1 °C), and the pH and Ca²⁺ concentration of the biotreatment solution were measured over time using a pH meter and the ethylenediaminetetraacetic acid (EDTA) titration method (see Section 3.3.2), respectively.

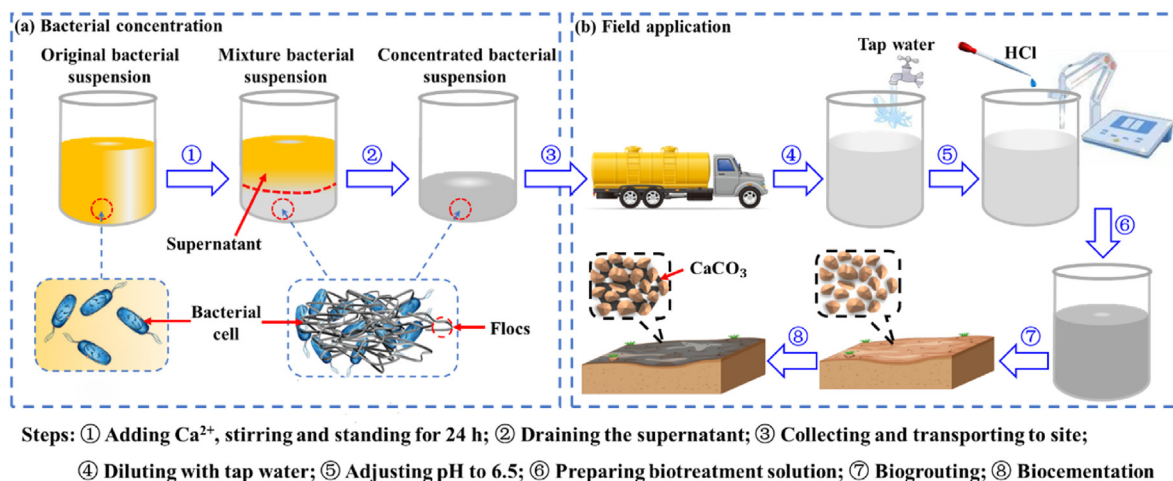


Fig. 1. Schematic diagram of bacterial concentration method: (a) Bacterial concentration method; and (b) Field applications.

Table 1
Details of bacterial concentration tests.

Test	OD ₆₀₀	Final calcium concentration (mol/L)
BCT1	0.56	0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.04, 0.05, 0.1, 0.15, 0.2
BCT2	1.12	
BCT3	1.68	
BCT4	2.24	
BCT5	2.8	
BCT6	3.36	

Note: The final calcium concentration is the Ca²⁺ concentration in the mixed solution.

Table 2
Mixing ratio of bacterial suspension and calcium chloride solution.

Final calcium concentration (mol/L)	Mixing ratio, V _b :V _{cc}
0.005	99:1
0.01	49:1
0.015	97:3
0.02	24:1
0.025	19:1
0.03	47:3
0.04	23:2
0.05	9:1
0.1	4:1
0.15	7:3
0.2	3:2

Note: V_b and V_{cc} are the volumes of bacterial suspension and calcium chloride solution (0.5 mol/L), respectively.

3.2.3. Sand column treatment test

The test program for the sand column treatment test is summarized in Table 4. All tests were performed in triplicate for repeatability. Sand samples were prepared using the polyvinyl chloride (PVC) tube as a mold with an internal diameter of 50 mm. A prescribed amount of dry sand was poured into the mold by dry

Table 3
Details of solution tests.

Test	Bacterial suspension type	Dilution	Mixing ratio, V _b :V _c	Calcium concentration, C _{Ca²⁺} (mol/L)
ST1	Concentrated	Medium	1:1	1
ST2	Concentrated	Tap water		
ST3	Original	–		

Note: V_c is the volume of cementation solution (i.e. urea and calcium chloride).

pulverization with compaction to a height of 100 mm. The relative density, D_r, of the sand sample was 50.5% and its initial void ratio, e, was approximately 0.92.

Prior to MICP injection, the concentrated bacterial suspension was first obtained by mixing the initial bacterial suspension (OD₆₀₀ = 3.36) with the calcium chloride solution (0.5 mol/L) at a volume ratio of 9:1 (i.e. the final calcium concentration is 0.05 mol/L), followed by settling for 24 h. Afterwards, the concentrated bacterial suspension was diluted using medium or tap water to the same concentration as the original bacterial suspension and used for MICP treatment. As the one-phase low pH injection method was adopted for MICP treatment, the low pH bacterial suspension was prepared using the hydrochloric acid at a concentration of 2 mol/L to adjust the pH of the bacterial suspension to 6.5 (Cui et al., 2021b). The biotreatment process of the sand column with the concentrated bacterial suspension is shown in Fig. 2. The low pH bacterial suspension was then mixed with the cementation solution to prepare the treatment solution, which was immediately injected into the sample. Here, the injection of the treatment solution was considered as one treatment. This injection was repeated until the required number of treatments, N, was achieved. After each biogrouting, all samples were incubated at room temperature (25 °C ± 1 °C) for 24 h.

3.3. Testing methods

3.3.1. Urease activity of the bacterial suspension

The urease activity (UA) of the bacterial suspension was determined by the conductivity method (Whiffin, 2004). Specifically, 1 mL of bacterial suspension was added to 9 mL of 1.11 mol/L urea, and the relative conductivity change (RCC) was recorded over 10 min at room temperature (25 °C ± 1 °C). The conductivity change of 1 mS/min corresponds to the urea hydrolysis amount of 11.1 mmol/L urea hydrolyzed per minute (Harkes et al., 2010). Considering that the bacterial suspension was diluted by a factor of

Table 4
Details of sand column treatment tests.

