Journal of Cleaner Production

Effects of grain size and treatment methods on the cementation of bio-improved calcareous sand

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original article
Keywords:	Calcareous sand; Microbially induced carbonate precipitation; Immersion method; Mix method; Failure pattern; Strength and stiffness
Corresponding Author:	Bo Zhou, Ph.D. Huazhong University of Science and Technology CHINA
First Author:	Xing Zhang
Order of Authors:	Xing Zhang
	Bo Zhou
	Ziyang Wu
	Huabin Wang
Abstract:	Calcareous sand, which is characterised by irregular morphologies and abundant intra-particle pores, poses difficulties for ocean construction. This study investigated the effects of microbially induced carbonate precipitation (MICP) treatment methods and grain size on the bio-cementation of calcareous sand. Calcareous sand specimens with a wide range of grain sizes were first treated by MICP with immersion and mix methods. Scanning electron microscopy analysis revealed the presence of air bubbles inside the specimens treated by the mix method, which hindered bio-cement production, further promoting the emergence of a gradual failure pattern and reducing the strength and stiffness of the sand specimens. A series of unconfined compressive strength tests indicated that the strength and stiffness of the calcareous sand specimens treated by the immersion method were considerably greater than those of the specimens treated by the mix method in the suitable grain size range. In addition, the immersion method was not suitable for sand specimens with a median grain size of <0.5 mm. In contrast, the mix method was suitable for sand specimens of various sizes. As with the immersion-treated specimens, the specimens treated by mix method displayed better mechanical properties at the early treatment stages, while the mix method-treated specimens exhibited much lower increase rates of strength and stiffness; moreover, the mix method-treated specimens exhibited lower strength and stiffness after long-term treatment than the immersion-treated specimens.
Suggested Reviewers:	Brina Mortensen Montoya, Doctor Assoc Professor, NC State University bmmorten@ncsu.edu
	Dilan Robert, Doctor Associate Professor, RMIT University dilan.robert@rmit.edu.au
	Chaosheng Tang, Doctor Professor, Nanjing University tangchaosheng@nju.edu.cn
	Zhen-Yu Yin, Doctor Professor, The Hong Kong Polytechnic University zhenyu.yin@polyu.edu.hk

School of Civil and Hydraulic Engineering
Huazhong University of Science and Technology
Wuhan, China

30 April 2023

Dear Editor:

I am delighted to submit an original research manuscript entitled “Effects of grain size and treatment methods on the cementation of bio-improved calcareous sand” by Xing Zhang, Bo Zhou, Ziyang Wu, and Huabin Wang. This manuscript has not been previously published nor is it currently under consideration for publication in English or any other language. We request that the paper be considered for review as an article for possible publication in *Journal of Cleaner Production*.

This study provides a comprehensive examination of the treatment efficacy and mechanical characteristics of two microbially induced carbonate precipitation (MICP) methods for marine calcareous sand and explores their application potential in environmental engineering. The insights and outcomes are likely to be valuable for researchers exploring MICP and soil improvement.

Correspondence regarding this paper should be directed to:

Dr Bo Zhou
School of Civil and Hydraulic Engineering
Huazhong University of Science and Technology
Please e-mail at zhoubohust@hust.edu.cn

Thank you for your consideration, and we look forward to hearing from you.

Yours sincerely,

Bo Zhou

Effects of grain size and treatment methods on the cementation of bio-improved calcareous sand

Xing Zhang[12], Bo Zhou[1*], Ziyang Wu[1], Huabin Wang[1]

[1]School of Civil and Hydraulic Engineering,
Huazhong University of Science and Technology,
Wuhan, China

[2] School of Computer Science and Engineering,
Nanyang Technological University,
Singapore

*Corresponding author:
Dr Bo Zhou, zhoubohust@hust.edu.cn

Highlights

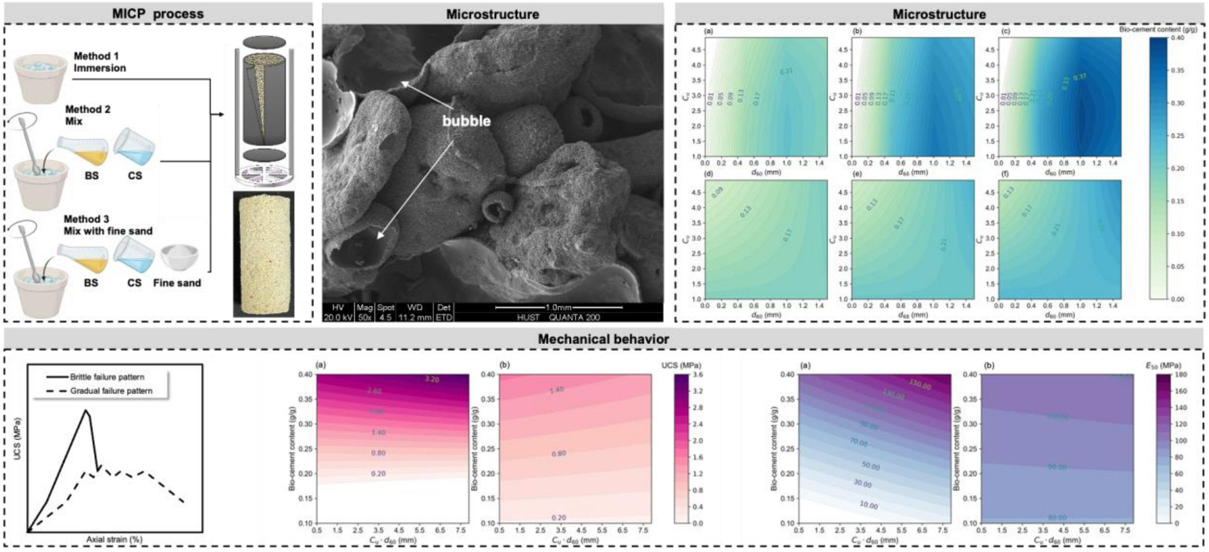
Mix method generated air bubbles, weakened MICP treatment effectiveness and reduced strength of sand specimen.

MICP-treated sand exhibited brittle and gradual failure patterns.

Mix method produced high initial strength but low rate of strength increase, while immersion method produced low initial strength but high rate of strength increase.

Mix method suits small-particle-size sand, while immersion method suits medium-particle-size sand.

Graphical abstract



Abstract

Calcareous sand, which is characterised by irregular morphologies and abundant intra-particle pores, poses difficulties for ocean construction. This study investigated the effects of microbially induced carbonate precipitation (MICP) treatment methods and grain size on the bio-cementation of calcareous sand. Calcareous sand specimens with a wide range of grain sizes were first treated by MICP with immersion and mix methods. Scanning electron microscopy analysis revealed the presence of air bubbles inside the specimens treated by the mix method, which hindered bio-cement production, further promoting the emergence of a gradual failure pattern and reducing the strength and stiffness of the sand specimens. A series of unconfined compressive strength tests indicated that the strength and stiffness of the calcareous sand specimens treated by the immersion method were considerably greater than those of the specimens treated by the mix method in the suitable grain size range. In addition, the immersion method was not suitable for sand specimens with a median grain size of <0.5 mm. In contrast, the mix method was suitable for sand specimens of various sizes. As with the immersion-treated specimens, the specimens treated by mix method displayed better mechanical properties at the early treatment stages, while the mix method-treated specimens exhibited much lower increase rates of strength and stiffness; moreover, the mix method-treated specimens exhibited lower strength and stiffness after long-term treatment than the immersion-treated specimens.

Keywords: Calcareous sand; Microbially induced carbonate precipitation; Immersion method; Mix method; Failure pattern; Strength and stiffness

1 Introduction

Calcareous sand, which is widely distributed in tropical and subtropical regions, is commonly used as a foundation material for coastal infrastructure construction or as a backfilling material for reclamation work in ocean engineering (Wang et al., 2019). Composed of debris and skeletal remnants of marine organisms, calcareous sand is characterised by irregular morphologies and abundant intra-particle pores (Zhou et al., 2020; Zhang et al., 2021). Thus, it is prone to grain breakage and degradation, leading to excessive settlement and the sudden loss of soil strength (Coop, 1990).

To meet engineering requirements, enhancing the mechanical properties of calcareous sand is vital. Traditional physical compaction methods (Wang et al., 2019) and chemical grouting methods (Xiao et al., 2018; Shen et al., 2020) have been proposed as possible solutions. However, physical compaction can cause an increase in grain breakage owing to the effect of dynamic loads, and chemical grouting can cause damage to the marine ecological environment. Thus, novel stabilisation techniques are urgently needed in ocean engineering to address these environmental concerns.

MICP has been recognised as a promising and eco-friendly soil improvement technique that involves the cementation of sand particles (van Paassen et al., 2010; Cheng et al., 2014; Montoya and DeJong, 2015; Xiao et al., 2019; Liu et al., 2021). MICP treatment has several applications, such as carbon emissions reduction (Almajed et al., 2020; Renjith et al., 2021), erosion control (Jiang et al., 2010; Liu et al., 2020; Wang et al., 2021c), permeability reduction(Ivanov et al., 2019; Xie et al., 2023), recycle optimization (Yang et al., 2020; Peng et al., 2022; Zhang et al., 2023), and the enhancement of soil bearing capacity and liquefaction resistance (He and Chu, 2014; Cheng et al., 2017; Wang et al., 2021; Liu et al., 2022). Among various MICP processes, the ureolysis-driven method has received significant attention owing to its ability to rapidly produce calcium carbonate crystals by urea hydrolyzation into carbonate ions in the presence of calcium (Cheng et al., 2013; Chu et al., 2014). Below are equations that describe the MICP process.

