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Original Research

Effect of Calcium Carbonate Deposition Induced by Microorganisms and Plant Urease on Sand Reinforcement

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Abstract

Soil solidification based on microbial mineralization is an environmentally friendly and sustainable technology. This study utilized microbial induced calcium carbonate precipitation (MICP) and urease-induced calcium carbonate precipitation (EICP) to solidify standard sand and silty sand. The physical and mechanical properties of the soil samples before and after solidification were tested, and the mechanisms of Sporosarcina pasteurii and urease-induced calcium carbonate solidification were analyzed. The results showed that the compressive strength of standard sand after MICP and EICP treatment was higher than that of silty sand. MICP treatment resulted in significantly higher compressive strength compared to EICP treatment. MICP formed a "skeleton" with calcium carbonate, enhancing shear strength and compressive strength but reducing permeability. EICP sealed the pores with calcium carbonate crystals, improving impermeability. The mechanical properties of solidified silty sand were worse due to particle shape and size, but it had better impermeability. During solidification, Sporosarcina pasteurii mainly stayed at the contact points between sand particles, with extracellular polymeric substances (EPS) containing negative ion groups. This enabled stronger adsorption capacity for calcium ions and facilitated the formation of "nucleation sites". Larger-sized, higher-strength calcium carbonate crystals were produced by MICP, aggregating at particle contact points. MICP treatment resulted in a sand microstructure resembling a "skeleton", enhancing shear strength, compressive strength, and permeability. In contrast, EICP directly used smaller-sized urease enzymes, which were more likely to be free in the pores. This caused the catalytically precipitated calcium carbonate to deposit between the pores, closing some of them and improving permeability. However, EICP often produced calcium carbonate in a disordered aggregate form with smaller size and brittle texture. The solidified samples were more brittle and prone to brittle failure. The research findings have certain guiding significance for sand soil solidification engineering.

Keywords: MIC, EICP, mechanical strength, calcium carbonate, microstructure

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Introduction

Desertification, as one of the greatest challenges facing humanity today in terms of environmental issues, is referred to as "Earth's cancer" [1]. It is urgent to address the solidification of soil. Traditional soil consolidation methods, such as compaction, preloading, vibration, and chemical grouting, use mechanical energy or artificial chemical materials to physically and chemically strengthen the soil [2, 3]. However, these methods not only consume a large amount of energy during the construction and material production processes, but also have hidden risks such as disturbing surrounding buildings, affecting municipal pipelines, and polluting the environment during the construction process and after project completion [4, 5]. Therefore, it is imperative to explore a more environmentally friendly and sustainable soil consolidation technology.

In the past decade, there has been increasing interest from civil engineering researchers in a soil consolidation technology that is based on the principle of microbial mineralization [6, 7]. This technology, which combines microbiology and engineering, is not only an innovation in theory and technology, but also contributes to long-term environmental protection [8, 9]. Microbial mineralization refers to the ability of certain microorganisms to produce inorganic compounds during their metabolism, which can fill and bond soil materials, thereby improving the engineering properties of the soil. Common microbial-induced mineralization processes include urea hydrolysis, denitrification, sulfate reduction, and sulfide reduction. The products of these processes are usually insoluble precipitates, such as calcium carbonate, iron hydroxide, and metal sulfide [10, 11]. When carbonate is the product, the process is called microbial-induced carbonate precipitation [12]. As early as the 18th century, Murray and Irvine (1891) [13] conducted research on microbial deposition of calcium carbonate. In 1995, Gollapudi et al. (1995) [14] firstly applied MICP to the field of geotechnical engineering. Zhang et al. (2016) [15] conducted research on bacteria selection for concrete crack self-healing and found that Sporosarcina pasteurii had the highest activity, reaching 94.8%. Salifu et al. (2016), Deng and Wang (2018) applied MICP technology to slopes with angles of 53º and 35º, respectively, and evaluated its effectiveness in mitigating erosion and stabilizing slopes through tidal cycle simulation tests. The results showed that 9.9% of the pores in the treated slope were filled with calcium carbonate, improving stability and erosion resistance [16, 17]. Wang et al. (2022) [18] discovered that Microbial Induced Calcite Precipitation (MICP) can protect porous cement-based materials and enhance concrete durability. The effectiveness of MICP relies on surface properties. Another approach, Enzyme-Induced Carbonate Precipitation (EICP), utilizes urease to induce carbonate precipitation through urea hydrolysis, which strengthens soil by generating calcium carbonate crystals. Unlike MICP, EICP does not involve bacteria; instead, free urease directly catalyzes the hydrolysis process. Johnson and Goody (2011) [19] proposed an enzyme catalysis kinetics model, which suggests that the reaction rate increases linearly with enzyme concentration. This model can monitor the reaction rate of EICP by continuously measuring hydrolysis product concentration after mixing urease with urea. Dilrukshi et al. (2018) [20] extracted urease from watermelon seeds and found that enzyme activity increases with temperature until 50ºC, after which it decreases significantly. Both microbially induced carbonate precipitation and enzyme-induced carbonate precipitation technologies have been extensively studied. Song et al. (2020) [21] determined that the optimal proportion of commercial urease and crude extracted urease in the cementation liquid is achieved when the molar ratio of urea to calcium chloride ($CaCl_2$) is 1.5, resulting in maximum $CaCO_3$ precipitation. Shibli et al. (2022) [22] investigated the effects of reactant concentrations, reaction medium, urease enzyme source, and calcium source on the precipitation rate of calcium carbonate. They concluded that using 4.5 g of sesame, 5 g of urea, and 6 g of jack bean in 60 mL of distilled water yields the best reaction conditions for precipitating 100.288 g of calcium carbonate. Based on current research, both technologies have their own advantages and disadvantages. The MICP method has a higher yield of precipitated calcium carbonate, larger crystal size, and higher strength. However, the MICP method is prone to blockage at the injection end during grouting, leading to uneven consolidation effects. On the other hand, the EICP technology has stronger penetration into soil particles, allowing for freer movement and reaction between pores. However, the size and strength of the calcium carbonate crystals formed by EICP technology are relatively small. In summary, the advantages and disadvantages of MICP and EICP technologies have resulted in significant differences in their soil consolidation effects [23-26]. Currently, there is a lack of relevant theoretical and experimental research in this area, which hinders the widespread application of MICP and EICP technologies in geotechnical engineering. Therefore, it is necessary to conduct comprehensive comparative studies on these two technologies, verify and analyze the consolidation mechanisms and engineering properties of the consolidated soil, and provide reference for the evaluation of soil consolidation schemes using MICP and EICP technologies.

This study used MICP and EICP methods to treat standard sand and silty sand, and tested the changes in calcium carbonate content and physical and mechanical properties of the soil before and after consolidation. The mechanisms of Sporosarcina pasteurii and urease-induced carbonate precipitation in soil consolidation were analyzed based on changes in chemical composition and microstructure.

Materials and Methods

Microorganism and Urease Culture

The microbial strain used in this experiment is Sporosarcina pasteurii, with the culture medium composition shown in Table 1. The pH of the liquid culture medium is 7.3. The strain is inoculated into the liquid culture medium at a volume ratio of 1:100, and incubated with shaking at 30ºC for 24 hours (Fig. 1). After three generations of cultivation, the OD600 value of the bacterial liquid is approximately 0.86, and the urease activity is approximately 6.77, as measured by UV spectrophotometry and conductivity testing method.

Plant urease uses soybeans as raw materials. The soybeans are dried in an oven at 40ºC, pulverized, and sieved through a 100-mesh sieve. 30 g of soybean powder is added to 100 ml of ultrapure water, thoroughly stirred on a magnetic stirrer for 30 minutes, and then allowed to stand in a low-temperature environment for 3 hours (Fig. 1). After standing, it is filtered and centrifuged at a speed of 3000 r/min for 15 minutes. The supernatant in the centrifuge tube is the crude extract of soybean urease. The activity of the enzyme solution is approximately 6.82, as measured by conductivity testing method.