Test	Bacterial suspension type	Dilution	Mixing ratio, $V_b:V_c$	Calcium concentration, $C_{Ca^{2+}}$ (mol/L)	Number of treatments, N
SCT1	Concentrated	Medium	1:1	1	2, 4, 6
SCT2	Concentrated	Tap water			
SCT3	Original	–			

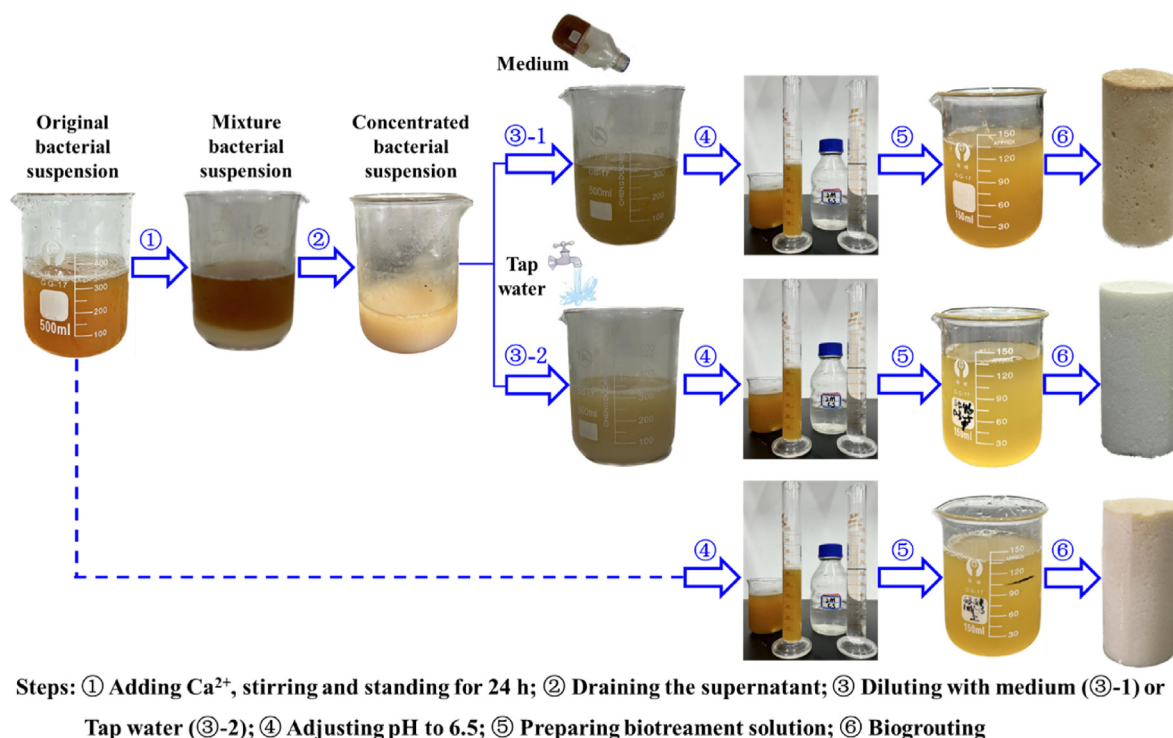


Fig. 2. Biotreatment process of the sand column with the concentrated bacterial suspension.

10-fold when mixed with the urea solution, the urease activity can be calculated as

$$UA = RCC \times 10 \times 11.1 \tag{1}$$

3.3.2. Calcium concentration of biotreatment solution

The calcium concentration, $C_{Ca^{2+}}$, in the biotreatment solution was determined by the EDTA titration method using an EDTA standard solution (Lai et al., 2021b). The specific steps are as follows: (1) adding a certain volume of solution (V_{dc}) to be tested to a beaker containing deionized water, a complexometric indicator and an ammonia buffer ($pH = 10$); and (2) adding 0.01 mol/L EDTA solution to the beaker drop by drop until the color of the solution changes from red to blue. Then, the calcium concentration $C_{Ca^{2+}}$ (in mol/L) can be obtained as

$$C_{Ca^{2+}} = 0.01V_{EDTA}/V_{dc} \tag{2}$$

where V_{EDTA} is the volume of EDTA solution consumed in the titration test.

3.3.3. Unconfined compressive strength of biocemented sand column

The unconfined compressive strength (UCS), q_{uc} , of the biocemented sand column was determined using the unconfined

compression test (UCT). Upon completion of the required treatments, the samples were rinsed with tap water to remove residual chemicals, followed by saturation for 24 h using the vacuum aspiration method (ASTM D7181–20, 2020). Subsequently, the samples then demolded to perform the UC test at an axial loading rate of 1 mm/min (ASTM D2166/D2166M–13, 2013). The axial peak during loading is taken as the q_{uc} of the sample.

3.3.4. Calcium carbonate content of biocemented sand column

The acid dissolving method was adopted to determine the calcium carbonate content (CCC) of the biocemented sand column (Xiao et al., 2020). After the UC test, all fractions of each sample were collected and fully dried in an oven at 105 °C. A specified quantity of the dried fractions was then dissolved in a 2 mol/L HCl solution and rinsed with tap water. This was followed by a further drying in the oven. The difference in the dry weight of the sample before and after the acid dissolution is the weight of the precipitated calcium carbonate. The calcium carbonate content (CCC) can then be determined as

$$CCC = \frac{M_1 - M_2}{M_2} \times 100\% \tag{3}$$

where M_1 and M_2 are the dry weight of the sample before and after acid dissolution, respectively.