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^{+} + CO_3^{2-} \tag{1}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(solid) \tag{2}$$

Permeation grouting is a common method used in MICP treatment, in which the cementation medium is transported into inter-particle pores via permeation. In some studies, bacterial suspension and cementation solutions were injected sequentially or together into the soil specimens (Qabany and Soga 2014; Wu et al., 2019; Pan et al., 2021). However, because the injection method requires numerous treatment rounds, researchers have adopted an immersion method as an alternative in lab-scale studies (Cheng et al., 2020; Lv et al., 2021; Zeng et al., 2021). Besides, both the injection and immersion methods are less effective for fine sand owing to the difficulty of the cementation medium in permeating through small pores, which are also prone to clogging, resulting in inhomogeneous bio-cement (Cui et al., 2017; Xiao et al., 2022). The mix method, which involves the mixing of bacterial suspension, soil grains, and cementation solutions to form a uniform bio-cement, has shown good treatment performance for fine sand (Montoya et al., 2019). However, the mix method is not efficient for treating coarse sand, as the wider inter-particle pores limit the bacteria's ability to adhere to sand grains.

In addition, the immersion and mix methods have been widely used to improve quartz sand soil (van Paassen et al., 2010; Qabany and Soga 2014; Chu et al., 2014; Terzis et al., 2016; Cui et al., 2017; Pan et al., 2020; Ma et al., 2022). However, calcareous sand, characterised by rich intra-particle pores and irregular morphologies, has the potential to absorb more cementation media, owing to its unique property. Moreover, the inter-particle pores and strength of calcareous sand vary significantly with particle size (Zhou et al., 2020), thus the treatment effects of the immersion and mix methods on calcareous sand are more complex than those on quartz sand (Dyer and Viganotti., 2016; Liu et al., 2019; Liu et al., 2021; Zeng et al., 2021; Cui et al., 2021; Zhou et al., 2023). Therefore, a comprehensive investigation of the treatment effects of both methods on calcareous sand and the optimisation of the methods is needed.

The objective of this study was to investigate the effect of the immersion and mix methods on the cementation of bio-cement in calcareous sand, and to recommend a suitable treatment optimisation approach. Calcareous sands of varying grain sizes, including coarse sand, very coarse sand, medium-fine sand, and mixture sand, were initially treated by the immersion and mix methods for multiple rounds. The impacts of these methods on the morphology and microstructures of the bio-cement were analysed using scanning electron microscopy (SEM). Additionally, the bio-cement content was studied as a function of grain size. Finally, a series of unconfined compressive strength (UCS) tests were conducted to evaluate the effects of the immersion and mix methods on the fracture strength and stiffness of calcareous sand specimens with different grain sizes.

2 Methodology

2.1 Test materials

The calcareous sand used in this study was obtained from the Spratly Islands in the South China Sea. Four types of calcareous sand were used: very coarse sand (1–2 mm), coarse sand (0.5–1.0 mm), medium-fine sand (0.1–0.5 mm), and mixture sand (0.1–2 mm), as shown in Fig. 1. The calcareous sand contained biological remains such as corals and shells and exhibited irregular shapes with rich intra-particle pores. The cumulative particle-size distribution curve for the sieved sand is presented in Fig. 2. The detailed parameters are listed in Table 1, with D_{60} and D_{10} representing particle sizes corresponding to 10% and 60% finer materials on the cumulative particle-size distribution curve, respectively. The coefficient of uniformity (C_u), calculated as D_{60}/D_{10}, is also provided in Table 1.

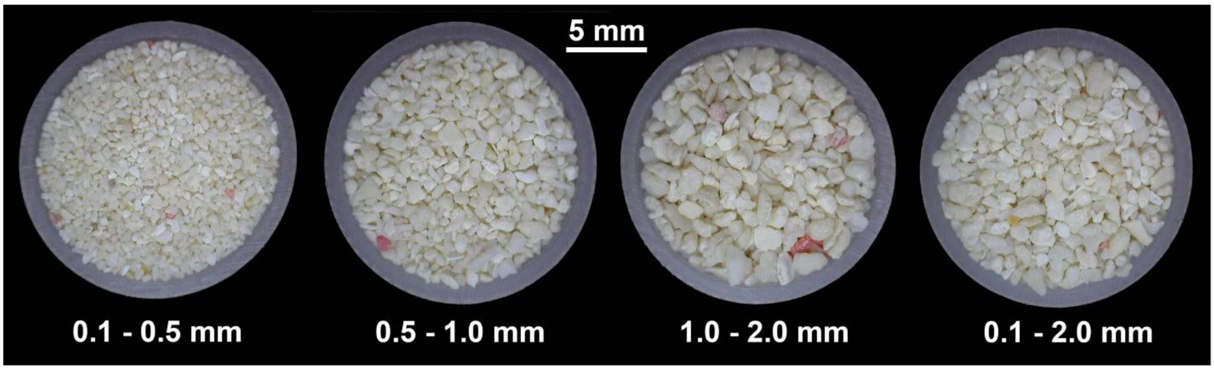


Fig. 1. Photographs of the tested calcareous sand

Table 1. Parameters of the three types of tested sand

Sand type	Grain size (mm)	D_{10}	D_{60}	C_u
Very coarse sand	1.0–2.0	1.1	1.6	1.45
Coarse sand	0.5–1.0	0.55	0.80	1.45
Medium-fine sand	0.1–0.5	0.14	0.33	2.35
Mixture sand	0.1–2.0	0.32	0.78	2.43

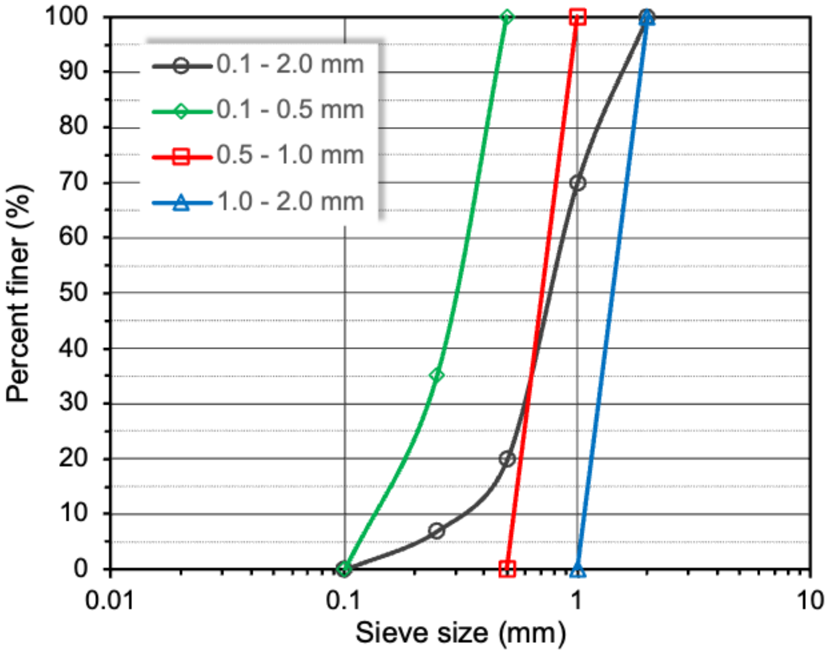


Fig. 2. Particle size distribution of the tested sand

2.2 MICP treatment process

In this study, *Sporosarcina pasteurii* (ATCC 11859), a commonly used bacterium, was selected for the MICP treatment. The bacterial cells were grown in a liquid medium containing 20 g/L yeast extract (NH_4-YE), 10 g/L NH_4Cl, 12 mg/L MnSO4·H_2O, and 24 mg/L $NiCl_2·6H_2O$ at a pH of 8.5 and a temperature of 30°C. The optical density at 600 nm (OD_{600}) of the bacterial suspension (BS) was measured using an ultraviolet spectrophotometer after 24 h of culture, and an OD_{600} range of 1.8–2.0 was obtained. The urease activity of the bacteria was determined by measuring its electrical conductivity, which averaged approximately 9.9–10.2 mM urea hydrolysed per minute. Based on a recent study (Qabany and Soga, 2014), a mixture of urea

(CON_2H_4) and $CaCl_2$ was adopted as the CS for MICP treatment, and the concentration of the mixture was ~0.5 mol/L.

Calcareous sand specimens with a diameter of 38 mm and a height of 76 mm were prepared using a specially designed mould made of 2 mm-thick geotextile and polyethylene brackets (Fig. 3). The geotextile helped to maintain the loose sand in a cylindrical shape while allowing the solution to permeate into the sample. The specimens were divided into 36 groups according to particle size (0.5–1.0 mm, 1.0–2.0 mm, and 0.1–2.0 mm), MICP preprocessing methods (immersion, mix, and mix + fine sand), and MICP treatment rounds (one, two, and three). The mould was filled with sand, which was then compacted to a controlled relative density of 0.62 ± 0.02. Each test group included three parallel specimens.