The cementing solution is prepared by mixing urea (purity≥99.0%) and anhydrous calcium chloride (purity≥96.0%) in different proportions. Under the conditions of calcium ion concentrations of 0.5, 1.0 and 1.5 mol/L, the ratios of calcium chloride to urea are 1:0.75, 1:1, 1:1.25, and 1:1.5, respectively, resulting in a total of 12 groups of cementing solutions with 3 parallel samples in each group. The bacterial liquid (soybean urease solution) and cementing solution are mixed in a 1:1 ratio, and after 1 day of reaction at 16±2ºC, the yield of calcium carbonate in the test tube is measured using acid washing method (Table 2). For MICP reaction, the formulation of group *D* cementing solution (calcium ion concentration of 0.5 mol/L, calcium chloride to urea ratio of 1:1) is optimal. For EICP reaction, the formulation of group G cementing solution (calcium ion concentration of 0.5 mol/L, calcium chloride to urea ratio of 1:1.25) is more advantageous. In the solid soil test, in order to obtain samples with significant strength, besides maintaining a high yield of calcium carbonate, it is also necessary to ensure the final yield of calcium carbonate deposited in the soil pores. Although the calcium carbonate yield of these two groups of cementing solutions reaches 92.78% to 99.48%, due to the limitation of calcium ion concentration of 0.5 mol/L, the calcium carbonate yield of 1 L of cementing solution is only 116 g to 124 g, which is insufficient to meet the requirements of soil stabilization. In addition, the proportion and concentration of the cementing solution can affect the crystal forms, morphology, particle size, and distribution characteristics of the deposited calcium carbonate in the pores. If different cementing solution formulations are used for the two different reinforcement methods, it is highly likely to have additional effects on the test results, which is not conducive to the evaluation of the experiment. In this study, the *K* group cementing solution with a calcium ion concentration of 1 mol/L and a calcium chloride to urea ratio of 1:1.5 was selected to conduct MICP grouting tests and EICP grouting tests. The calcium carbonate yield after MICP and EICP reactions in the test tube is approximately 70% to 77%, corresponding to a calcium carbonate yield of approximately 175 g to 192 g.

Sample Preparation

The object solidified in this experiment are standard sand silty sand. The sand is dried and crushed in a constant temperature oven at 108ºC. Then, the powdered sand is placed into the corresponding test molds using the method of layered compaction (direct shear test, unconfined compressive strength test, permeability test). The molds are wrapped with water-stop tape and wrapping film to prevent the reagents from seeping out through the peripheral gaps during the grouting process. The grouting molds are then assembled with peristaltic pumps, silicone tubes, glass tubes, and other equipment. Using the peristaltic pump, a microbial solution (or soy urease solution) with a flow rate of 5 mL/min is injected from the bottom of the sample, filling approximately 1.2 times the pore volume between the sand particles. The sample is then allowed to stand for 4 hours to ensure that the microbial solution (or soy urease solution) fills the pores between the sand particles. Next, a urea-calcium chloride binder fluid with an equal volume is injected at a flow rate of 10 mL/min using the peristaltic pump. The reason for selecting the above grouting parameters is that the lower injection rate of the microbial solution or enzyme solution (5 mL/min) allows for a more uniform distribution of microorganisms in the sand particles, while the higher injection rate of the binder fluid (10 mL/min) can prevent premature deposition of calcium carbonate near the injection port and resulting blockage. This process is repeated daily for one cycle of grouting, with curing times of 7, 14, 21 and 28 d, respectively. In order to prevent water evaporation, all remoulded sand samples should be stored in the curing box, the curing temperature is 20±5ºC, and the humidity is above 95%.

Testing Methods

The cured soil samples are subjected to mechanical tests, including shear strength, unconfined compressive strength, and permeability coefficient tests. After curing, a portion of the cured samples is dried in a low-temperature oven until a constant weight is reached. Small samples are then taken from the representative middle positions, and the change in calcium carbonate content before and after curing is analyzed using X-ray diffraction analysis. After drying, the samples are cut into SEM scanning samples with a diameter of 1cm

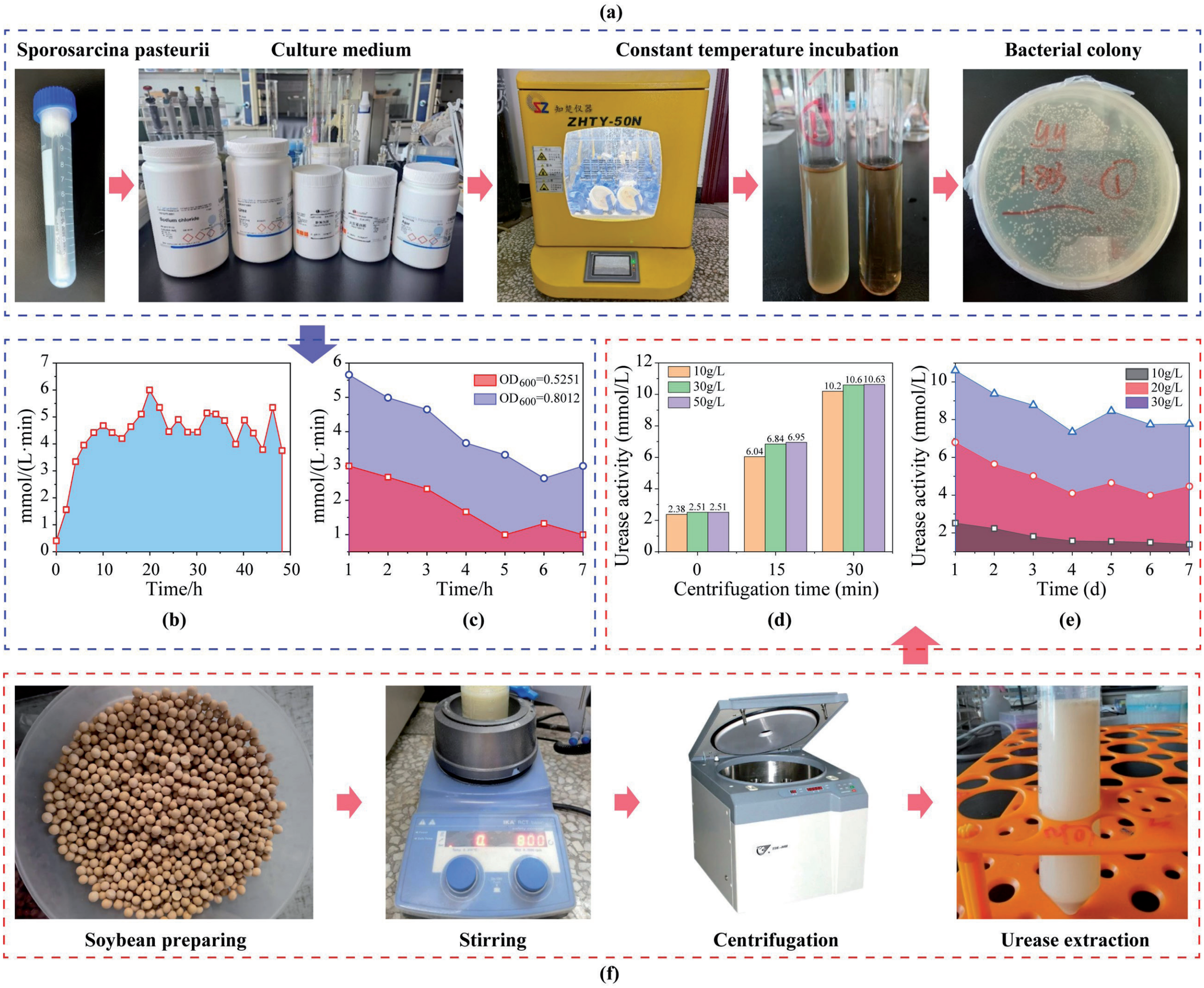

Fig. 1. Sporosarcina pasteurii and urease preparation. a) Sporosarcina pasteurii culturing; b) Urease activity changes with time; c) Activity variation of Sporosarcina pasteurii at 4°C; d) Urease activity under different centrifugation time; e) Activity variation of urease at 4°C; f) Urease culturing.

and a thickness of 5mm using a knife. After gold coating the surface of the samples, the microstructure is observed using a scanning electron microscope (ΣIGMATM) at a magnification of 1000 times. PCAS software was used to quantitatively analyse the SEM images, including the morphological characteristics of pores and particles [27].