3.3.5. Microscopic characteristics of biocemented sand

(1) Scanning electron microscopic test

A scanning electron microscope (SEM) was used to observe the distribution and morphology of the precipitated calcium carbonate crystals in the biocemented sand column. The fractions collected after the UC test were completely dried in an oven at a temperature of 105 °C and then prepared into samples (approximately 50 mm × 50 mm × 30 mm) for the SEM test. Prior to the SEM test, the sample surface was subjected to a gold spray treatment for at least 3 min to eliminate the potential electronic charging effect.

(2) X-ray diffraction test

X-ray diffraction (XRD) was employed to determine the polymorph of the precipitated calcium carbonate crystals. The fractions collected after the UC test were completely dried in an oven at a temperature of 105 °C, and then carefully ground and repeatedly sieved to obtain the powder with particle sizes smaller than 0.075 mm for the XRD test. Then the test was carried out on a multifunctional X-ray polycrystalline diffractometer.

4. Results and discussion

4.1. Concentration effect of bacterial suspension

To quickly understand the concentration process of the method proposed in this study, the deposition of flocs at different time was examined. The photos shown in Fig. 3, which were taken for the case with OD_{600} of 3.36 and Ca^{2+} concentration of 0.05 mol/L, serve as an illustrative example for analysis, as a similar was observed in the other cases. It can be observed that the flocs were formed immediately (as illustrated in Fig. 3a) after the bacterial suspension was mixed with the calcium chloride solution. Thereafter, the flocs deposited with time (Fig. 3b–g) and the supernatant became clear with an OD_{600} value that was ignorable (data are shown in Fig. 4b). This indicates that almost all bacterial cells were adsorbed on the flocs. In other words, the flocs serve as carriers in the process of bacterial concentration.

To characterize the concentration effect, the concentration ratio R_c (Eq. (4)), defined as the volume ratio of the mixture to the flocs, is proposed and adopted in this study. Generally, a larger concentration ratio R_c indicates a more effective concentration effect.

$$R_c = \frac{V}{V_f} \tag{4}$$

where V is the volume of the mixture, and V_f is the volume of the flocs.

The variations of R_c and OD_{600} of the supernatant with calcium concentration for all cases under various OD_{600} are plotted in Fig. 4. As shown in Fig. 4a, R_c decreases gradually with the increase in the calcium concentration, and this relationship does not seem to be affected much by the OD_{600} of the bacterial suspension. In other words, the concentration effect of the proposed method depends mainly on the calcium concentration. It seems that the lower the calcium concentration, the more pronounced the concentration effect. However, the concentration of calcium ions should not be too low to completely concentrate bacterial cells. In this regard, the OD_{600} of the supernatant was measured for each test. The relationship between the OD_{600} of the supernatant and the calcium concentration is shown in Fig. 4b. It can be observed that the OD_{600} of the supernatant tends to decrease with an increase in the calcium concentration for each case, which is almost zero once the concentration of calcium ions exceeds a certain value. This suggests that there is a threshold calcium concentration required for complete bacterial cell concentration. As illustrated in the inset of Fig. 4b, the threshold value appears to increase linearly with the OD_{600} of the bacterial suspension. Consequently, the concentration of bacterial cells requires an increased concentration of calcium ions.

To investigate whether the concentration method proposed in this study affects the urease activity of the bacterial cells, the concentrated bacterial suspension was diluted to the same concentration as the original bacterial suspension using sterilized medium or tap water, and then the urease activity of the bacterial suspension was measured. The test results are shown in Fig. 5. It can be observed that the urease activity of the bacterial suspension is similar in each case. This indicates that the concentration method proposed in this study has a minimal effect on the urease activity of the bacterial cells.

4.2. MICP process in the biotreatment solution

The photos of the biotreatment solution at different time stages of the solution test are shown in Fig. 6. It can be seen that a large amount of the flocs appeared in the solution in each case when the bacterial suspension was mixed with the cementation solution. The flocs then gradually settled to the bottom of the beaker and a large amount of precipitates can be observed at the bottom of the beaker. Finally, the biotreatment solution became clear and almost no bacterial cells were detected in the supernatant (both OD_{600} and urease activity were almost zero). That is, the bacterial cells are almost deposited to the bottom of the beaker along with the flocs, which further demonstrates the mechanism of the bacterial concentration method proposed in this study.