The adopted MICP process consisted of two main steps: preprocessing treatment and cementation treatment. The MICP preprocessing methods are detailed below:

Method 1 (immersion): The mould was filled with dry sand to create a cylindrical specimen, and the specimen was then placed in a polystyrene container. BS (300 mL/sample) was then added to the container to fully immerse the specimens, and the container was placed in an incubator at 30°C for 2 h.

Method 2 (mix): Dry sand, BS, and CS were mixed at a mass ratio of 10:2:2. This ratio ensures that the sample will not be oversaturated. Afterwards, the mould was filled with wet sand to create the specimen, and the specimen was then placed in an incubator at 30°C for 2 h.

Method 3 (mix + fine sand): Fine sand with a grain size of 0.1–0.5 mm was added to the mixture obtained by method 2 such that the mass ratio of sand, BS, CS, and fine sand was 10:2:2:2.

After the completion of the MICP preprocessing treatment, the specimens were subjected to cementation treatment, which involved the following steps: (1) The specimens were transferred into a new container, to which CS (~500 mL/specimen) was added, and the container was then placed in an incubator for 24 h to trigger carbonate precipitation within each specimen. (2) Step (1) was repeated to complete one round of MICP cementation treatment, which included a total immersion time of 48 h in CS.

After a specific round of MICP treatment was completed, the moulds covering the specimens were removed. The specimens were then carefully washed with deionised water to remove any soluble salts (e.g., calcium chloride) that may have accumulated during the treatment process. Subsequently, the specimens were thoroughly oven-dried at 60°C to ensure complete moisture removal. Finally, the bio-cemented samples were prepared for UCS testing.

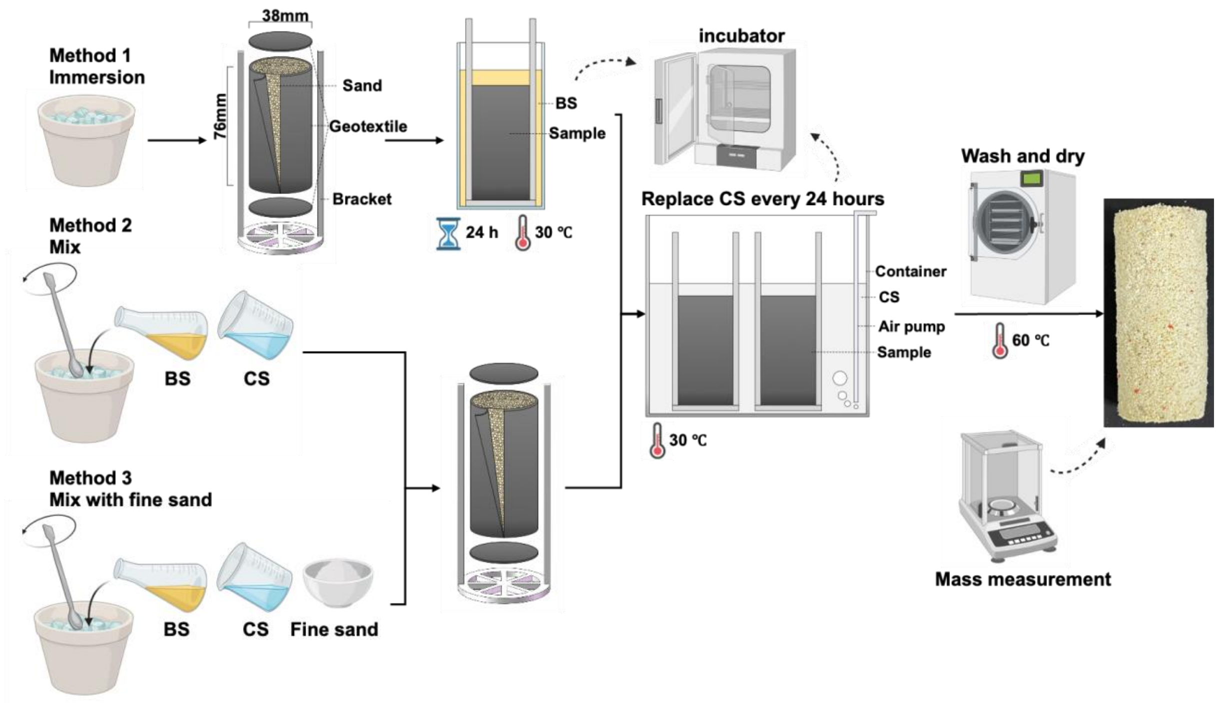


Fig. 3. Schematic of specimen preparation and the MICP process

2.3 Bio-cement analysis

The bio-cement content, which serves as a significant indicator of the extent of calcium carbonate precipitation, can be determined using various techniques. Acid washing is a popular method for determining the bio-cement content of quartz sand (Qabany and Soga, 2014; Cui et al., 2021). However, for calcareous sand, which contains a substantial proportion of calcium carbonate, acid corrosion is a concern. In this study, the bio-cement contents (C_c) of both calcareous sand and quartz sand were calculated using Equation (3):

$$C_c = \frac{M_1 - M_0}{M_0} \times 100\% \quad (3)$$

where M_0 is the weight of the untreated dry sand, and M_1 is the weight of the bio-cemented sand in the dry state.

To measure the UCS, a series of UCS tests were conducted in accordance with ASTM D2166 (2016) standards. The sand samples were subjected to uniaxial compression at a rate of 0.1 mm/s until failure occurred. Real-time compressive load and sample deformation in the vertical direction were captured using a load sensor and a displacement sensor, respectively. After the UCS test, the bio-cemented samples were cut into cubes of ~8 mm. The oven-dried cubes were examined by SEM and X-ray diffraction (XRD).

3 Results and discussion

3.1 Bio-cement content

In general, the bio-cement content of specimens increased with the number of cementation treatment rounds, regardless of the adopted preprocessing method. The bio-cement content was significantly affected by both the type of preprocessing method and the sand particle size. As shown in Fig. 4, except for the sand treated by the mix method, the sand specimens treated by the other methods exhibited increasing bio-cement content with increasing particle size; the bio-cement content reached a peak at ~1 mm before slightly decreasing. In contrast, the specimens preprocessed by the mix method showed decreasing bio-cement content with increasing particle size.

The medium-fine sand (grain size: 0.1–0.5 mm) preprocessed by the immersion method exhibited 0.04 less bio-cement content than the specimens preprocessed by the mix method. However, with increasing particle size, the immersion method-processed specimens exhibited a higher bio-cement content than the mix method-processed specimens, particularly for coarse sand (grain size: 0.5–1.0 mm) and very coarse sand (grain size: 1.0–2.0 mm). Interestingly, the mixture sand specimens (grain size: 0.1–2.0 mm) preprocessed by the immersion method showed a comparable bio-cement content to the corresponding coarse and very coarse sands, while the mixture sand specimens preprocessed by the mix method exhibited a bio-cement content almost as high as that processed by the immersion method, but higher than those of the coarse sand and very coarse sand preprocessed by the mix method.

According to the above findings, the immersion method tended to result in a higher bio-cement content in moderate-porosity sand specimens, while the mix method yielded a higher bio-cement content in small-porosity sand specimens. Thus, the mix method could be enhanced through the incorporation of fine sand to reduce the porosity of larger-size sand specimens. This improved method, referred to as the mix + fine sand method, as described in Section 2.2, has the potential to enhance bio-cementation in sand.

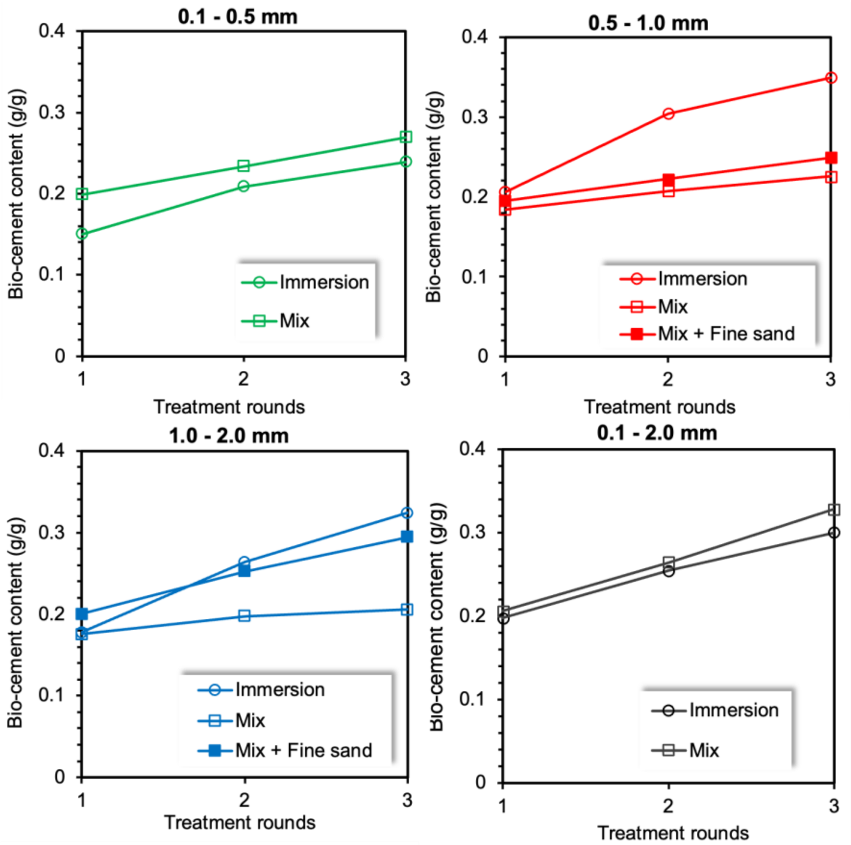


Fig. 4. Evolution of bio-cement content with the number of treatment rounds for samples treated by different methods

The mix + fine sand method improved the bio-cement content in the coarse sand and very coarse sand specimens. It more significantly enhanced the bio-cement content in very coarse sand compared with the mix method. This suggests that the average particle size and the particle size distribution influenced the bio-cement content of the treated sand. To elucidate the underlying mechanisms, the relationship between the continuous independent variables (D_{60} and C_u) and the continuous dependent variable (C_c) was investigated by the least squares method to fit a quadratic surface. The fine sand addition in the mix + fine sand method was considered to increase the C_u value of the sand specimens. For brevity, the mix + fine sand method was merged with the mix method. The results are presented below.