Results and Analysis

Shear Strength

The shear strength of silty sand and standard sand after treatment with MICP and EICP is shown in Fig. 2. The term "Original sample" represents untreated soil samples. Both the standard sand and the silty sand samples treated with MICP and EICP show a significant increase in shear strength. With an increase in curing days, the shear strength of the soil samples improves to varying degrees, although the rate of improvement gradually diminishes. Compared to the untreated standard sand, the shear strength of the samples treated with MICP shows the largest increase (four times higher, reaching approximately 1400 kPa), which is approximately 1.5 times higher than that of the samples treated with EICP. In the case of untreated silty sand, there is little difference in shear strength between samples treated with MICP and EICP, but compared to standard sand, its shear strength is relatively low (with a maximum value of about 650 kPa), approximately half of that of standard sand. After treatment with MICP and EICP, the cohesion and internal friction angle values of the standard sand samples also increase with curing days. The increase in cohesion is more significant since the pure sand has a cohesion of 0. This phenomenon also occurs in silty sand samples treated with MICP and EICP, although the increase in cohesion is not as apparent as the internal friction angle. Overall, the internal friction angle of the treated standard sand is lower than that of the silty sand, while the cohesion is higher than that of the silty sand.

Table 1. Components of the culture medium.

Composition	Casein peptone	Soy peptone	Sodium chloride	Urea
Unit g/L	15	5	5	20

Table 2. Scheme of the cementing fluid ratio.

No.	Calcium chloride / (mol/L)	Urea / (mol/L)	Calcium chloride: Urea	Productivity of calcium carbonate yield under MICP	Productivity of calcium carbonate yield under EICP
A	0.5	0.375	1:0.75	94.99	70.10
B	1.0	0.75	1:0.75	77.24	53.94
C	1.5	1.125	1:0.75	67.22	40.02
D	0.5	0.5	1:1	99.48	89.94
E	1.0	1.0	1:1	78.10	59.26
F	1.5	1.5	1:1	64.41	36.25
G	0.5	0.625	1:1.25	95.82	92.78
H	1.0	1.25	1:1.25	77.39	58.54
I	1.5	1.875	1:1.25	63.90	43.30
J	0.5	0.75	1:1.5	95.19	66.46
K	1.0	1.5	1:1.5	77.66	70.09
L	1.5	2.25	1:1.5	62.22	42.60

Unconfined Compressive Strength

The compressive strength of standard sand and silty sand after treatment with MICP and EICP is shown in Fig. 3. According to the variation of the stress-strain relationship curve, all specimens reached their peak axial stress within a short period of time. With an increase in curing days, the peak compressive strength becomes larger. However, there are differences in the strength peak values of the soil samples treated with MICP and EICP. Specifically, after 28 days of MICP treatment, the peak strength of the soil sample increased by nearly 500 kPa compared to the sample cured for 7 days. Furthermore, when the curing days are 21 days and 28 days, the peak compressive strength of the soil sample is relatively close. On the other hand, the peak strength of the soil sample treated with EICP is approximately 800 kPa, which is about half of the peak value of the MICP-treated soil sample at 28 days, and even lower than the peak value of the MICP-treated soil sample cured for 7 days. However, the peak strength of the EICP-treated soil sample after 28 days of curing is significantly higher than that of the sample cured for 21 days. For silty sand, the variation pattern of the compressive strength of the samples treated with MICP and EICP is similar to that of the standard sand, with the difference being that the peak strength values are much lower (approximately half) than those of the standard sand. Overall, the compressive strength of the standard sand after treatment with MICP and EICP is significantly higher than that of the silty sand, while the compressive strength of the soil sample treated with MICP is much higher than that of the soil sample treated with EICP.

Permeability Coefficient

The results of the variable head permeability tests for standard sand and silty sand after MICP and EICP treatments are shown in Fig. 4. Both methods result in a reduction in the permeability coefficient of both standard sand and silty sand to varying degrees. In comparison, the permeability coefficient of silty sand is generally smaller (with a difference of approximately 2×10^{-4} cm/s). The permeability coefficient of the soil sample treated with MICP is higher than that of the soil sample treated with EICP, and this difference is quite significant (with a difference of approximately 1.5×10^{-4} cm/s), which is closely related to the particle composition of the two types of sand. In summary, both MICP and EICP techniques can reduce the permeability coefficient of standard sand and silty sand to some extent, thereby enhancing the soil's resistance to seepage. However, the EICP technique shows better anti-seepage effectiveness in treating sandy soil compared to the MICP technique.

Calcium Carbonate Content

The calcium carbonate content in different parts of standard sand and silty sand samples after MICP and EICP treatments is shown in Fig. 5. For the original