Fig. 7 shows the pH and calcium concentration ($C_{Ca^{2+}}$) of the biotreatment solution over time in each case. As shown in Fig. 7a, there are no significant differences in the pH and $C_{Ca^{2+}}$ of the

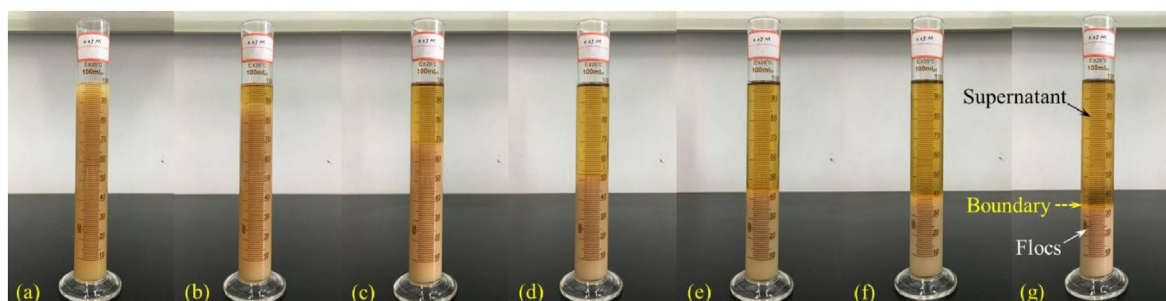


Fig. 3. Photos of bacterial concentration test at different times ($C_{Ca^{2+}} = 0.05$ mol/L): (a) $t = 0$ h; (b) $t = 1$ h; (c) $t = 3$ h; (d) $t = 6$ h; (e) $t = 9$ h; (f) $t = 12$ h; and (g) $t = 24$ h.

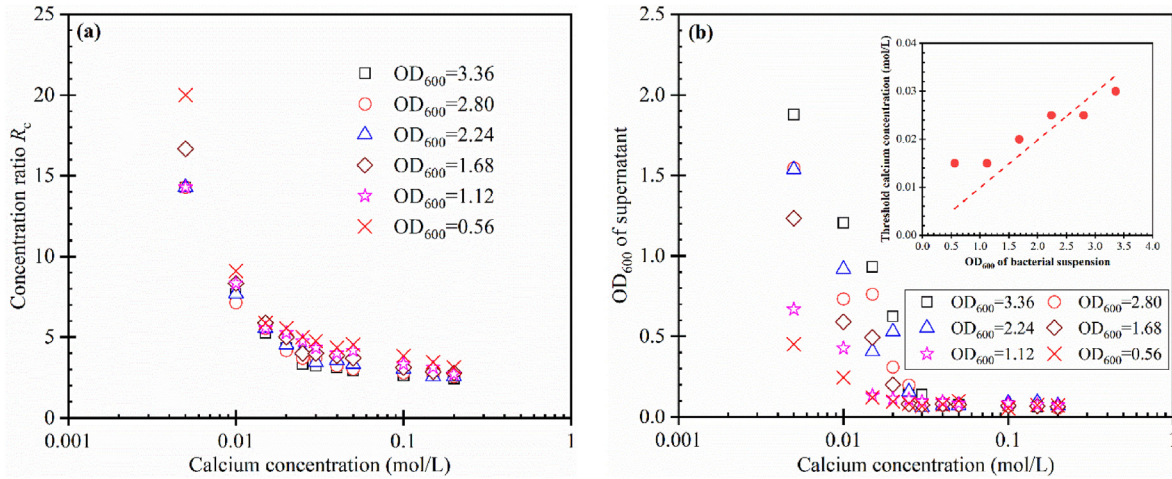


Fig. 4. Concentration effect of the bacterial suspension: (a) Variation of the concentration ratio (R_c) with the calcium concentration; and (b) Variation of the OD_{600} of the supernatant with the calcium concentration.

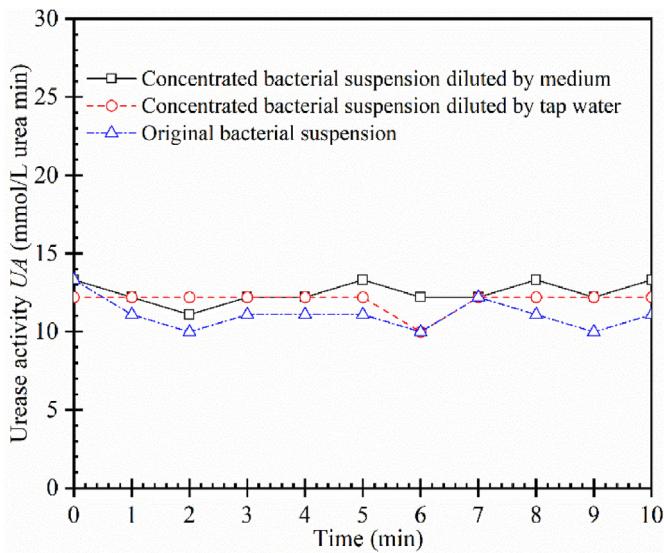
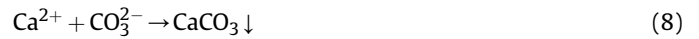
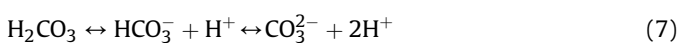
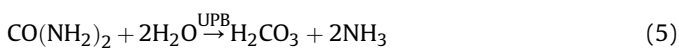


Fig. 5. Urease activity of bacterial suspension.

biotreatment solution among the different cases. The pH of the biotreatment solution is initially weakly alkaline ($7 < \text{pH} < 8.5$). The initial pH of the biotreatment solution of the ST1 case is higher than that of the ST2 and ST3 cases, which is primarily due to the higher pH of the sterilized medium used to dilute the concentrated bacterial suspension. As the reaction progressed, the pH of the biotreatment solution decreased and eventually approached neutrality in all cases. Similar results have also been reported by Hu et al. (2021) and Lai et al. (2023). This is closely related to the conversion and consumption of reactants caused by biochemical reactions during the MICP process:



As expressed in Eq. (5), the urea will be hydrolyzed into H_2CO_3 and NH_3 by UPB. The released NH_3 will then be hydrolyzed and produce NH_4^+ and OH^- (Eq. (6)), leading to an increase in the pH. Conversely, H_2CO_3 will decompose to form CO_3^{2-} and produce H^+ (Eq. (7)), resulting in a decrease in the pH. In the presence of Ca^{2+} , CaCO_3 will be precipitated (Eq. (8)). In alkaline conditions, the hydrolysis of NH_3 (Eq. (6)) is inhibited to some extent, with some NH_3 escaping from the solution as the NH_3 content in the solution increases with the hydrolysis of urea. In contrast, the alkaline condition favors the decomposition of H_2CO_3 (Eq. (7)). In addition, the consumption of CO_3^{2-} due to the precipitation of CaCO_3 (Eq. (8)) will also further promote the decomposition of H_2CO_3 . This means that the amount of H^+ produced in the MICP process will be greater than that of OH^- under alkaline conditions. This can be used to explain why the pH of the biotreatment solution decreases over time in all cases.

With regard to $C_{\text{Ca}^{2+}}$, as illustrated in Fig. 7b, it decreases gradually over time in all cases. The main difference among the cases is that, after 24 h of reaction, the Ca^{2+} consumption of the ST2 case is significantly lower than that of the ST1 and ST3 cases. This is thought to be caused by the premature coating of bacterial cells by precipitated calcium carbonate (Lai et al., 2021b). As shown in Fig. 6, flocs deposition was completed at approximately 0.5 h in the ST2 case, while it was completed at approximately 2 h in the ST1 and ST3 cases. Due to the limited area at the bottom of the beaker, the earlier the flocs are deposited, the more likely it is that more bacterial cells will be prematurely encapsulated by the precipitated calcium carbonate crystals, resulting in the inability of the bacterial cells to participate in urea hydrolysis continuously.

4.3. Biocementation of sand column

Fig. 8 shows the calcium carbonate content (CCC) and UCS (q_{uc}) of biocemented sand columns treated with different bacterial suspensions, including the original bacterial suspension, and the concentrated bacterial suspension diluted by sterilized medium and tap water, respectively. As shown in Fig. 8a, the CCC increases with the number of treatments, N , for all tests. Overall, there is no significant difference in the CCC of the sand treated with different methods. This indicates that the proposed concentration method is

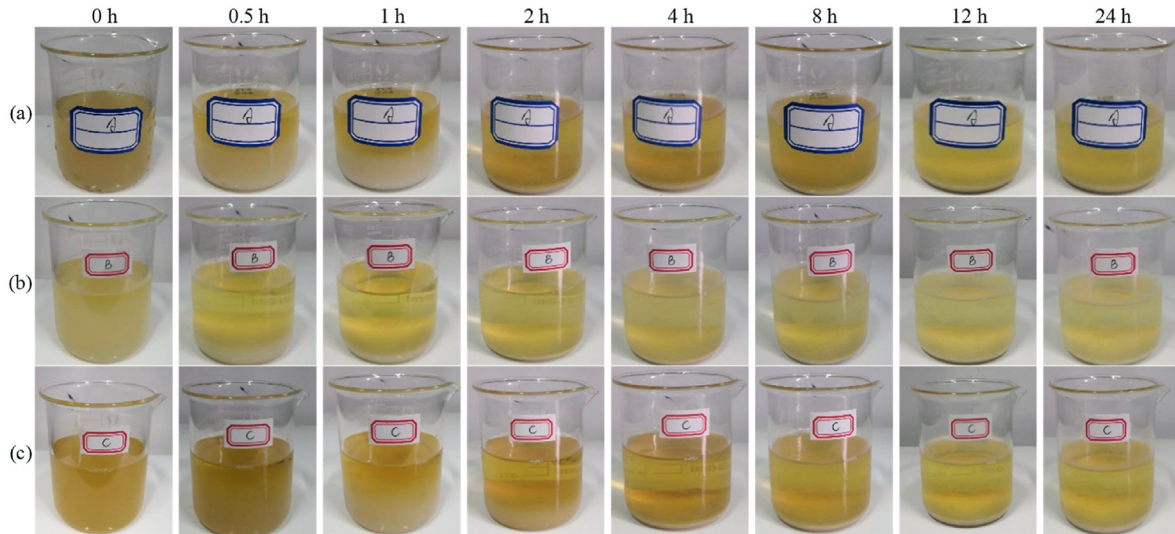


Fig. 6. Photos of solution tests: (a) ST1 case with concentrated bacterial suspension diluted by sterilized medium; (b) ST2 case with concentrated bacterial suspension diluted by tap water; and (c) ST3 case with original bacterial suspension.