Immersion method with one treatment round:

$$C_{\mathrm{c}} = -0.192D_{60}^2 - 0.003C_{\mathrm{u}}^2 + 0.01D_{60} \cdot C_{\mathrm{u}} + 0.395D_{60} + 0.013 \quad (R^2 = 0.82) \tag{4}$$

Immersion method with two treatment rounds:

$$C_{\mathrm{c}} = -0.277D_{60}^2 - 0.002C_{\mathrm{u}}^2 + 0.006D_{60} \cdot C_{\mathrm{u}} + 0.596D_{60} - 0.006C_{\mathrm{u}} + 0.01 \quad (R^2 = 0.86) \tag{5}$$

Immersion method with three treatment rounds:

$$C_{\mathrm{c}} = -0.318D_{60}^2 - 0.008C_{\mathrm{u}}^2 + 0.013D_{60} \cdot C_{\mathrm{u}} + 0.685D_{60} + 0.022C_{\mathrm{u}} - 0.018 \quad (R^2 = 0.9) \tag{6}$$

Mix method with one treatment round:

$$C_{\mathrm{c}} = -0.318D_{60}^2 - 0.008C_{\mathrm{u}}^2 + 0.013D_{60} \cdot C_{\mathrm{u}} + 0.685D_{60} + 0.022C_{\mathrm{u}} - 0.018 \quad (R^2 = 0.9) \tag{7}$$

Mix method with two treatment rounds:

$$C_{\mathrm{c}} = 0.029D_{60} \cdot C_{\mathrm{u}} - 0.029D_{60} - 0.034C_{\mathrm{u}} + 0.247 \quad (R^2 = 0.62) \tag{8}$$

Mix method with three treatment rounds:

$$C_{\mathrm{c}} = 0.034D_{60} \cdot C_{\mathrm{u}} - 0.038D_{60} - 0.036C_{\mathrm{u}} + 0.276 \quad (R^2 = 0.47) \tag{9}$$

where R^2 is the coefficient of determination, which indicates how well a particular model predicts the outcome of a given dataset. It is defined as follows:

$$R^2 = 1 - \frac{SS_{\mathrm{res}}}{SS_{\mathrm{tot}}} = 1 - \frac{\sum (z - \hat{z})^2}{\sum (z - \bar{z})^2} \tag{10}$$

where SS_{tot} is the total sum of squares and SS_{res} is the residual sum of squares. The R^2 value normally ranges from 0 to 1, where a value of 1 indicates a perfect fit between the modelled values and the observed values, resulting in $SS_{res} = 0$ and. Higher R^2 values suggest a better fit of the model to the data, indicating that the model is more capable of predicting the outcome.

The fitting equation was used to draw the fitting surface, and the corresponding contour maps are shown in Fig. 5. A comparison between the mix method and the immersion method revealed that the immersion method was more sensitive to changes in D_{60} and C_u. The samples treated by the immersion method exhibited a sharper bio-cement content distribution, with a peak observed at $D_{60} = 1.0$ mm and $C_u = 1–3$. In contrast, the samples treated by the immersion method exhibited a flatter bio-cement content distribution, with a plateau formed at conditions of near low D_{60} and low C_u or high D_{60} and high C_u.

Achieving a high bio-cement content requires maximising the amount of BS inside the sand specimens and ensuring that the CS can permeate into the sand. Because bacteria typically range in size from 1 to 3 μm, which is much larger than the solute size in CS, a larger pore diameter is needed in the sand for bacteria to permeate into and wash out compared to CS. In the immersion method treatment, BS permeate into the sand specimens from the outside and adhered to the particles. Under a moderate particle size and balanced size distribution, the combined effect of the surface area and inter-particle pores of the sand samples provided an optimal environment for BS penetration and adhesion, resulting in a high bio-cement content. In contrast, the mix method treatment involved mixing loose sand and a small amount of CS with BS before the specimen was moulded. The smaller inter-particle pores in the sand prevented the loss of BS and ensured a uniform distribution within the sand. Therefore, under high or low C_u and D_{60} conditions, the inter-particle pores were small, resulting in a higher bio-cement content.

Furthermore, as observed in Fig. 5, as the number of treatment rounds increased, the immersion-treated sample exhibited rapidly increasing bio-cement content, but within narrow ranges of C_u and D_{60}. Excellent performance was observed within the ranges of $D_{60} = 0.5–1.5$ mm and C_u =

1–4. In contrast, the mix method-treated sample showed a slower increase in bio-cement content, but the method was more robust and applicable to wider C_u and D_{60} ranges.

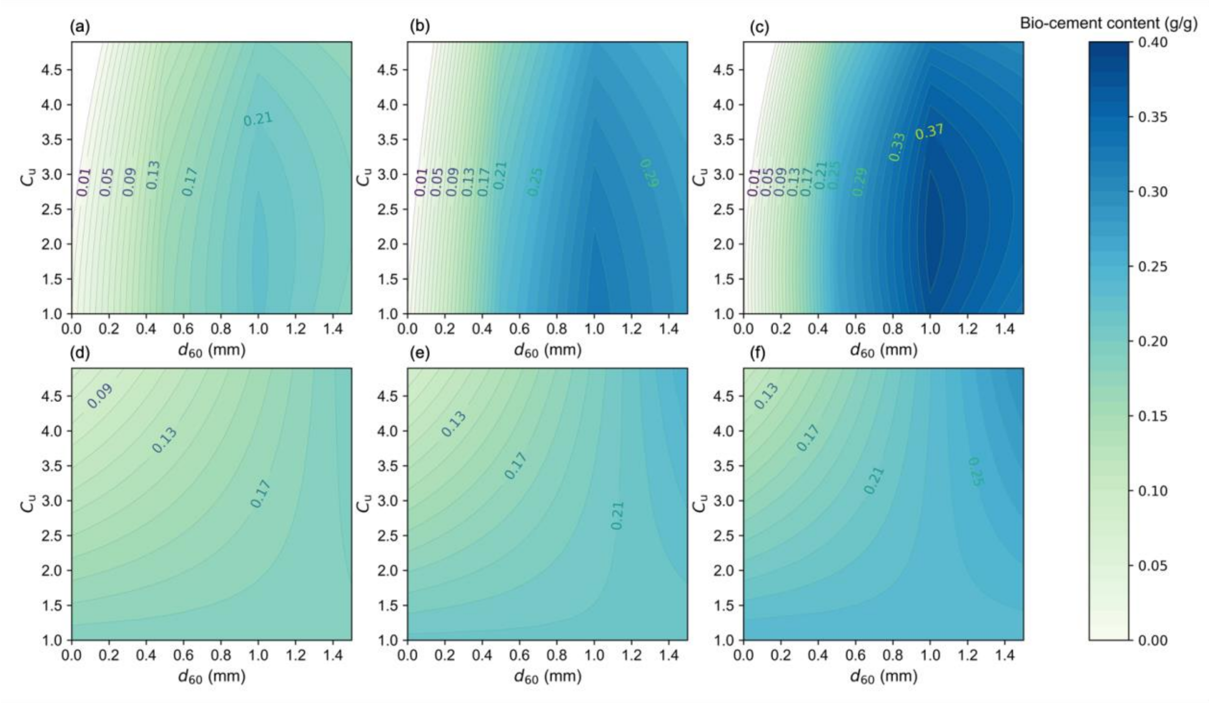


Fig. 5. Bio-cement distributions of specimens under various conditions: (a, b, c) immersion method; (d, e, f) mix method; (a, d) one treatment round; (b, e) two treatment rounds; (c, f) three treatment rounds.

3.2 Failure pattern

UCS tests were conducted on the bio-cement samples. Figure 6 depicts the stress–strain curves of the bio-cement samples subjected to different treatment methods and grading conditions. The UCS of all of the samples gradually increased with the number of treatment rounds. The curves showed two failure patterns: brittle failure and gradual failure, which are illustrated in Figure 6 (dashed-line box).