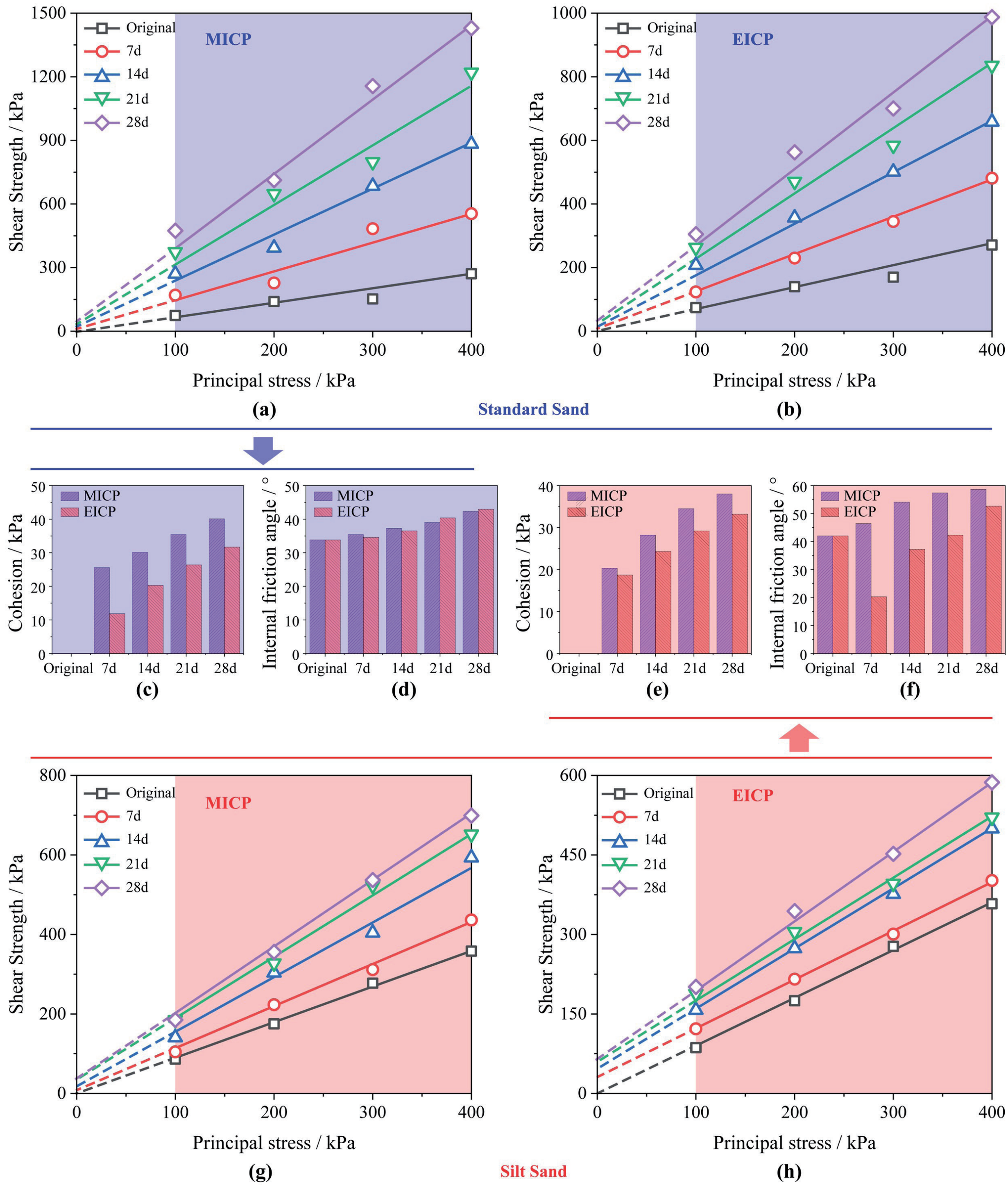

Fig. 2. Shear strength after solidification respectively with MICP and EICP methods.

standard sand, the calcium carbonate content in the upper, middle, and lower parts is approximately 3%. After MICP treatment, the calcium carbonate content of the samples significantly increases, and it continues to increase with the curing days. Comparatively, the calcium carbonate content of the soil samples treated with EICP also increases, but the magnitude is lower than that of the samples treated with MICP. For silty sand, although the trend of calcium carbonate content changes with curing days is similar to that of standard sand, the calcium carbonate content in silty sand is higher than that in standard sand when the curing days are the same. For example, when the curing days are 28 days, the average calcium carbonate content in the silty sand sample treated with MICP is 13%, while it is 11% in the standard sand sample. Similarly, the average calcium carbonate content in the silty sand sample treated with EICP is 10%, while it is 8% in the standard sand sample.

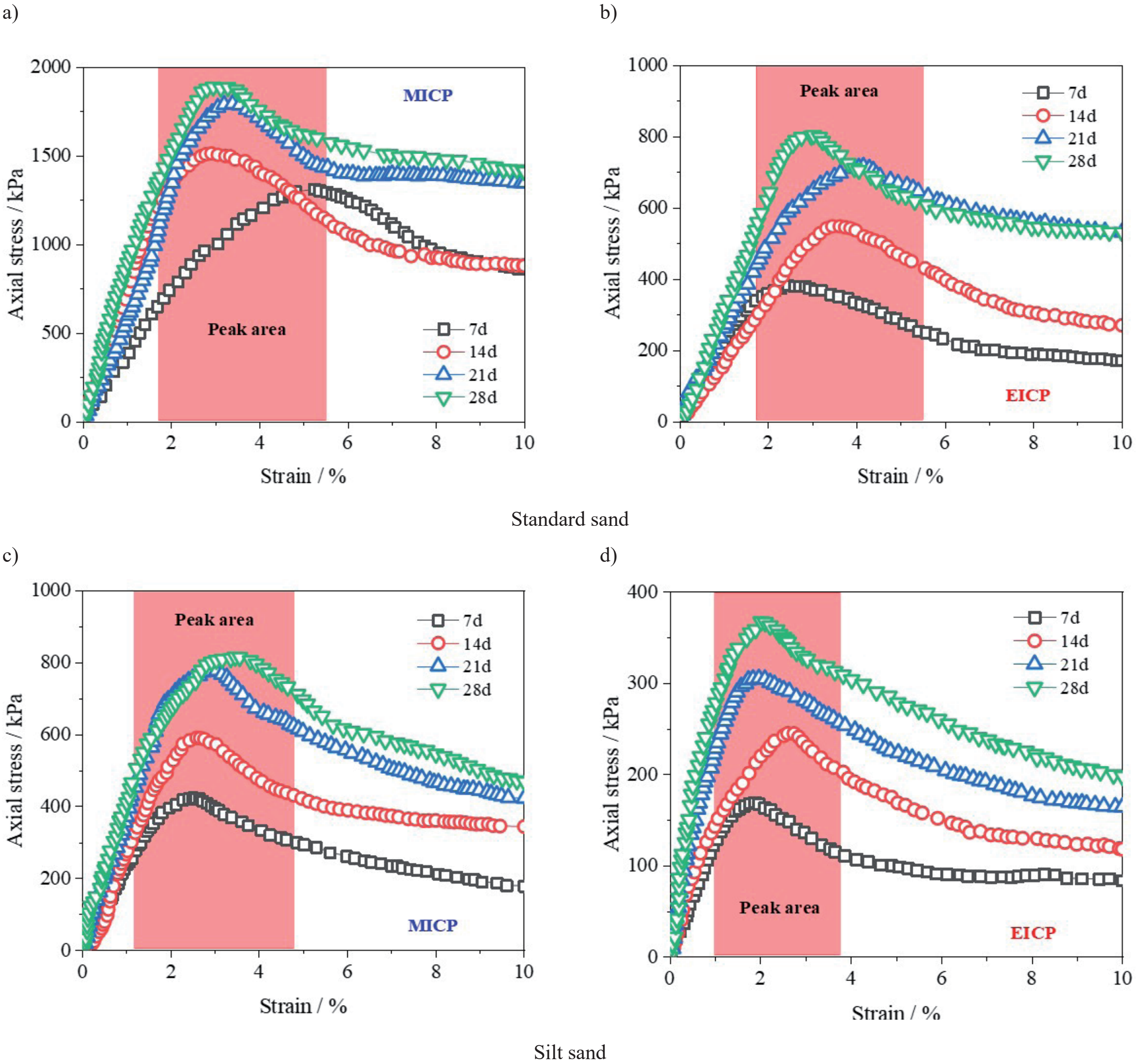

Fig. 3. Compressive strength after solidification respectively with MICP and EICP methods.

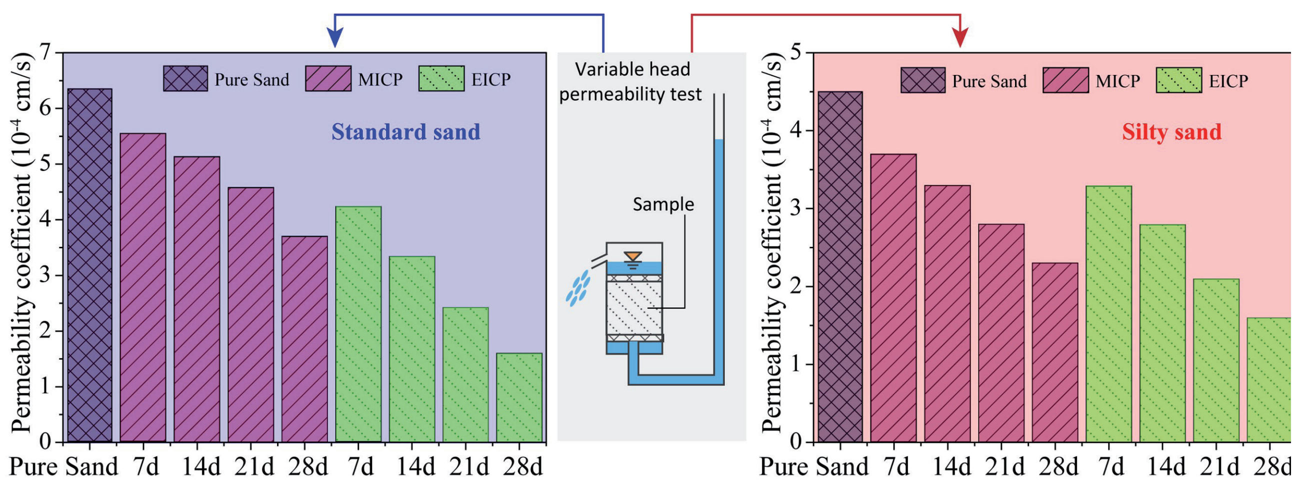

Fig. 4. Changes of permeability coefficient of standard sand and silty sand samples after solidification respectively with MICP and EICP methods.