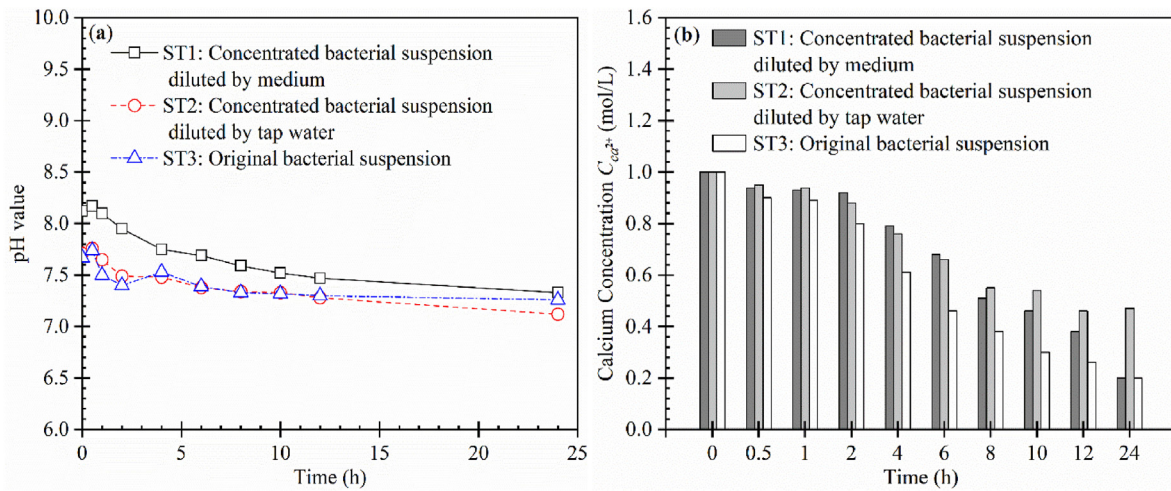


Fig. 7. Monitoring results of microbially induced carbonate precipitation (MICP) process in solution tests: (a) pH value; and (b) Ca^{2+} concentration.

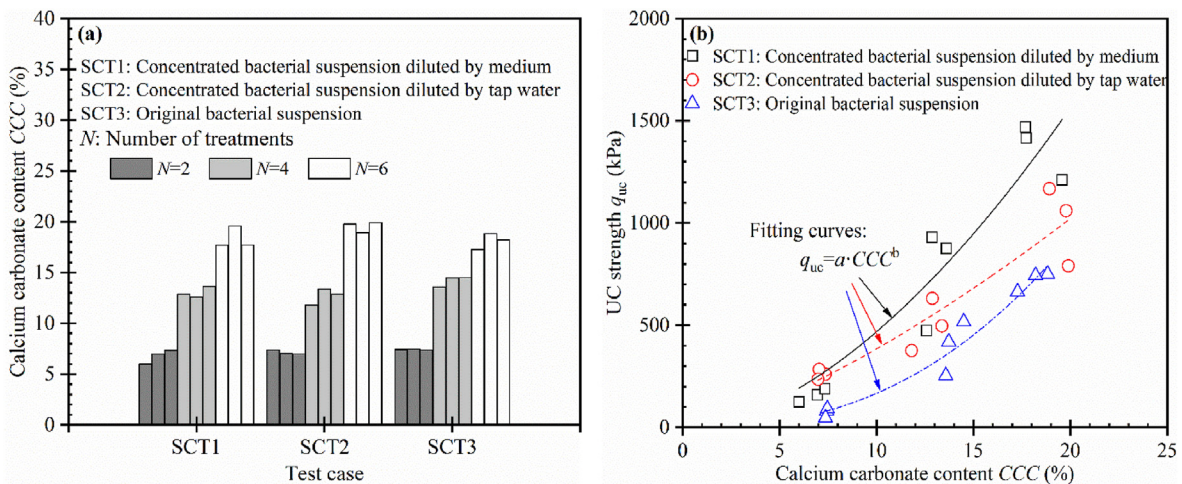


Fig. 8. Sand column treatment tests: (a) Calcium carbonate content (CCC); and (b) Variations of UCS (q_{uc}) with CCC.

effective and its ability to hydrolyze urea is not affected by the concentration process. It can be further observed in Fig. 8b that a higher q_{uc} is obtained for sand treated using the concentrated bacterial suspension, particularly for the case of the concentrated bacterial suspension diluted using sterilized medium. This suggests that the Ca^{2+} -based bacterial concentration method could enhance the biocementation effect of sand to some extent.

Fig. 9 presents the SEM images of the biocemented sand column samples subjected to 6 treatments. In all cases, a considerable quantity of rhombic and/or spherical precipitates can be observed on the surface of the sand particles and at the interparticle contacts. As illustrated in Fig. 10, these precipitates have been identified by XRD analysis as $CaCO_3$ crystals in the morphology of calcite and/or vaterite. In general, calcite is rhombic, while vaterite is spherical (Sarkar and Mahapatra, 2010; Okhrimenko et al., 2013). Furthermore, calcite is a more stable morphology of calcium carbonate crystal than that of vaterite (Gabrielli et al., 1999). As reported by Gebauer et al. (2008), a more stable morphology of the $CaCO_3$ crystal is more likely to have a greater bonding strength. The proportion of vaterite in the SCT3 case is obviously greater than that in the SCT1 and SCT2 cases, as can be observed from the SEM images shown in Fig. 9. The discrepancy in the relationship between q_{uc} and CCC, as illustrated in Fig. 8, can be attributed to the differential prevalence of distinct morphologies of precipitated $CaCO_3$ crystals. However, the precise determination of the proportion of these morphologies and the underlying mechanisms that govern their formation necessitate further investigation. Nevertheless, the results have demonstrated that the concentration method of bacterial suspension proposed in this paper will have a negligible effect on

the biomineralization of UPB.