The brittle failure pattern was characterised by a rapid decrease in the UCS of the bio-cement sample after it reached its peak value. Conversely, in the gradual failure pattern, the UCS of the sample reached a peak, followed by a plateau with wavering UCS, and then slowly decreased. The immersion-treated samples exhibited a brittle failure pattern, which became more pronounced as the number of treatment rounds increased. The samples treated by the mix method exhibited both brittle and gradual failure patterns. The samples graded 0.1–0.5 mm and

0.1–2.0 mm showed a brittle failure pattern, with increasing brittleness as the number of treatment rounds increased. In contrast, the samples graded 0.5–1.0 mm and 1.0–2.0 mm showed a gradual failure pattern. Moreover, the sample treated by the mix + fine sand method tended to transition from a gradual failure pattern to a brittle failure pattern as the number of treatment rounds increased. Notably, this sample displayed a higher UCS than the sample treated without fine sand addition.

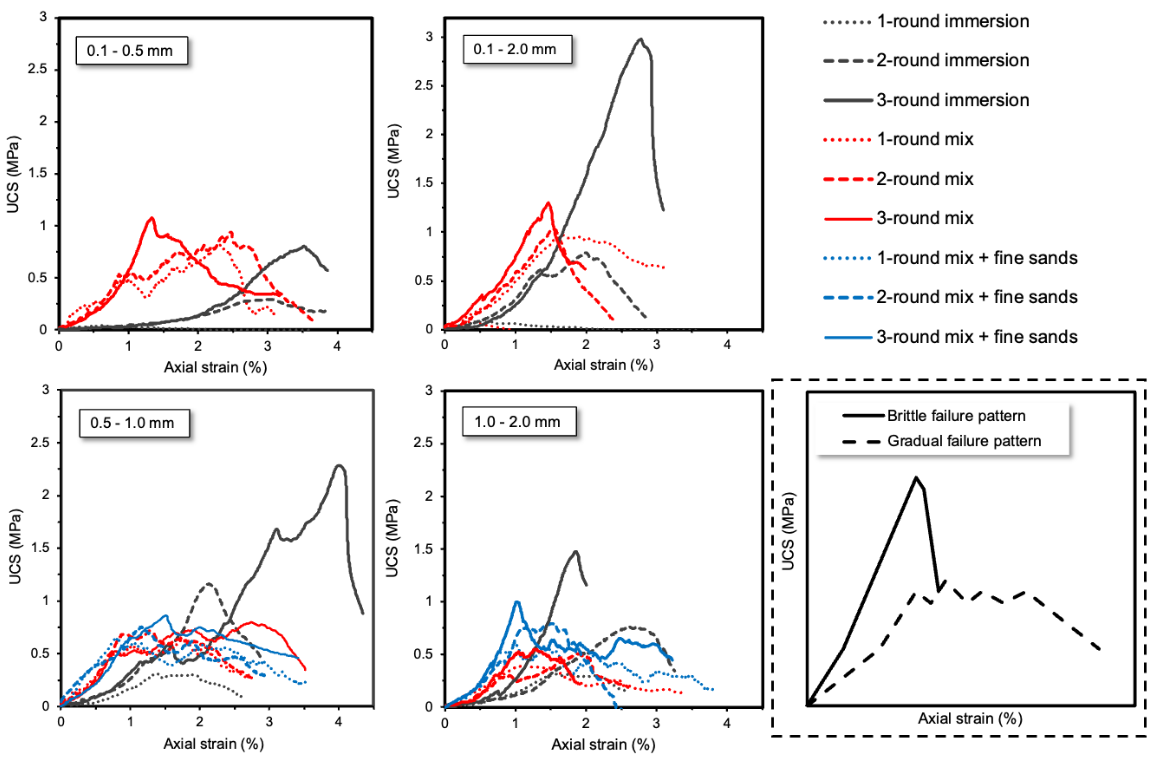


Fig. 6. Stress–strain curves of bio-cement specimens and failure patterns (dashed box)

3.3 Strength and stiffness

The mechanical behaviour of bio-cemented sand was investigated using two commonly used indicators: strength and stiffness. Strength was determined as the maximum peak stress on a stress–strain curve, while stiffness was calculated as E_{50}, that is, the tangent modulus at 50% of the peak stress. These values are graphically represented on stress–strain curves (Van Paassen et al., 2010; Liu et al., 2019). Figure 7 illustrates the curves of strength versus bio-cement content and stiffness versus bio-cement content for different treatment methods and particle sizes.

The particle size inhomogeneity significantly influenced the UCS and E_{50} of all of the treated bio-cemented sand specimens. Well-graded sand specimens with a more balanced particle size distribution exhibited improved inter-particle contact compared with poorly graded sand specimens, resulting in denser and tighter bio-cement and leading to higher UCS and E_{50}.

The immersion-treated specimens exhibited similar UCS and E_{50} trends. The mixture sand specimens showed the highest UCS and E_{50}, followed by coarse sand and very coarse sand. The coarse and very coarse sand exhibited comparable UCS and E_{50}. The medium-fine sand exhibited the lowest UCS and E_{50}. This indicates that the bio-cemented sand specimens with smaller average particle sizes were likely to have lower strength and stiffness. The small inter-particle pores in the medium-fine sand hindered BS penetration into the sand specimens during the immersion process, resulting in an inhomogeneous distribution of bio-cement and lower UCS and E_{50}.

The well-graded sand specimens treated by the mix method outperformed the corresponding poorly graded sand specimens in terms of both UCS and E_{50}. The occurrence of smaller-size particles tended to increase the UCS of the poorly graded sand specimens. Specifically, the medium-fine sand exhibited the highest UCS, followed by the coarse sand, and then the very coarse sand. However, the E_{50} trend was opposite to the UCS trend; the coarse sand exhibited the highest E_{50}, followed by the very coarse sand, and then the medium-fine sand. The UCS trend is attributable to the mixing of the BS, CS, and loose sand, resulting in the uniform adhesion of BS to the sand particles. Sand specimens with smaller particle sizes had smaller inter-particle pores, which prevented the loss of BS and led to the formation of denser bio-cement, resulting in increased UCS. The E50 trend observed in the immersion-treated specimens was also observed in this case and appeared to be positively correlated with the maximum particle size of the specimen.

For the mix + fine sand method, fine sand addition increased the C_u of the sand specimens, which in turn improved their strength and E_{50} compared with those of the mix method-treated sand.

A comparison of the mechanical behaviour of sand specimens preprocessed by different methods revealed that the UCS and E_{50} of specimens preprocessed by the immersion method were slightly lower than those obtained with the mix method at the beginning of the treatment period. However, with the extension of the treatment period, the UCS and E_{50} of the specimens preprocessed by the immersion method significantly increased, surpassing those of the mix method-treated specimens, except for samples with a particle size of 0.1–0.5 mm.

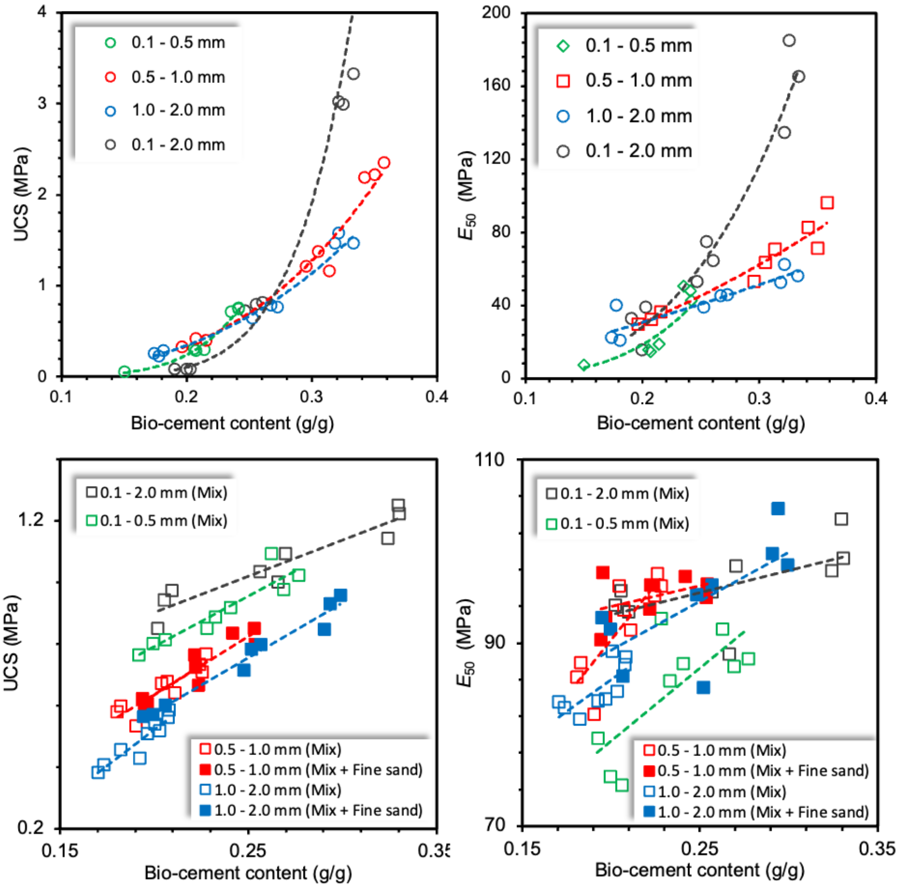


Fig. 7. Strength and stiffness of bio-cement specimens treated by the immersion method (first row) and the mix method (second row)