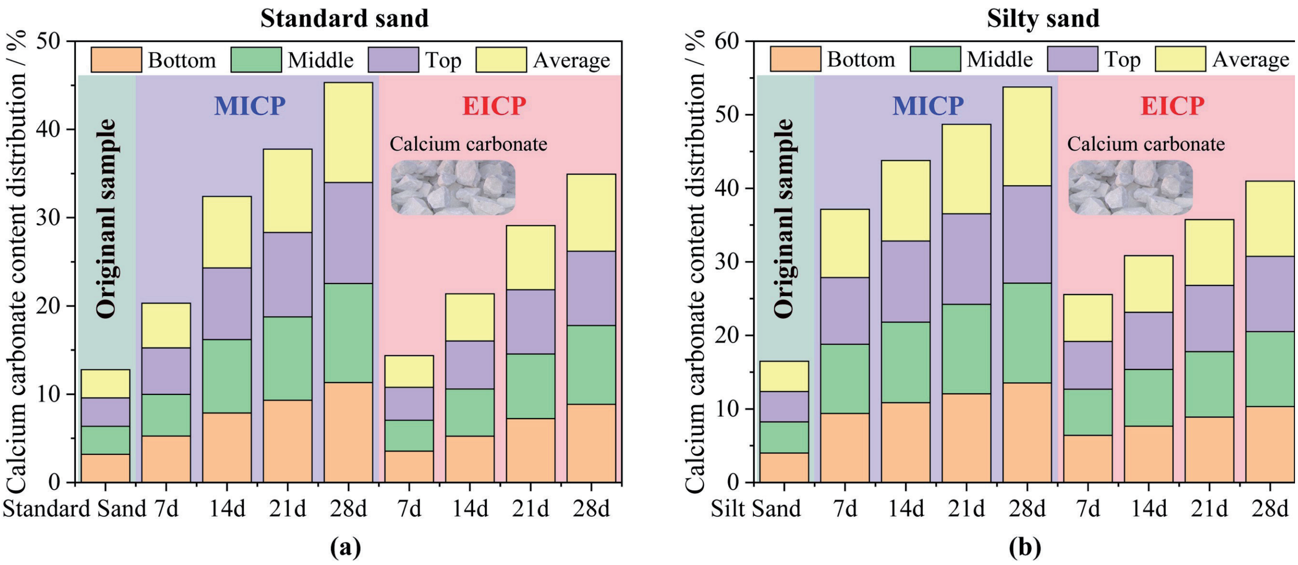

Fig. 5. Changes of the calcium carbonate content of standard sand and silty sand samples after solidification respectively with MICP and EICP methods.

Discussion

Relationship Between Microstructure and Mechanical Properties

The SEM images were analyzed using PCAS software, and the microscopic analysis results of different treatment methods are shown in Fig. 6. The pore area of the solidified standard sand particles, as determined by the MICP method, is larger, and the average pore width and length are also larger. However, there is a large amount of crystal formation on the surface and contact points, and these crystals are mostly agglomerated. These agglomerates are composed of several small cubic crystal particles that are relatively regular in shape. The particles are in face-face contact or edge-edge contact, resulting in a larger effective contact area. Due to the action of these crystals distributed on the surface and contact points of the sand particles, the loose standard sand is cemented into a "framework," greatly enhancing its shear strength and unconfined compressive strength. The solidified standard sand sample still maintains connectivity between pores, similar to the results of Rong et al. [28]. This characteristic weakens the effect of enhancing the impermeability of the MICP grouting sample but ensures that MICP grouting can achieve low-pressure, long-distance, and multiple-cycle infusion. On the other hand, the pore area of the solidified standard sand particles, as determined by the EICP method, is greatly reduced. Moreover, there is a large amount of crystal formation on the particle surfaces and within the pores. These crystals are smaller in size and have irregular polyhedral shapes, with a few being elliptical or needle-like. The particles are mostly in spherical contact, resulting in a smaller effective contact area. A considerable portion of the pores in the solidified standard sand sample are blocked by these irregular crystals, which confirms the results of the permeability test, namely that the impermeability of the sample is significantly enhanced by EICP grouting.

Different from standard sand, the particle size of the silt sand is smaller, and there are some organic impurities on the surface of the sand particles. After MICP grouting treatment, a large number of stacked crystals cover the surfaces and contact points of the sand particles. These crystals are mostly cubic in shape, with a few being plate-like, and the particles are mostly in face-face contact. These crystals firmly bond the loose sand particles together, significantly enhancing the strength of the silt sand sample. However, the porosity of the sample is not greatly changed, so the permeability of the MICP sample is reduced to a lesser extent. The pore area of the solidified silt sand particles, as determined by the EICP method, is similar to that of the soil sample treated with MICP grouting. However, the surfaces and pores of the particles are covered with a large number of micro-layered particle-like crystals. These particles have a layered structure, and the particles are mostly in spherical contact. On one hand, the small size, smooth shape, and stronger dispersibility of these crystals result in weaker mechanical strength of the silt sand sample treated with EICP grouting. On the other hand, these crystals have a larger specific surface area and are mostly distributed in the pores between the sand particles, leading to a significant reduction in the porosity of the silt sand sample and a noticeable enhancement in impermeability.

Relationship Between Calcium Carbonate Crystals and Mechanical Properties

MICP and EICP consolidation of soil both rely on the generation of calcium carbonate crystals. The yield of calcium carbonate inside the samples to some