4.4. Discussion

4.4.1. Bacterial suspension concentration

Bacterial suspension concentration is typically achieved by centrifugation (Cuthbert et al., 2013; Nayanthara et al., 2019). This approach requires a high-speed centrifuge and is difficult to be applied to the treatment of large volumes of bacterial suspensions. The bacterial suspension concentration method proposed in this study is to concentrate the bacterial suspension using the flocs formed by the addition of calcium ions to the bacterial suspension as carriers. The *Sporosarcina pasteurii* used in this study is a type of gram-positive bacterium (Sharma et al., 2019). Its cell wall is negatively charged (Hall-Stoodley et al., 2004). Divalent metal cations, such as calcium ions, can be attracted to the negatively-charged bacterial cell wall, as the functional groups of the cell wall contribute to metal binding (Thomas and Rice, 2014). During this ion adsorption process, flocs can form (as illustrated in Fig. 1). This is primarily due to the flocculation of the extracellular polymeric substances (EPS), which mainly comprises macromolecular substances such as polysaccharides, proteins and nucleic acids (Flemming and Wingender, 2010). These substances can physically connect neighboring bacterial cells to each other and settle as flocs with other inert materials (Shen et al., 1993). Calcium ions may cause conformational changes in certain surface proteins that interact with both surfaces and link them together (Papahadjopoulos et al., 1990). Moreover, as the bacterial cells are negatively charged, resulting in a barrier to bacterial cells to approach each other, calcium ions are

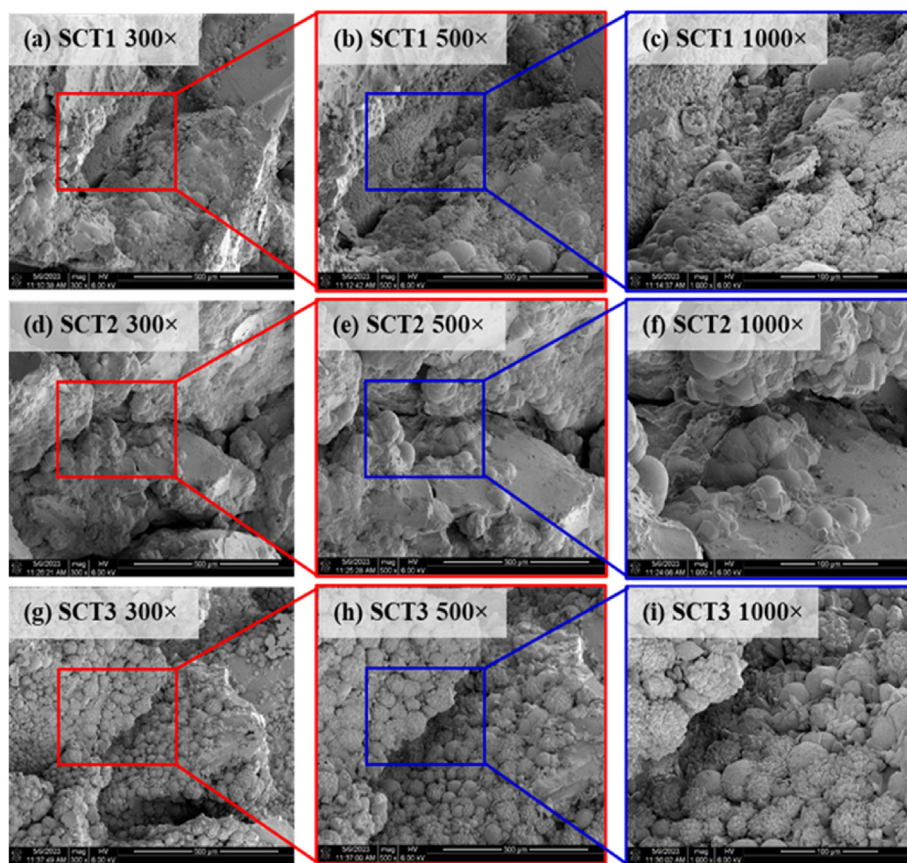


Fig. 9. SEM images of the sand column samples subjected to 6 treatments: (a)–(c) SCT1 case with concentrated bacterial suspension diluted by medium; (d)–(f) SCT2 case with concentrated bacterial suspension diluted by tap water; and (g)–(i) SCT3 case with original bacterial suspension.