In a manner similar to the curve fitting approach described in Section 3.1, the relationship between the independent variable ($C_u \cdot D_{60}$) and the dependent variable (C_c) was explored, and the fit surface was obtained. Both C_u and D_{60} influenced the strength and stiffness of the specimens, and thus, their product $C_u \cdot D_{60}$ was used as the independent variable in the analysis. Additionally, in the case of the mix + fine sand method, the inclusion of fine sand was considered to potentially increase the C_u value of the sand specimens, and therefore this method was merged with the mix method for the analysis. The results showed that the quadratic coefficient was close to zero, hence, the quadratic terms were neglected in the analysis. The findings are as follows:

Immersion method:

$$UCS = 0.598D_{60} \cdot C_u \cdot C_c - 0.139D_{60} \cdot C_u + 12.384C_c - 2.195 \quad (R^2 = 0.77) \tag{11}$$

$$E_{50} = 8.467D_{60} \cdot C_u \cdot C_c + 4.267D_{60} \cdot C_u + 465.269C_c - 73.94 \quad (R^2 = 0.54) \tag{12}$$

Mix method:

$$UCS = -0.122D_{60} \cdot C_u \cdot C_c + 0.007D_{60} \cdot C_u + 5.054C_c - 0.334 \quad (R^2 = 0.79) \tag{13}$$

$$E_{50} = 2.341D_{60} \cdot C_u \cdot C_c - 0.263D_{60} \cdot C_u + 88.321C_c + 70.292 \quad (R^2 = 0.42) \tag{14}$$

The fitting surface was drawn based on the fitting equation, and the corresponding contour maps are presented in Fig. 8 and Fig. 9. The contour map of the immersion method was more sensitive to D_{60} and C_u than that of the mix method, consistent with the findings in Section 3.1. Overall, the strength and stiffness of the sand specimens treated by all of the methods increased with increasing bio-cement content. Specifically, among the immersion-treated specimens, the specimens with smaller $C_u \cdot D_{60}$ values tended to exhibit lower strength and stiffness. In contrast, among the mix method-treated specimens, the specimens with smaller $C_u \cdot D_{60}$ values tended to exhibit higher strength but lower stiffness.

Interestingly, the contour maps of the immersion-treated specimens showed regions in which the strength and stiffness were equal to zero, despite the presence of bio-cement in the sand. This suggests that the bio-cement in these regions did not contribute to the specimen strength or stiffness; this phenomenon has been referred to as non-effective cementation in some studies. However, with increasing bio-cement content, the strength and stiffness of the immersion-treated sand specimens increased sharply, reaching two to three times that of the mix method-treated specimens. In contrast, the contour map of the mix method-treated specimens exhibited no blank areas, indicating a low level of non-effective cementation. Furthermore, the mix method-treated specimens with a small bio-cement content exhibited a higher initial strength and stiffness. Overall, the immersion method may be suitable for cases in which high strength and stiffness are required and sufficient treatment time is allowed, whereas the mix method may be suitable for temporary cases that do not require high strength and stiffness.

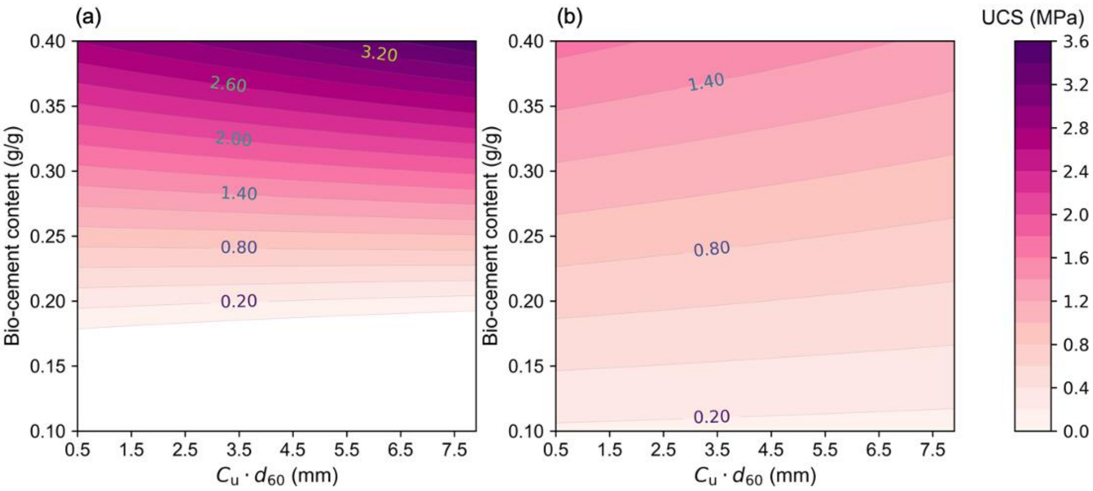


Fig. 8. Strength distribution of bio-cement specimens treated by (a) the immersion method and (b) the mix method

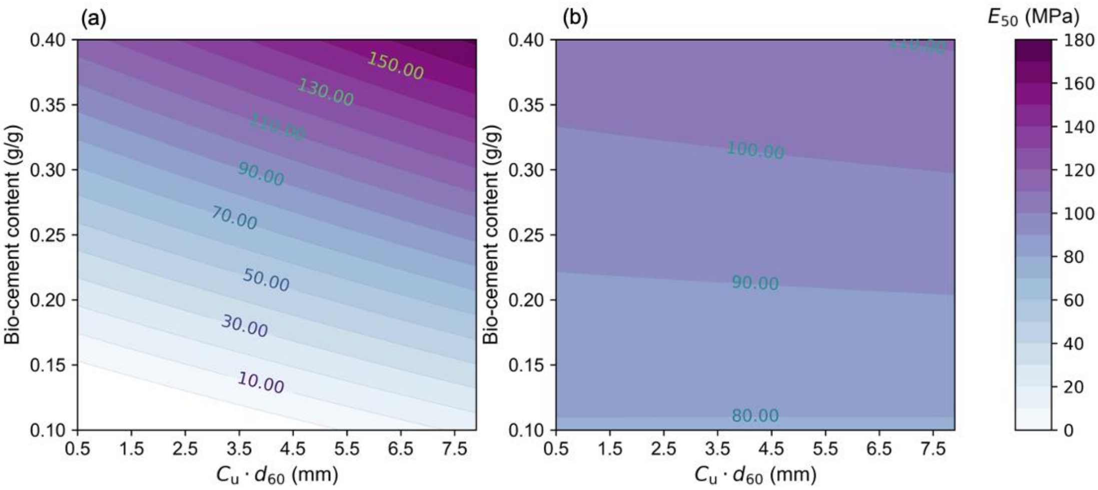


Fig. 9. Stiffness distribution of bio-cement specimens treated by (a) the immersion method and (b) the mix method

3.4 Microstructure characteristics

The previous analyses revealed that the sand specimens treated by the three methods (i.e., immersion [method 1], mix [method 2], and mix + fine sand [method 3]) differed in bio-cement content and mechanical behaviour. This study also investigated whether the differences in the composition and structure of the bio-cement contributed to these results. Because mix + fine sand (method 3) was similar to mix (method 2), the MICP preprocessing methods can be classified as immersion and mix. After UCS tests were conducted, the failure specimens were cut into small cubes for SEM observations. Additionally, three samples, untreated calcareous sand, calcareous sand treated by the immersion method, and calcareous sand treated by the mix method, were ground separately to powder for XRD testing to identify the phases present. The XRD results (Fig. 10) indicated that the calcareous sand contained aragonite, while the bio-cements produced by both the immersion and mix methods contained calcite. Therefore, the bio-cement composition did not contribute to the observed differences in the above-discussed mechanical behaviour.

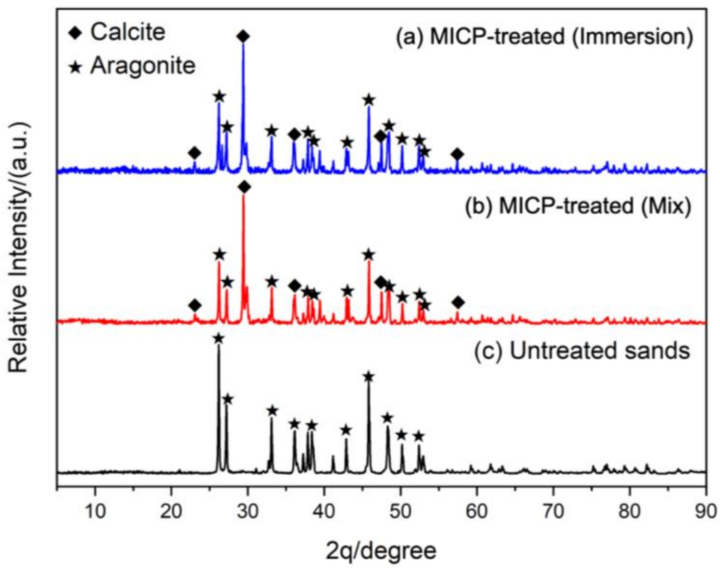


Fig. 10. XRD patterns showing the phases of the test materials

The SEM observation results (Fig. 11) revealed that bio-cement covered the surfaces of the calcareous sand and filled the inter-particle pores, resulting in effective cementation and the construction of inter-particle bridges. Specimens with smaller-size particles had more effective cementation, and well-graded sand exhibited more tightly connected cementation compared with poorly graded sand, which may explain the stronger mechanical behaviour illustrated in Fig. 7. In addition, the mix method produced thin shell-like cementation in the sand specimens owing to the rapid production of ammonia gas during the mixing of sand, BS, and CS. The ammonia gas could not be rapidly transported outside, resulting in air bubble formation in the inter-particle pores. The cement then slowly encircled the bubbles during MICP cementation, so that thin shells of cement were formed around the bubbles. These bubbles occupied the inter-particle pores, reducing sand permeability and ion exchange efficiency. Moreover, they compressed the space available for bio-cement growth, resulting in slower bio-cement growth compared with the specimens treated by the immersion method (Fig. 4 and Fig. 5).