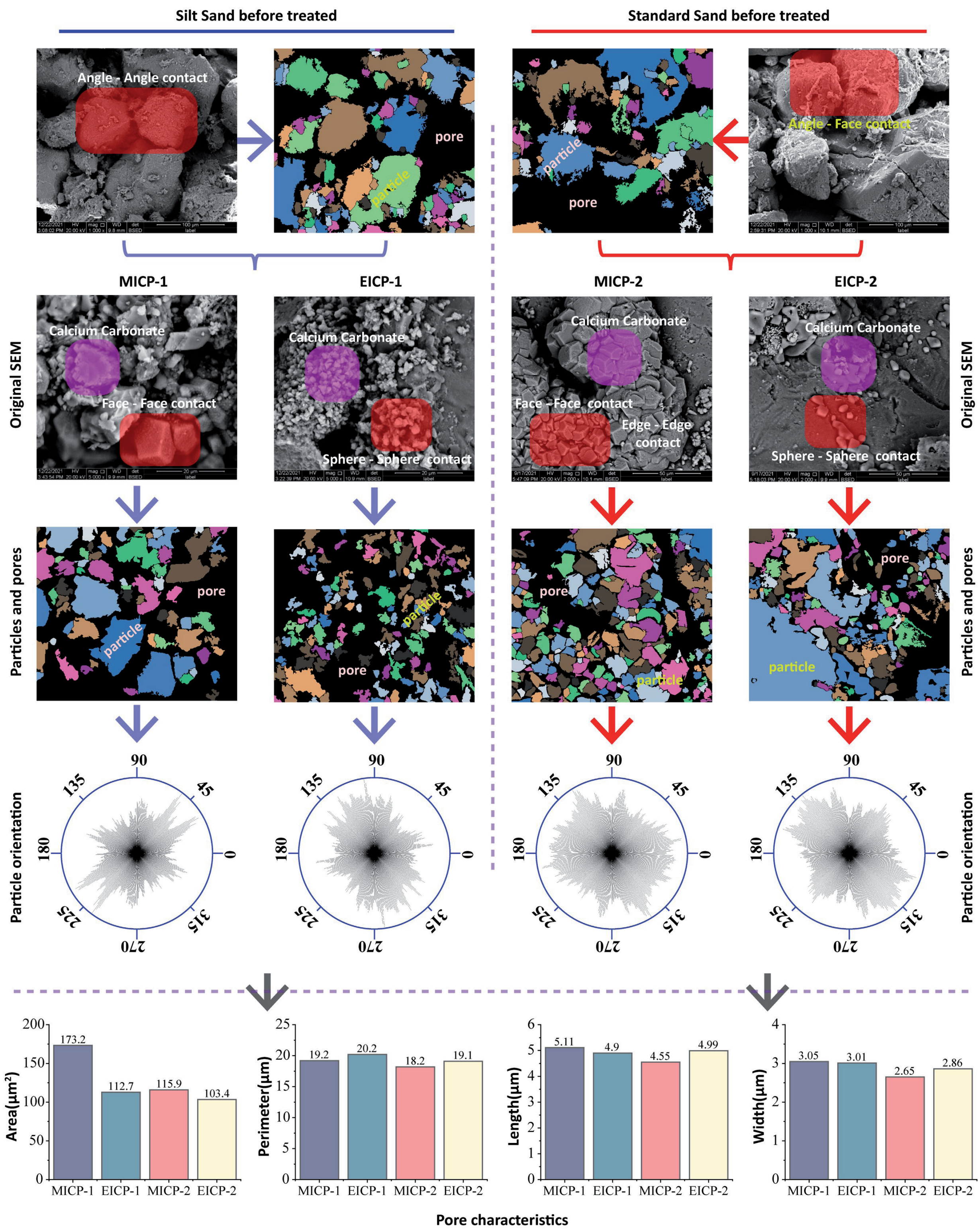

Fig. 6. Changes of the micro-structure of standard sand and silty sand samples after solidification respectively with MICP and EICP methods.

extent reflects the injection effect of MICP and EICP. Whether it is standard sand or silty sand, the calcium carbonate content in the solidified samples of MICP injection is higher than that of EICP injection samples (Fig. 7). Correspondingly, the mechanical strength of the solidified samples of MICP injection is higher than that of the EICP injection samples, which also reflects that the mechanical properties of the samples after injection and solidification are to some extent related to the calcium carbonate content. The increase in calcium carbonate content will cause an increase in the density of cemented sand, a decrease in the porosity

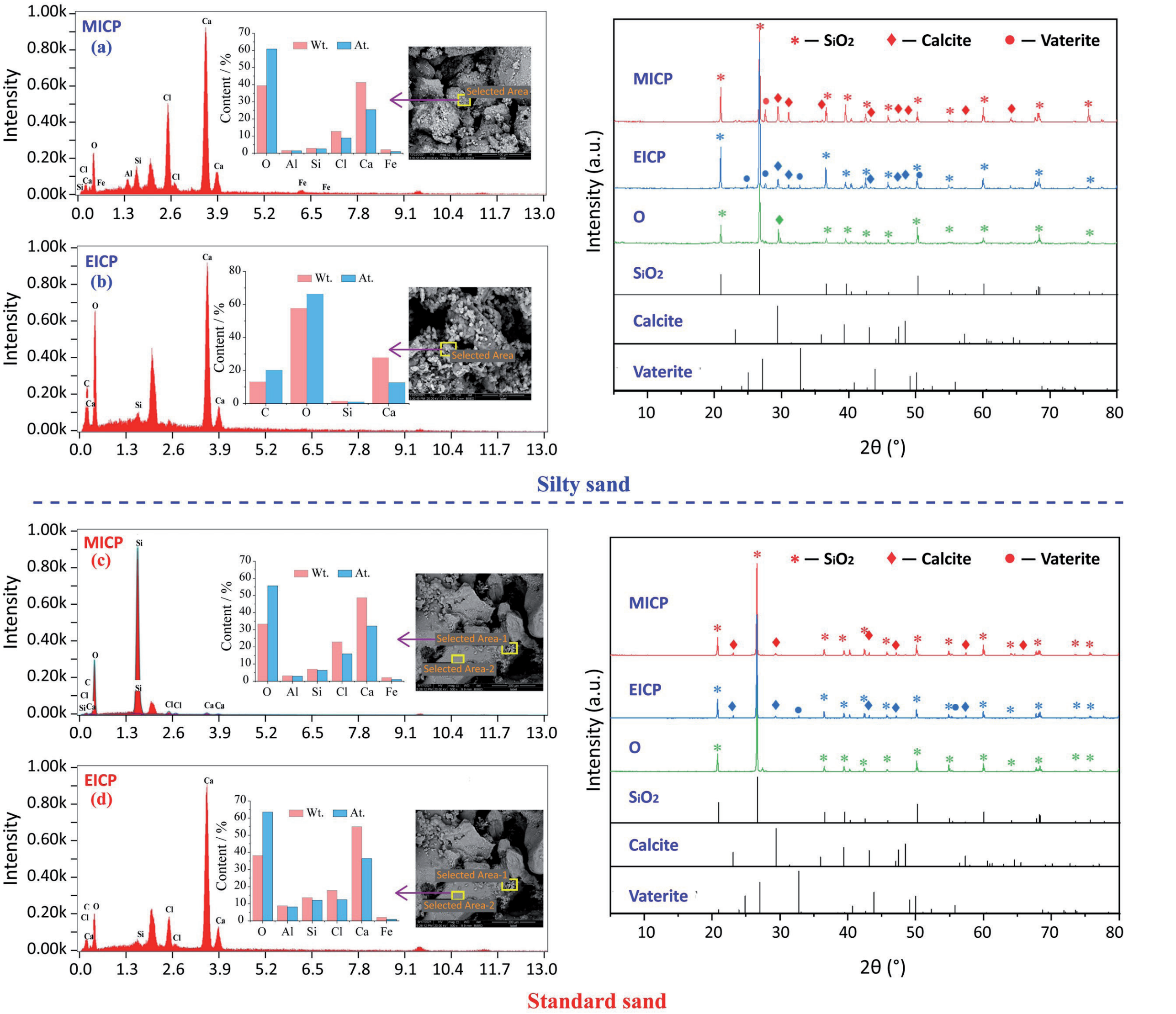

Fig. 7. Changes of the main components of standard sand and silty sand samples after solidification respectively with MICP and EICP methods.

of the sand body, and an increase in the amount of calcium carbonate that acts as a cementing agent for soil particles. The cohesion and internal friction angle of the samples increase. At the same time, the calcium carbonate adhering to the surface of the sand grains can also improve the roughness of the sand grains.

The larger the particle roughness, the more energy is required to overcome the "interlocking" of the particles during the sample's failure process. Therefore, the increase in the total calcium carbonate content helps to improve the mechanical strength of the samples. Under similar urease activity and the same urea-calcium chloride cementing liquid conditions, the amount of calcium carbonate produced by MICP reaction is higher than that of EICP reaction due to the following reasons: First, in the process of cultivating Sporosarcina pasteurii, ASO AGAR + 20g/L urea culture medium is used to provide carbon and nitrogen sources for bacterial growth and reproduction, so the urea concentration in the MICP reagent is actually higher than that in the EICP reagent. A higher urea concentration can not only increase the theoretical yield of carbonate ions but also accelerate the precipitation rate of calcium carbonate within a certain range. Second, during the MICP and EICP reactions, the byproduct monohydrate ammonia continuously ionizes into hydroxide ions, causing the pH of the environment to rise continuously, which affects the activity of urease. Therefore, the reaction rate of EICP continues to slow down, while urease in MICP reaction can be continuously produced by Sporosarcina pasteurii with less negative impact. In addition, the urease auxiliary protein of Sporosarcina pasteurii also plays an important role in maintaining the activity of urease.

Reinforcement Mechanism

For sandy soil, the MICP grouting method focuses more on enhancing the mechanical performance of the soil, while the EICP grouting method is more effective

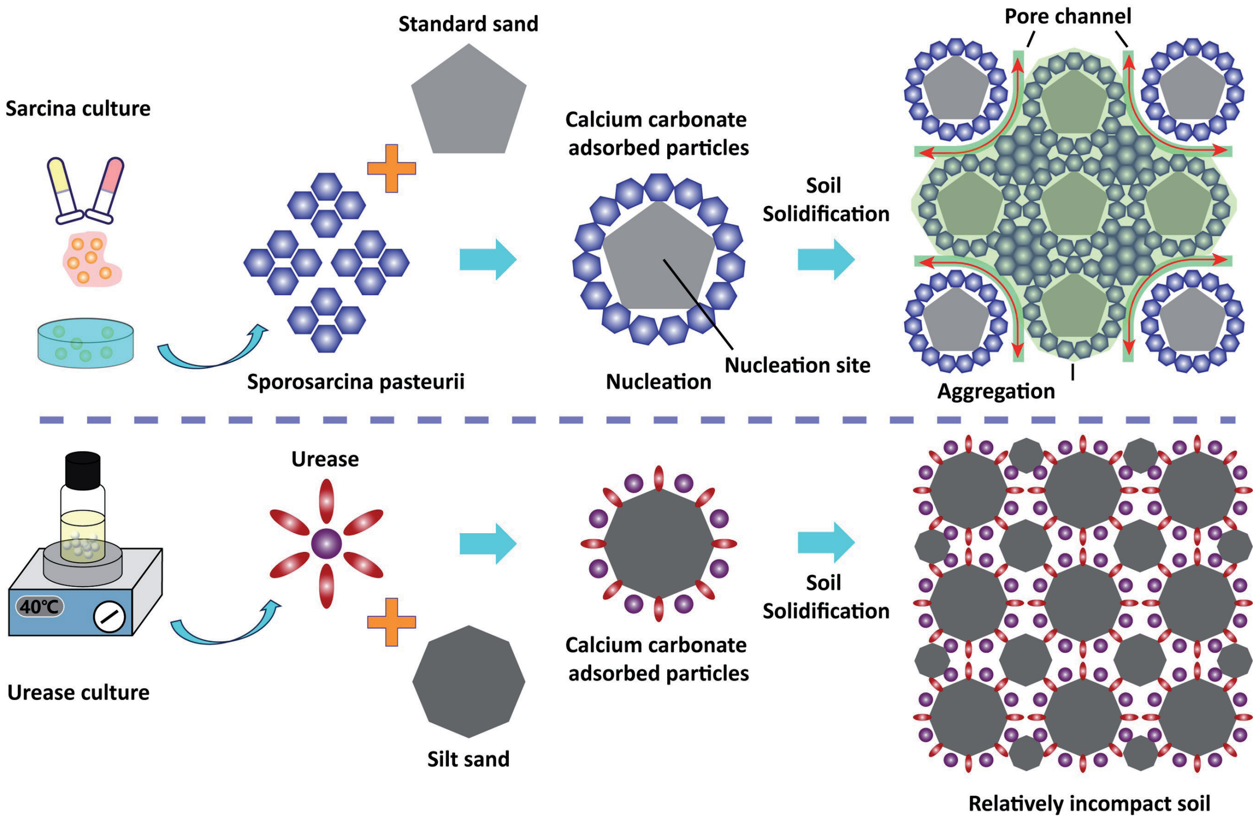

Fig. 8. Reinforcement mechanisms of MICP and EICP methods.

in terms of impermeability. This is related to the size of bacteria and urease enzymes, as well as the precipitation of calcium carbonate. The sporosarcina pasteurii used in this study has a length of about 2-3 μm, even larger than the size of some clay particles, which makes it easier for the bacteria to stay at the contact points between sand particles during grouting (Fig. 8). The extracellular polymeric substance (EPS) produced by the metabolism of sporosarcina pasteurii contains negatively charged groups, which usually results in a negative charge on the bacterial surface. This enables stronger adsorption of calcium ions and, under conditions of high concentration of carbonate and alkaline, calcium ions serve as "nucleation sites" on the bacteria, leading to the precipitation of calcium carbonate crystals around the bacteria. The bacterial solution also contains a large amount of organic matter, which may be encapsulated within the calcium carbonate crystals [29-31]. Therefore, the calcium carbonate crystals produced by the MICP method are often larger in size, higher in strength, and tend to aggregate at the contact points between particles. After MICP grouting treatment, the microstructure of the sandy soil resembles a "skeleton", enhancing the shear and compressive strength of the soil while maintaining connectivity and permeability of the pores. On the other hand, EICP technology directly uses smaller-sized urease enzymes to catalyze the reaction. These enzymes are more likely to remain free in the pores, so the catalyzed calcium carbonate deposition occurs more between the pores of the soil, resulting in the partial closure or semi-enclosure of the pores and reducing the permeability of the specimen. The crude extract of soybean urease used in EICP also contains some plant protein components, which can further help improve permeability [32]. However, due to the lack of "nucleation sites", the calcium carbonate precipitated by the EICP method tends to have a disorderly and smaller size, with a more brittle texture. The cured specimen is more brittle and prone to brittle failure.

Conclusions

This paper used MICP and EICP methods to treat standard sand and silty sand respectively. The physical and mechanical properties of the soil samples before and after consolidation were tested. The mechanism of sporosarcina pasteurii and urease-induced calcium carbonate on sand consolidation was analyzed based on changes in chemical composition and microstructure. The following conclusions were drawn:

(1) The calcium carbonate content in standard sand treated with MICP and EICP is 11% and 8% respectively, while for silty sand it is 13% and 10%. The compressive strength of standard sand after MICP and EICP treatment is much higher than that of silty sand. The soil samples treated with MICP also exhibit significantly higher compressive strength compared to those treated with EICP.

(2) MICP treatment results in the formation of a calcium carbonate “skeleton” that binds the loose sand particles together, greatly enhancing shear strength and unconfined compressive strength, but reducing permeability. On the other hand, EICP treatment blocks the pores in the sand with calcium carbonate crystals generated by urease, thereby improving permeability. The mechanical properties of consolidated silty sand are inferior to standard sand due to differences in particle shape and size, but it has better permeability.

(3) Increasing the calcium carbonate content increases the density of cemented sand and decreases porosity. The increased amount of calcium carbonate acting as a binder for soil particles leads to higher cohesion and internal friction angle in the soil sample. Additionally, the calcium carbonate adhered to the surface of sand grains improves their roughness. The increased particle roughness requires more energy to overcome interlocking during the sample’s failure. This explains why the mechanical properties of sand treated with MICP are superior to those treated with EICP.

(4) During the consolidation process, sporosarcina pasteurii tends to accumulate at the contact points between sand particles. The extracellular polymeric substances produced by the bacteria have negatively charged groups, resulting in a negative charge on the bacterial surface and enhanced adsorption capacity for calcium ions. This facilitates the formation of “nucleation sites,” leading to larger-sized and higher-strength calcium carbonate crystals generated by MICP. These crystals tend to aggregate at the particle contact points, giving the sand soil a “framework” microstructure that enhances shear strength, compressive strength, and maintains pore connectivity and permeability. EICP, on the other hand, utilizes smaller-sized urease directly, which is more likely to be free in the pores. This results in more calcium carbonate deposition between the soil pores, transforming some of them into closed or semi-closed states, and improving permeability. However, due to the absence of “nucleation sites,” the calcium carbonate produced by EICP often forms randomly scattered aggregates with smaller size and a more brittle texture, resulting in a higher brittleness of the consolidated samples and a higher tendency of brittle failure.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

References

1. XU E., ZHANG H.A. stratified environmental reference system for better understanding of the relationship between remote sensing observations and ground monitoring of karst rocky desertification. Land Degradation and Development. **33** (9), 1366, **2022**.
2. LU Y., MEI G., HU M.P. A Deep Learning Approach for Predicting Two-Dimensional Soil Consolidation Using Physics-Informed Neural Networks (PINN). Mathematics. **10** (16), 18, **2022**.
3. JÓZEFIAK K., ZBICIAK A., BRZEZIŃSKI K., MAŚLAKOWSKI M. Novel Approach to the Analysis of the Soil Consolidation Problem by Using Non-Classical Rheological Schemes. Applied Sciences. **11**, 12, **2021**.
4. DEY D., SRINIVAS D., PANDA B., SURANENI P., SITHARAM T.G. Use of industrial waste materials for 3D printing of sustainable concrete, A review. Journal of cleaner production. **340** (15), 130749, **2022**.
5. NGUYEN B.P., MAN A., PRADHAN S., NGUYEN T.H., HUYNH T.C. Large-strain consolidation analysis of PVD-installed soft soil considering the discharge capacity variation according to depth and time Discharge capacity variation. Engineering Computations. **38** (4), 1652, **2021**.
6. MAHAPATRA S., YADAV R., RAMAKRISHNA W. Bacillus subtilis impact on plant growth, soil health and environment, Dr. Jekyll and Mr. Hyde. Journal of Applied Microbiology. **132** (5), 3543, **2022**.
7. IKRAM H., KHAN H.A., ALI H., LIU Y., KIRAN J., ULLAH A., AHMAD Y., SARDAR S., GUL A. Evaluation, Characterization and Molecular Analysis of Cellulolytic Bacteria from Soil in Peshawar, Pakistan. Microbiology and biotechnology letters. **50** (2), 245, **2022**.
8. WANG A., YI H., ZHAN Q., ZHANG Y., ZHOU J. Study on Improving the Properties of Recycled Aggregate by Microbial Mineralization. Science of advanced materials. **14** (2), 333, **2022**.
9. ANDREA M., ELIGIO M., FLAVIA P., LOREDANA C. Quantification of nitrogen cycle functional genes from viable archaea and bacteria in paddy soil. Journal of Applied Microbiology. **134** (8), 13, **2023**.
10. COZZOLINO M., FUSARO M., CICERI P., GASPERONI L., CIANCIOLO G. The Role of Vitamin K in Vascular Calcification. Advances in Chronic Kidney Disease. **26** (6), 437, **2019**.
11. YADAV V.K., BHAGAT N., SHARMA S.K. Modulation in Plant Growth and Drought Tolerance of Wheat Crop upon Inoculation of Drought-tolerant-Bacillus Species Isolated from Hot Arid Soil of India. Journal of Pure & Applied Microbiology. **16** (1), 246, **2022**.
12. DUPRAZ C., REID R.P., BRAISSANT O., DECHO A.W., NORMAN R.S., VISSCHER P.T. Processes of carbonate precipitation in modern microbial mats. Earth Science Reviews. **96** (3), 141, **2009**.
13. MURRAY J., IRVINE R. On Coral Reefs and other Carbonate of Lime Formations in Modern Seas. Proceedings of the Royal Society of Edinburgh. **17**, 79, **1891**.
14. GOLLAPUDI U.K., KNUTSON C.L., BANG S.S. A new method for controlling leaching through permeable channels. Chemosphere. **30** (4), 695, **1995**.
15. ZHANG J.L., WU R.S., LI Y.M., ZHONG J.Y., DENG X., LIU B., HAN N.X., XING F. Screening of bacteria for self-healing of concrete cracks and optimization of

the microbial calcium precipitation process. Applied Microbiology and Biotechnology. **100** (15), 6661, **2016**.
16. SALIFU E., MACLACHLAN E., IYER K.R., KNAPP C.W., TARANTINO A. Application of microbially induced calcite precipitation in erosion mitigation and stabilisation of sandy soil foreshore slopes: A preliminary investigation. Engineering Geology. **201**, 96, **2016**.
17. DENG W., WANG Y. Investigating the Factors Affecting the Properties of Coral Sand Treated with Microbially Induced Calcite Precipitation. Advances in Civil Engineering, (9), 6, **2018**.
18. WANG L., REN Z., WANG H., LIANG X., LIU S., REN J., HE Y., ZHANG M. Microstructure-property relationships in cement mortar with surface treatment of microbial induced carbonate precipitation. Composites, Part B. Engineering. **239** (15), 11, **2022**.
19. JOHNSON K.A., GOODY R.S. The Original Michaelis Constant, Translation of the 1913 Michaelis–Menten Paper. Biochemistry. **50** (39), 8264, **2011**.
20. DILRUKSHI R.A.N., NAKASHIMA K., KAWASAKI S. Soil improvement using plant-derived urease-induced calcium carbonate precipitation. Soils and Foundations. **58** (4), 894, **2018**.
21. SONG J.Y., SIM Y., JANG J., HONG W.T., YUN T.S. Near-surface soil stabilization by enzyme-induced carbonate precipitation for fugitive dust suppression. Acta Geotechnica. **15** (7), 1967, **2020**.
22. SHIBLI U.A., ALSAIDI M.S., AKHTAR A., QURESHI M.U., ZHAO Y., KHALID N. Enzyme Induced Calcite Precipitation (EICP) for Engineering Application by Using Plant Based Biomaterials. Materials Science Forum. **13** (1), 1059, **2022**.
23. ISAAC A., ENVELOPE M.R., RAJIBUL K., SIMON B. Unconfined compressive strength of MICP and EICP treated sands subjected to cycles of wetting-drying, freezing-thawing and elevated temperature, Experimental and EPR modelling. Journal of Rock Mechanics and Geotechnical Engineering. **15** (5), 1126, **2022**.
24. CUI M.J., LAI H.J., HOANG T., CHU J. One-phase-low-pH enzyme induced carbonate precipitation (EICP) method for soil improvement. Acta Geotechnica. **16** (2), 481, **2021**.
25. ISAAC A., MIZANUR R., RAJIBUL K., SIMON B. Unconfined compressive strength of MICP and EICP treated sands subjected to cycles of wetting-drying, freezing-thawing and elevated temperature, Experimental and EPR modelling. Journal of Rock Mechanics and Geotechnical Engineering. **15** (5), 1226, **2022**.
26. PISANI W.A., JENNESS G.R., SCHUTT T.C., LARSON S.L., SHUKLA M.K. Preferential Adsorption of Prominent Amino Acids in the Urease Enzyme of Sporosarcina pasteurii on Arid Soil Components: A Periodic DFT Study. Langmuir. **38** (44), 414, **2022**.
27. CHEN D., QIANG X.U., ZHENG G., PENG S., WANG Z., PAN H.E. Particle identification and statistical methods of a rock avalanche accumulation body based on the particle analysis system. Hydrogeology & Engineering Geology. **48** (1), 60, **2022**.
28. QIAN C., RONG H., YU X., WANG X. Experiments on and predictions about properties of sand bonded by microbe cement. Science China (Technological Sciences), **59** (8), 1186, **2016**.
29. MA L., PANG A.P., LUO Y. Beneficial factors for biomineralization by ureolytic bacterium Sporosarcina pasteurii. Microbial Cell Factories. **19** (1), 12, **2020**.
30. SARACHO A.C, HAIGH S.K., JORAT M.E. Flume study on the effects of microbial induced calcium carbonate precipitation (MICP) on the erosional behaviour of fine sand. Géotechnique. **71** (12), 1135, **2020**.
31. MUTITU K.D., MUNYAO M.O., WACHIRA M.J., MWIRICHIA R., MARANGU M.J. Effects of biocementation on some properties of cement-based materials incorporating Bacillus Species bacteria – a review. Journal of Sustainable Cement-Based Materials. **8** (5), 17, **2019**.
32. DILRUKSHI R., WATANABE K. Strengthening of sand cemented with calcium phosphate compounds using plantderived urease. INT J GEOMATE, **11** (3), 2461, **2016**.