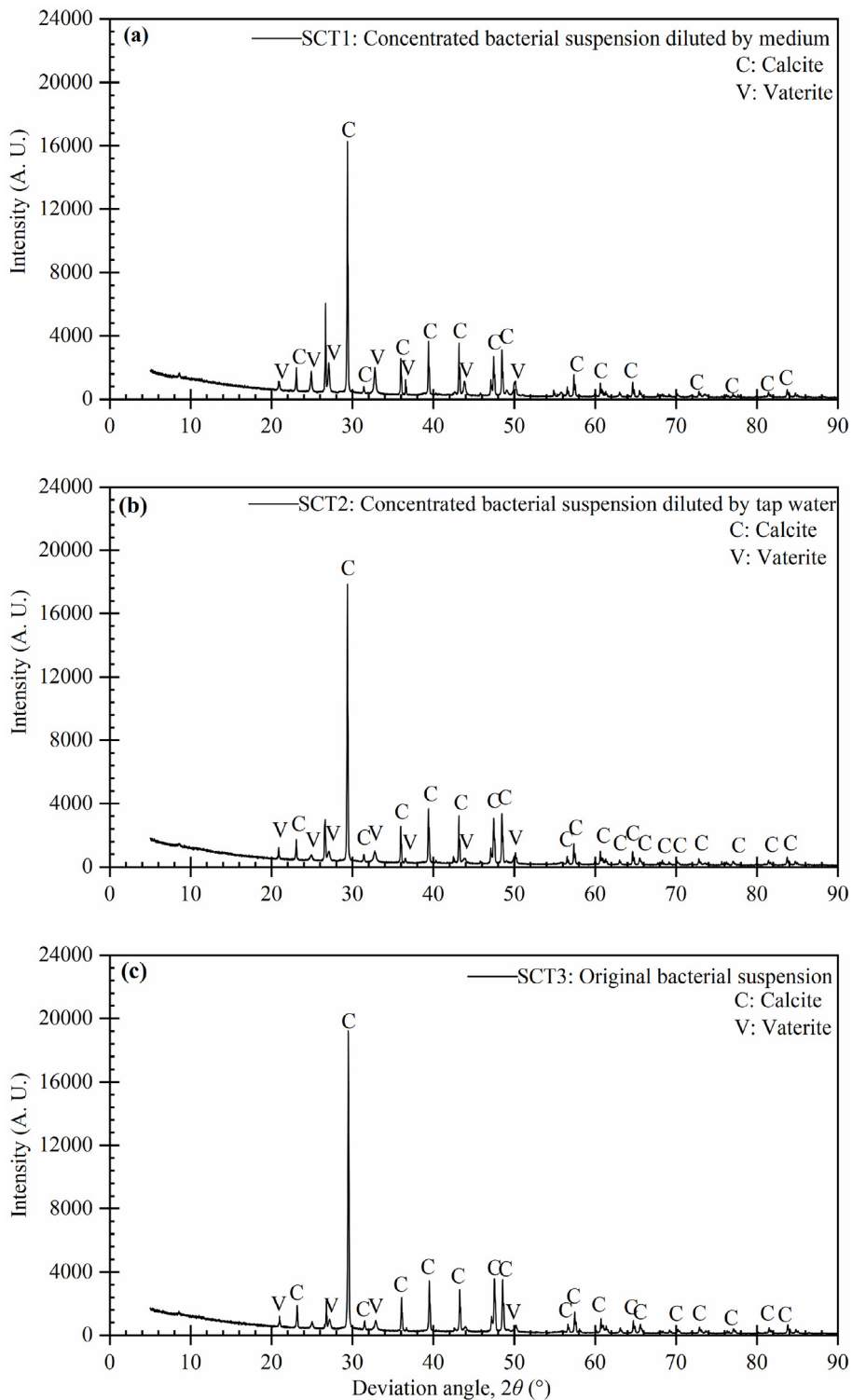


Fig. 10. XRD analysis of the sand column samples subjected to 6 treatments: (a) SCT1 case with concentrated bacterial suspension diluted by medium; (b) SCT2 case with concentrated bacterial suspension diluted by tap water; and (c) SCT3 case with original bacterial suspension.

considered to act as cationic bridges between bacterial cells by neutralizing the negative charges on bacterial surfaces (Schmidt and Ahring, 1993). This results in a decrease in electrical repulsion and an increase in bacterial cell attraction (Liu et al., 2002). The coupling of the EPS bonding and calcium ion-bridge results in the flocculation and precipitation of bacterial cells.

4.4.2. Cost analysis

Test results demonstrate that the volume of bacterial suspension can be significantly reduced by adding a small amount of calcium ions. As illustrated in Fig. 2, the volume of bacterial suspension can be reduced to one third of the original suspension when 0.05 mol/L calcium ions are employed for concentrating the

bacterial suspension with OD_{600} of 3.36. This suggests that two thirds of the cost of transporting the bacterial suspension can be saved.

4.4.3. Significance and limitation

The bacterial suspension concentration method proposed in this study facilitates the transportation of the bacterial suspension for large-scale MICP grouting in geotechnical engineering. By concentrating the bacterial suspension, its volume can be reduced to save the transportation costs. The experimental results show that the bacterial concentration method proposed in this paper does not affect the cementation effect of UPB on the soil. Therefore, the application of this method in geotechnical engineering will result in cost savings in bacterial transport. This study mainly aims to demonstrate the feasibility and effectiveness of the bacterial concentration method in soil biocementation. However, there are some underlying mechanisms that have not yet been clarified, such as the differences in strength enhancement (Fig. 8b) and the effect on the polymorph and crystallinity of the precipitated calcium carbonate crystal (Figs. 9 and 10). Further studies are required in the future to address the aforementioned limitations.

5. Conclusions

A new bacterial concentration method is proposed to facilitate the production of a large quantity of bacterial suspension for large-scale field application. The effectiveness of the proposed method is validated through the data presented. It is demonstrated that the concentration ratio defined as the volume ratio of the mixture to the flocs decreases with increasing calcium concentration. There is a threshold concentration of calcium ions for the bacterial concentration method, which tends to increase linearly with the OD_{600} of the bacterial suspension. The solution tests demonstrate that this method does not affect the ability of bacteria to hydrolyze urea. Moreover, the data from sand column tests also indicate that the use of the concentrated bacteria enhances the biocementation effect. However, the potential mechanisms underlying this effect require further studies.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Mingjuan Cui is presently working as an associate professor at the College of Civil Engineering, Fuzhou University, China. She obtained her MSc degree from Yantai University, China and PhD degree in Geotechnical Engineering from Huazhong University of Science and Technology, China. She was a postdoctor at Huazhong University of Science and Technology, China, from 2017 to 2019, a research fellow at Nanyang Technological University, Singapore, from 2019 to 2021. Her research mainly focuses on the basic theory and technology development of bio-geotechnics. Dr. Cui has been participated in several National Natural Science Foundation of China (NSFC) projects. She has published research papers in authoritative international and national journals.