Figure 11 shows that larger particle sizes led to larger-volume bubbles, which were more likely to connect and cause a loose sample quality with a gradual failure pattern during the UCS test (Fig. 6). This failure pattern originated from the UCS wavering process, which gradually compressed the bubble structure. In contrast, the occurrence of smaller-size particles in samples led to the generation of smaller and denser bubbles. The addition of fine sand to the sample limited the space available for bubble generation, leading to smaller bubble volumes. During the UCS test, the compression of small-volume bubbles corresponded to weaker UCS waves, resulting in a brittle failure pattern (0.1–2.0 mm) or a transition from a gradual to a brittle failure pattern (1.0–2.0 mm, Fig. 6). In summary, the bubbles generated in the mix method-treated samples play a key role in the differences in bio-cementation content and mechanical properties between samples treated by the immersion and mix methods.

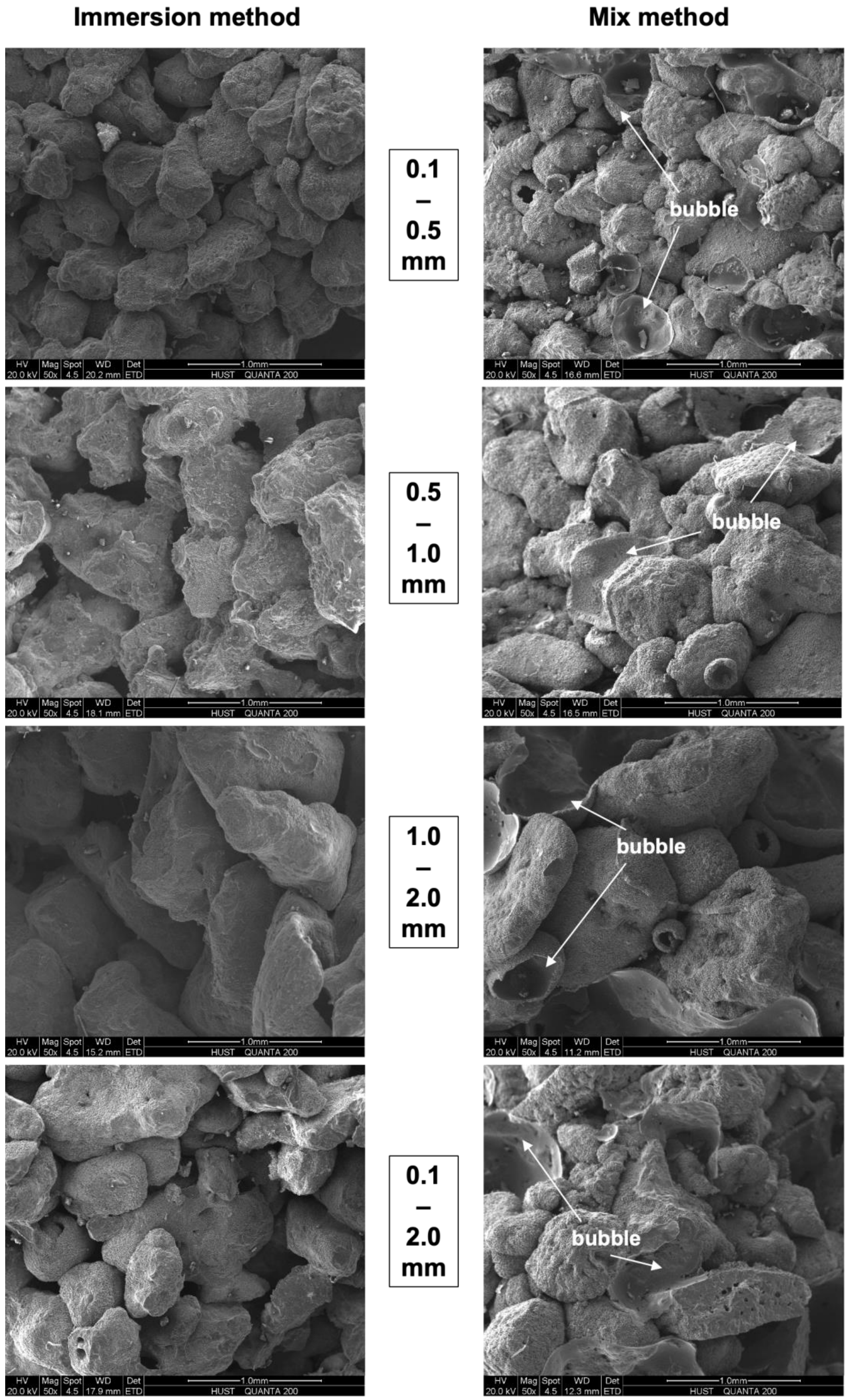


Fig. 11. SEM image of the bio-cement morphology

4. Conclusions

Based on the UCS test results and microstructures of the bio-cemented sand specimens, the following primary conclusions are drawn:

(1) The immersion method was unsuitable for sand specimens with an average particle size of <0.5 mm, but the immersion-treated samples showed a peak in bio-cement content at particle sizes of 0.5–1.5 mm and C_u of 1–4. In contrast, the mix method was suitable for sand specimens of all particle sizes, and the treated specimens exhibited a plateau in bio-cement content at small particle sizes and low C_u or large particle sizes and high C_u.

(2) The mix method produced dense air bubbles inside the sand specimens, and specimens with larger particles exhibited larger bubbles. These air bubbles hindered bio-cement production, further promoting the emergence of a gradual failure pattern and reducing the strength and stiffness of the sand specimens. In particular, the UCS and E_{50} of sand specimens preprocessed by the immersion method increased with higher bio-cement content and $C_u \cdot D_{60}$. In contrast, the UCS and E_{50} of specimens preprocessed by the mix method decreased and slightly increased, respectively, with higher bio-cement content and $C_u \cdot D_{60}$.

(3) The strength and stiffness of the immersion-treated sand specimens were low at the early stage of the treatment but rapidly increased after longer treatment. In contrast, the specimens preprocessed by the mix method exhibited higher strength and stiffness during the early stage of the treatment. However, due to the limitation of air bubbles, the rates of increase of the bio-cement content, strength, and stiffness were lower than those of the immersion-treated specimens.

Acknowledgments

This study was supported by Research Grants 42072298 and 41931286 from the National Natural Science Foundation of China.

References

Almajed, A., Abbas, H., Arab, M., Alsabhan, A., Hamid, W., Al-Salloum, Y., 2020. Enzyme-Induced Carbonate Precipitation (EICP)-Based methods for ecofriendly stabilization of different types of natural sands. Journal of Cleaner Production 274, 122627. https://doi.org/10.1016/j.jclepro.2020.122627

Cheng, L., Cord-Ruwisch, R., Shahin, M.A., 2013. Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Canadian Geotechnical Journal 50(1), 81-90. https://doi.org/10.1139/cgj-2012-0023

Cheng, L., Shahin, M., Cord-Ruwisch, R., 2014. Bio-cementation of sandy soil using microbially induced carbonate precipitation for marine environments. Géotechnique 64(12), 1010-1013. https://doi.org/10.1680/geot.14.T.025

Cheng, L., Shahin, M.A., Mujah, D., 2017. Influence of key environmental conditions on microbially induced cementation for soil stabilization. Journal of Geotechnical and Geoenvironmental Engineering 143(1), 04016083. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001586

Cheng, L., Kobayashi, T., Shahin, M.A., 2020. Microbially induced calcite precipitation for production of “bio-bricks” treated at partial saturation condition. Construction and Building Materials 231, 117095. https://doi.org/10.1016/j.conbuildmat.2019.117095

Chu, J., Ivanov, V., Naeimi, M., Stabnikov, V., Liu, H.-L., 2014. Optimization of calcium-based bioclogging and biocementation of sand. Acta Geotechnica 9, 277-285. https://doi.org/10.1007/s11440-013-0278-8

Coop, M., 1990. The mechanics of uncemented carbonate sands. Géotechnique 40(4), 607-626. https://doi.org/10.1680/geot.1990.40.4.607

Cui, M.-J., Zheng, J.-J., Zhang, R.-J., Lai, H.-J., Zhang, J., 2017. Influence of cementation level on the strength behaviour of bio-cemented sand. Acta Geotechnica 12, 971-986. https://doi.org/10.1007/s11440-017-0574-9

Cui, M.-J., Zheng, J.-J., Chu, J., Wu, C.-C., Lai, H.-J., 2021. Bio-mediated calcium carbonate precipitation and its effect on the shear behaviour of calcareous sand. Acta Geotechnica 16, 1377-1389. https://doi.org/10.1007/s11440-020-01099-0

Dyer, M., Viganotti, M., 2016. Oligotrophic and eutrophic MICP treatment for silica and carbonate sands. Bioinspired, Biomimetic and Nanobiomaterials 6(3), 168-183. https://doi.org/10.1680/jbibn.16.00002

He, J., Chu, J., 2014. Undrained responses of microbially desaturated sand under monotonic loading. Journal of Geotechnical and Geoenvironmental Engineering 140(5), 04014003. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001082

Ivanov, V., Stabnikov, V., Kawasaki, S., 2019. Ecofriendly calcium phosphate and calcium bicarbonate biogrouts. Journal of cleaner production 218, 328-334. https://doi.org/10.1016/j.jclepro.2019.01.315

Liu, L., Liu, H., Stuedlein, A.W., Evans, T.M., Xiao, Y., 2019. Strength, stiffness, and microstructure characteristics of biocemented calcareous sand. Canadian Geotechnical Journal 56(10), 1502-1513. https://doi.org/10.1139/cgj-2018-0007

Liu, S., Wang, R., Yu, J., Peng, X., Cai, Y., Tu, B., 2020. Effectiveness of the anti-erosion of an MICP coating on the surfaces of ancient clay roof tiles. Construction and Building Materials 243, 118202. https://doi.org/10.1016/j.conbuildmat.2020.118202

Liu, B., Xie, Y.-H., Tang, C.-S., Pan, X.-H., Jiang, N.-J., Singh, D.N., Cheng, Y.-J., Shi, B., 2021. Bio-mediated method for improving surface erosion resistance of clayey soils. Engineering Geology 293, 106295. https://doi.org/10.1016/j.enggeo.2021.106295

Liu, K., Ouyang, J., Sun, D., Jiang, N., Wang, A., Huang, N., Liang, P., 2022. Enhancement mechanism of different recycled fine aggregates by microbial induced carbonate precipitation. Journal of Cleaner Production 379, 134783. https://doi.org/10.1016/j.jclepro.2022.134783

Lv, C., Zhu, C., Tang, C.-S., Cheng, Q., Yin, L.-Y., Shi, B., 2021. Effect of fiber reinforcement on the mechanical behavior of bio-cemented sand. Geosynthetics International 28(2), 195-205. https://doi.org/10.1680/jgein.20.00037

Ma, G., Xiao, Y., Fan, W., Chu, J., Liu, H., 2022. Mechanical properties of biocement formed by microbially induced carbonate precipitation. Acta Geotechnica 17(11), 4905-4919. https://doi.org/10.1007/s11440-022-01584-8

Montoya, B., DeJong, J., 2015. Stress-strain behavior of sands cemented by microbially induced calcite precipitation. Journal of Geotechnical and Geoenvironmental Engineering 141(6), 04015019. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001302

Montoya, B.M., Safavizadeh, S., Gabr, M.A., 2019. Enhancement of coal ash compressibility parameters using microbial-induced carbonate precipitation. Journal of Geotechnical and Geoenvironmental Engineering 145(5), 04019018. https://doi.org/10.1061/(ASCE)GT.1943-5606.0002036

Pan, X., Chu, J., Yang, Y., Cheng, L., 2020. A new biogrouting method for fine to coarse sand. Acta Geotechnica 15, 1-16. https://doi.org/10.1007/s11440-019-00872-0

Peng, E., Hu, X., Chou, Y., Sheng, Y., Liu, S., Zhou, F., Wu, J., Cao, W., 2022. Study of microbially-induced carbonate precipitation for improving coarse-grained salty soil. Journal of Cleaner Production 365, 132788. https://doi.org/10.1016/j.jclepro.2022.132788

Qabany, A.A., Soga, K., 2014. Effect of chemical treatment used in MICP on engineering properties of cemented soils, Bio-and Chemo-Mechanical Processes in Geotechnical Engineering: Géotechnique Symposium in Print 2013. ICE Publishing, pp. 107-115.

https://doi.org/10.1680/bcmpge.60531.010

Renjith, R., Robert, D., Setunge, S., Costa, S., Mohajerani, A., 2021. Optimization of fly ash based soil stabilization using secondary admixtures for sustainable road construction. Journal of Cleaner Production 294, 126264. https://doi.org/10.1016/j.jclepro.2021.126264

Shen, J., Dongsheng, X., Liu, Z., Wei, H., 2020. Effect of particle characteristics stress on the mechanical properties of cement mortar with coral sand. Construction and Building Materials 260, 119836. https://doi.org/10.1016/j.conbuildmat.2020.119836

Terzis, D., Bernier-Latmani, R., Laloui, L., 2016. Fabric characteristics and mechanical response of bio-improved sand to various treatment conditions. Géotechnique Letters 6(1), 50-57. https://doi.org/10.1680/jgele.15.00134

van Paassen, L.A., Ghose, R., van der Linden, T.J., van der Star, W.R., van Loosdrecht, M.C., 2010. Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. Journal of geotechnical and geoenvironmental engineering 136(12), 1721-1728. https://doi.org/10.1061/(ASCE)GT.1943-5606.0000382

Wang, X., Weng, Y., Wei, H., Meng, Q., Hu, M., 2019. Particle obstruction and crushing of dredged calcareous soil in the Nansha Islands, South China Sea. Engineering Geology 261, 105274. https://doi.org/10.1016/j.enggeo.2019.105274

Wang, H.-L., Yin, Z.-Y., 2021. Unconfined compressive strength of bio-cemented sand: state-of-the-art review and MEP-MC-based model development. Journal of Cleaner Production 315, 128205. https://doi.org/10.1016/j.jclepro.2021.128205

Wang, K., Chu, J., Wu, S., He, J., 2021. Stress–strain behaviour of bio-desaturated sand under undrained monotonic and cyclic loading. Géotechnique 71(6), 521-533. https://doi.org/10.1680/jgeot.19.P.080

Wang, R., Jin, P., Ding, Z., Zhang, W., 2021c. Surface modification of recycled coarse aggregate based on Microbial Induced Carbonate Precipitation. Journal of Cleaner Production 328, 129537. https://doi.org/10.1016/j.jclepro.2021.129537

Wu, C., Chu, J., Cheng, L., Wu, S., 2019. Biogrouting of aggregates using premixed injection method with or without pH adjustment. Journal of Materials in Civil Engineering 31(9), 06019008. https://doi.org/10.1061/(ASCE)MT.1943-5533.0002874

Xiao, Y., Stuedlein, A.W., Chen, Q., Liu, H., Liu, P., 2018. Stress-strain-strength response and ductility of gravels improved by polyurethane foam adhesive. Journal of Geotechnical and Geoenvironmental Engineering 144(2), 04017108. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001812

Xiao, Y., He, X., Evans, T.M., Stuedlein, A.W., Liu, H., 2019. Unconfined compressive and splitting tensile strength of basalt fiber–reinforced biocemented sand. Journal of Geotechnical and Geoenvironmental Engineering 145(9), 04019048.

https://doi.org/10.1061/(ASCE)GT.1943-5606.0002108

Xiao, Y., He, X., Zaman, M., Ma, G., Zhao, C., 2022. Review of strength improvements of biocemented soils. International Journal of Geomechanics 22(11), 03122001. https://doi.org/10.1061/(ASCE)GM.1943-5622.0002565

Xie, D., Zhang, R., Wang, J., 2023. The influence of environmental factors and precipitation precursors on enzyme-induced carbonate precipitation (EICP) process and its application on modification of recycled concrete aggregates. Journal of Cleaner Production 395, 136444. https://doi.org/10.1016/j.jclepro.2023.136444

Yang, Y., Chu, J., Cao, B., Liu, H., Cheng, L., 2020. Biocementation of soil using non-sterile enriched urease-producing bacteria from activated sludge. Journal of Cleaner Production 262, 121315. https://doi.org/10.1016/j.jclepro.2020.121315

Zeng, H., Yin, L.-Y., Tang, C.-S., Zhu, C., Cheng, Q., Li, H., Lv, C., Shi, B., 2021. Tensile behavior of bio-cemented, fiber-reinforced calcareous sand from coastal zone. Engineering Geology 294, 106390. https://doi.org/10.1016/j.enggeo.2021.106390

Zhang, X., Zuo, J., Wang, Z., Cai, Y., Zhou, Y., 2021. The evolution of the microstructure and mechanical properties of coral aggregate mortar under uniaxial compression using ultrasonic analysis. Construction and Building Materials 300, 124000. https://doi.org/10.1016/j.jclepro.2023.136982

Zhang, J., Su, P., Li, L., 2023. Microbial induced carbonate precipitation modified steel slag: Mechanical improvement and erosion resistance to sulfate attack. Journal of Cleaner Production 405, 136982. https://doi.org/10.1016/j.conbuildmat.2021.124000

Zhou, B., Ku, Q., Wang, H., Wang, J., 2020. Particle classification and intra-particle pore structure of carbonate sands. Engineering Geology 279, 105889. https://doi.org/10.1016/j.enggeo.2020.105889

Zhou, B., Zhang, X., Wang, J., Wang, H., Shen, J., 2023. Insight into the mechanism of microbially induced carbonate precipitation treatment of bio-improved calcareous sand particles. Acta Geotechnica 18(2), 985-999. https://doi.org/10.1007/s11440-022-01625-2

CRediT authorship contribution statement

Xing Zhang: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft. **Bo Zhou**: Resources, Methodology, Writing – review & editing, Supervision. **Ziyang Wu:** Data curation, Investigation. **Huabin Wang**: Resources.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: