Structural Monitoring and Maintenance, *Vol. 11, No. 3 (2024) 187-202*
https://doi.org/10.12989/smm.2024.11.3.187

Comparative study of calcium carbonate deposition induced by microorganisms and plant ureases in fortified peat soils

Chao Wang[1a], Jianbin Xie[*1], Yinlei Sun[1], Jianjun Li[1], Jie Li[1] and Ronggu Jia[2]

[1]School of Architecture and Planning, Yunnan University, Kunming 650500, P.R. China
[2]Yunnan Construction and Investment Holding Group No.1 Engineering Survey and Design Co., Ltd., Kunming 650500, P.R. China

(*Received March 21, 2024, Revised June 17, 2024, Accepted June 25, 2024*)

Abstract. For the problems of high compressibility and low strength of peat soil formed by lake-phase deposition in Dianchi Lake, microbial-induced calcium carbonate deposition (MICP), phyto-urease-induced calcium carbonate deposition (EICP) and phyto-urease-induced calcium carbonate deposition combined with lignin (EICP combined with lignin) were used to reinforce the peat soil, the changes in mechanical properties of the soil before and after the reinforcement of the peat soil were experimentally investigated, and the effect and mechanism of peat soil reinforcing by the three reinforcing techniques were tested and analyzed using X-ray diffraction (XRD) and scanning electron microscope (SEM). The results show that: compared to the unreinforced remolded peat soil specimens, the unconfined compressive strength (UCS), cohesion and internal friction angle of the specimens reinforced by MICP, EICP and EICP combined with lignin techniques have been greatly improved, and the permeability resistance has been improved by two, two and three orders of magnitude, respectively; the different methods of reinforcing generate different calcium carbonate crystalline phases, with the EICP combined with lignin technique generating the most stable calcite, and the MICP and EICP techniques generating a mixed phase of calcite and spherulitic chalcocite. Analyses showed that for peat soil reinforcement, the acidic environment of peat soil inhibited the growth and reproduction of bacteria, EICP technology was superior to MICP technology, and the addition of lignin solved the defect of the EICP technology that did not have a "nucleation site", so EICP combined with lignin reinforcement was preferred for the improvement of peat soil.

Keywords: EICP; EICP combined lignin; MICP; reinforcement mechanism; the peat soil

1. Introduction

Peat soil, due to varying formation and development conditions, exhibits distinct regional characteristics. Peat soil formed by lacustrine sedimentation in DianChi Lake has poor structural integrity, high organic matter content, and high moisture content, among other unfavorable engineering properties. If not improved in advance, directly using it for building foundations will likely fail to meet strength design requirements and may lead to adverse consequences such as foundation settlement (Cai *et al*. 2022, Gui *et al*. 2016). Currently, the area surrounding DianChi

*Corresponding author, Professor, E-mail: kmxiejb@sina.com
[a]Master degree student, E-mail: 545205688@qq.com

http://www.techno-press.org/?journal=smm&subpage=7 ISSN: 2288-6605 (Print), 2288-6613 (Online)

Lake has gradually become the core economic zone of Kunming, with varying thicknesses of peat soil distributed throughout. There is an urgent need for a green and environmentally friendly soil reinforcement method to support the healthy development of the city.

Traditional methods of improvement involve permeating soil with materials such as cement, lime, and polypropylene to harden soil particles, aiming to achieve the desired strength and stability in design (Peng *et al*. 2023, Avramenko *et al*. 2022). However, such methods can cause environmental pollution and incur high costs, making them less conducive to widespread adoption. Sand well drainage, another method, is not practical due to the loss of moisture from organic matter forming a water film. The use of dynamic compaction and replacement methods can increase project volume, escalate economic costs, and the strong vibrations can adversely affect surrounding structures. Given these challenges, green ecological soil reinforcement methods have become a research hotspot. Among them, microbial-induced carbonate precipitation (MICP) and enzyme-induced carbonate precipitation (EICP) techniques utilizing urease enzyme hydrolysis of urea have been used for soil improvement (Dong *et al.* 2021, He *et al*. 2023, Li *et al*. 2021, Liu *et al*. 2019, Song *et al*. 2020, Wang *et al*. 2023). The mineralization model of microbial urease hydrolyzing urea can rapidly generate large amounts of calcium carbonate precipitation in a short time, making it the most direct and easily controllable reinforcement technique (Iamchaturapatr *et al.* 2022, Jiang *et al*. 2016, Johnson and Goody 2011, Peng *et al*. 2016). However, most bacteria used in engineering are alkali-loving bacteria, while peat soil is acidic and contains a high level of active microorganisms. Therefore, the application of MICP technology for peat soil reinforcement requires further research.

EICP differs from MICP in that urease directly participates in the hydrolysis reaction of urea without the involvement of microorganisms. Its urease can be purified or crudely extracted from plants (Ahenkorah *et al.* 2021, Dilrukshi *et al.* 2018, Gowthaman *et al.* 2022, Meng *et al.* 2021, Nam *et al*. 2015, Wang *et al.* 2023). Since the size of urease is on the nanometer scale, it is more effective in reinforcing peat soil. However, the urease in EICP technology exists in a free state and cannot provide "nucleation sites" for calcium carbonate precipitation, thus failing to achieve the expected reinforcement effect (Dong and Liu 2022, Ghasemi *et al.* 2022, Gitanjali *et al*. 2024, Xie *et al.* 2024, Yang *et al*. 2022). Lignin, as an environmentally friendly material, can effectively enhance soil strength (Dong *et al.* 2023, Gao *et al.* 2023, Shu *et al*. 2023). By adding lignin to EICP technology, "nucleation sites" can be provided for the formation of calcium carbonate precipitation, enabling orderly distribution and enhancing reinforcement effectiveness (Zhang *et al*. 2021, 2022). Currently, the application of MICP, EICP, and permeation of biopolymers mainly focuses on silty sand, loess, sandy soil, and repairing cracks in concrete, yielding significant research results (Chen *et al*. 2019, Liu *et al.* 2024, Oh *et al.* 2024, Ren *et al*. 2024, Zhang *et al*. 2019). However, there is limited research on reinforcing peat soil.

In summary, this paper compares three reinforcement techniques: MICP, EICP, and EICP combined with lignin-for reinforcing peat soil. It evaluates the macroscopic strength of reinforced samples through mechanical property experiments and analyzes the microscopic structure using XRD and SEM tests. From both macroscopic and microscopic perspectives, the study investigates the strength characteristics and reinforcement mechanisms of these three techniques for peat soil. This research can provide theoretical basis and technical guidance for selecting soil improvement methods in regions with widespread distribution of peat soil, such as Yunnan.

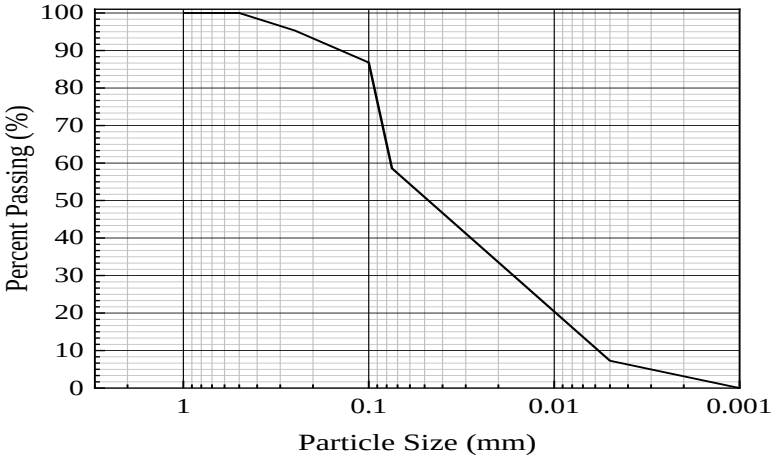


Fig. 1 Grain size grading curve of peat soil

Table 1 Physical indicators of peat soil

ω (%)	ρ(g.cm^{-3})	ρ_d(g.cm^{-3})	e	ω_P(%)	ω_L(%)	pH	Organic content(%)
303	1.096	0.254	5.472	203.6	130.8	5.1	58

2. Materials and methods

2.1 Experimental soils

The experimental soils used in this study were sourced from a project in Dingjia Village, Guandu District, Kunming City, which is formed by lacustrine sedimentation in the Dianchi Lake area. The peat soil obtained appears black-brown or black in color, with a relatively hard texture. The physical properties of the soil are summarized in Table 1. Test analysis of the undisturbed soil samples showed a high organic matter content, indicating it belongs to the category of strong peat soil. The soil exhibits weak acidity and a high natural moisture content. Loss of moisture from the organic matter content forms a water film on the surface of soil particles, hindering the penetration of solution into the soil during subsequent grouting processes. Moreover, the abundant microorganisms present in the soil are unfavorable for the metabolism of Bacillus subtilis, necessitating the reshaping of the peat soil. The air-dried peat soil was passed through a 2 mm sieve and then dried in a constant temperature drying oven at 65°C before being stored in a sealed drying oven. To obtain the particle size distribution curve of remolded peat soil, the organic matter in the peat soil is first removed using incineration. Then, the cohesive soil clumps are thoroughly crushed using a rubber hammer and a wooden pestle. Subsequently, the particle size distribution curve is determined using sieving and density measurement methods, as illustrated in Fig. 1.

2.2 Extraction of urease

2.2.1 Bacillus subtilis urease

Table 2 Medium Composition Table

$CO(NH_2)_2$ (L)	Casein Peptone (g)	Soy Peptone (g)	NaCl (g)	Distilled Water (L)	pH
20	15	5	5	1	7.3

Experimental scheme one involves using microorganisms to induce calcium carbonate precipitation. The microorganism chosen is Bacillus subtilis, purchased from the Microbial Culture Collection Center of Guangdong Province, with dimensions approximately 2 to 3 micrometers. The liquid culture medium was prepared by mixing the components listed in Table 2 to make 100 ml of medium. The mixture was then sterilized in a high-temperature autoclave at 121°C for 25 minutes and then transferred to a sterile operation room at room temperature for further procedures.

Add 1-2 ml of sterile water into an ampoule containing dry powder, and thoroughly shake and mix to form a suspension. Transfer the suspended bacterial solution into 10 ml of liquid culture medium using a sterile pipette. Incubate the mixture at 30°C, 120 rpm, for 48 hours to cultivate the first-generation bacterial liquid. To prepare the second-generation bacterial liquid, transfer the activated first-generation bacterial liquid into fresh medium at a ratio of 1:100 (bacterial liquid to culture medium volume). Incubate this mixture at constant temperature and shake for 24 hours. Repeat the above steps to culture it into the third-generation bacterial liquid.

The third-generation bacterial liquid was transferred to the culture medium at the ratio of 1:100 (bacterial liquid volume to culture medium volume), and then incubated with continuous shaking at a constant temperature for 72 hours. After inoculation, the bacterial culture was subjected to absorbance and activity measurements. Absorbance was measured using a UV spectrophotometer (model: *UV9000*) by taking 3 ml of the bacterial suspension every 2 hours and measuring the absorbance at a wavelength of 600 nm. Urease activity was determined using the conductivity method (Omoregie *et al*. 2017). The conductivity measurements were performed using a conductivity meter (model *DDS-307A*) with a conductivity range of 0.00 to 99.9 mS/cm and a TDS range of 0.000 to 2000 mg/L. This method relies on the urease-mediated hydrolysis of urea, which changes the concentration of ions in the solution and results in a change in conductivity. The change in conductivity of 1 mS per minute is equivalent to the hydrolysis of 11.11 mmol of urea. Urease activity was expressed as the ratio of total urease activity to OD600 value, representing the unit urease activity.

2.2.2 Soybean urease

Experimental scheme two involves preparing crude extracts of urease solution from soybeans. Edible soybeans were dried in an oven at 45°C for 8 hours, then ground with a grinder and sieved through a 100-mesh sieve. Portions of 10 g, 20 g, 30 g, and 50 g of the sieved soybean powder were placed separately in beakers, and 100 ml of ultrapure water was added to each beaker and stirred until turbid. The mixtures were then stirred for 30 minutes at 20°C using a constant-temperature magnetic stirrer, followed by allowing them to stand at room temperature for 6 hours. After standing, the mixtures were filtered to remove impurities. Subsequently, the filtered solutions were evenly poured into 45 ml centrifuge tubes and centrifuged at 3000 rpm for 10 minutes, 20 minutes, and 30 minutes respectively. A control group without centrifugation was also set up. The centrifuged soybean powder solutions were filtered through sterile gauze to remove impurities. After standing for a period of time, the supernatant was collected as the urease solution.

2.3 Lignin

The experimental material used is dealkalized lignin purchased from the Internet, which is soluble in water and is a naturally occurring composite biopolymer with biodegradability and biocompatibility. The active groups present in its tissue structure have polymerization effects and play a role in condensing the formed mineral particles. The main components are listed in Table 3.

2.4 Tube test

Relevant studies have indicated that as the concentration of calcium ions increases, calcium production initially rises, then decreases, reaching a peak at 0.75 mol/L (Zhang *et al*. 2023). Lower concentrations of the cementing solution were found to be more effective for calcium carbonate precipitation compared to higher concentrations (Gitanjali *et al.* 2024). The optimal concentration was determined to be 1 mol/L. Concentrations exceeding 1 mol/L may lead to a Retarding effect, which could impact the cementation performance of calcium carbonate crystals (Cui *et al*. 2024, Lai *et al.* 2021, Liu *et al*. 2024, Xu *et al.* 2023).

Considering that the pH increased by urea hydrolysis during the conversion of calcium ions to calcium carbonate will decrease due to the precipitation of calcium carbonate as well as the acidic nature of peaty soils, it is necessary to moderately increase the concentration of calcium ions (Cui *et al.* 2024). To investigate the effect of different urea and calcium chloride mixing ratios on calcium ion mineralization efficiency, this experiment selected three calcium ion concentrations: 0.5 mol/L, 1.0 mol/L, and 1.5 mol/L. For each calcium ion concentration, three parallel experiments were conducted. The mixing ratios of calcium chloride and urea (concentration of the cementitious solution) were 1 : 0.75, 1 : 1, and 1 : 1.25. 5 ml of the cementitious solution with different mixing ratios is taken in test tubes. In the MICP reaction, 5 ml of Bacillus subtilis bacterial suspension is added to the test tubes, while in the EICP reaction, 5 ml of soybean urease solution is added. The reactions are allowed to proceed for 24 hours until a noticeable white precipitate forms at the bottom of the test tubes. After filtration to remove the turbid liquid from the test tubes, the calcium carbonate crystals were washed with distilled water. The test tubes containing calcium carbonate precipitates were dried in an oven until a constant weight was achieved, denoted as W_1. Subsequently, the calcium carbonate crystals were dissolved in 5% dilute hydrochloric acid until no obvious bubbles were produced in the test tubes. The tubes with complete reactions were then heated to evaporate the hydrochloric acid completely, and dried again in an oven until a constant weight was achieved, denoted as W_2. The precipitation rate of calcium carbonate (m) is calculated as follows

$$m = \frac{W_1 - W_2}{W_1} \times 100\% \tag{1}$$

2.5 Grouting experiment

The grouting schematic is shown in Fig. 2. During the experiment, Vaseline should be applied to the inner sides of the molds to prevent difficulty in removing the specimens after grouting. The areas where the glass tube contacts the rubber stopper holes, as well as where the molds meet the rubber stoppers, need to be sealed with the raw material tape to prevent liquid leakage during grouting. Two layers of gauze should be placed at the bottom and top of the molds to prevent

Table 3 Main components of lignin

CAS	moisture	sulphate	pH
9005-53-2	≤ 20 %	10~20 %	3~4

Table 4 Grouting process processing table

Experimental program	Grouting Step	Injecting slurry	Injection rate ($mL \cdot min^{-1}$)	retention period (h)
1	1	Bacterial urease solution	5	6
	2	Urea-calcium chloride cemented solution	10	18
2	1	Soybean urease liquid	5	6
	2	Urea-calcium chloride cemented solution	10	18
3	1	Soybean urease liquid	5	6
	2	Mixture of lignin and gelling solution	10	18

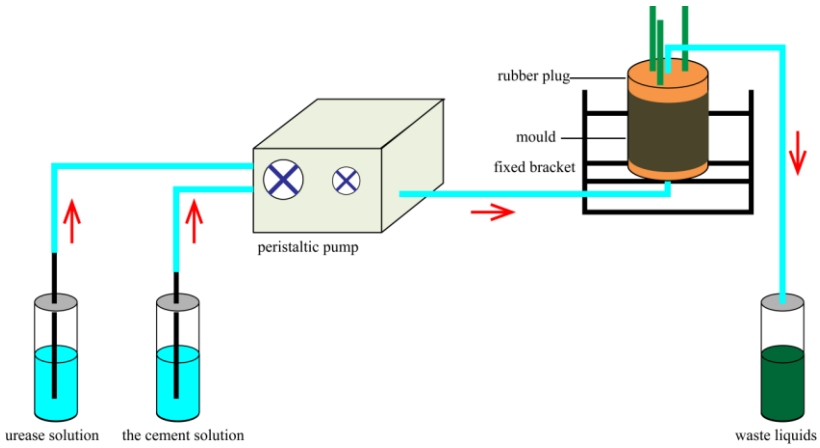
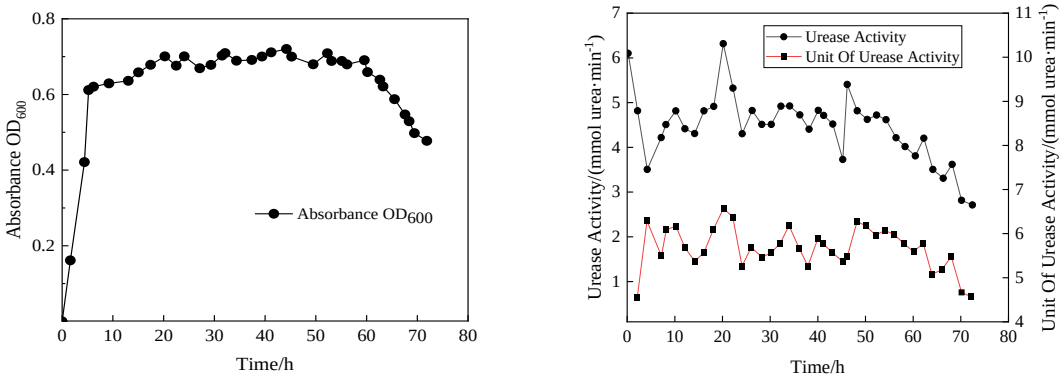


Fig. 2 Grouting Schematic

particle blockage of the glass tube. After assembling the apparatus, ultra-pure water is injected into the molds from the bottom to saturate the soil and expel air bubbles from the specimen. Subsequently, taking 150 ml of urease solution respectively and grouting according to Table 4.

When infusing bacteria urease solution and soybean urease solution, choosing a slower injection rate can evenly distribute the urease among the soil particles. However, when infusing cementing solution, selecting a faster injection rate can largely mitigate the phenomenon of precipitation reactions occurring at the injection port and blocking the injection orifice. When infusing the sample with the combination of EICP and lignin technology, lignin is added to the cementing solution at 3% of the sample mass to ensure thorough mixing. After the infusion of soybean urease solution is complete, the lignin-containing cementing solution is injected into



(a) Growth curve of Bacillus subtilis (b) Change curve of urease activity

Fig. 3 Growth curve of Bacillus subtilis and variation

the sample to ensure that lignin can be evenly distributed in the pores of the soil mass, maximizing the reinforcement effect. Repeat the above steps for 7 days, complete the entire infusion experiment, then let it stand for 24 hours to allow the cementing solution and urease solution to react fully, and to cure the sample. After curing, conduct mechanical performance tests.

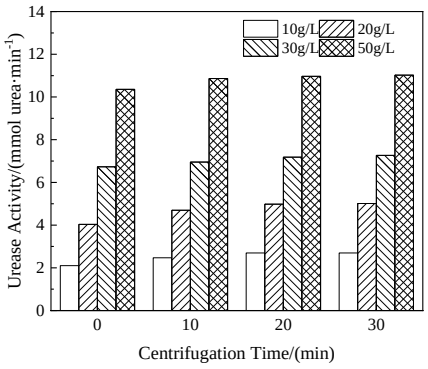
2.6 Microscopic tests

After reinforcement is completed, select typical locations for microstructural test sampling (cubes with a side length of 1 cm). Dry the samples in a constant temperature oven at 65°C for 48 hours, then thoroughly grind the fragments and sieve them through a 200-mesh sieve. Some samples are subjected to mineral composition analysis using an X-ray diffractometer (*TTIII-18KW*). The experiment employs a Cu target and scans the diffraction angle range from 5°to 80°at a rate of 10°/min. The data obtained from the scans are analyzed using *MDI-Jade6* software. Other samples undergo SEM testing (*Quanta-200*) at a magnification of 2000 times.

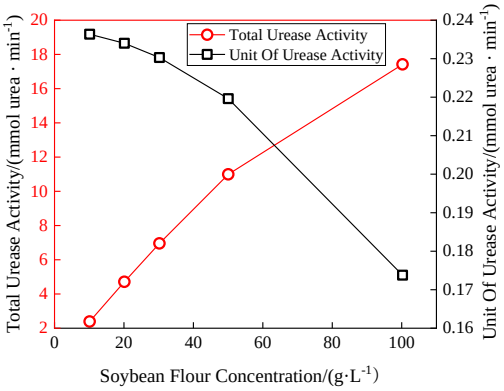
3. Results and analysis

3.1 Activity of Bacillus subtilis urease and soybean urease

The growth curve of Bacillus subtilis and the curve depicting the change in urease activity are shown in Fig. 3. At 20 hours, both the absorbance (OD) value and urease activity of the bacterial culture reached their maximum values, measuring 0.716 and 6.375, respectively. The relationship between urease activity and centrifugation time and concentration is illustrated in Fig. 4. The urease activity of soybean urease solution after centrifugation is greater than that of the non-centrifuged solution, indicating that thorough centrifugation can yield higher soybean urease activity. Additionally, soybean urease activity increases with the concentration of soybean powder. To compare and analyze the reinforcement effects of MICP technology and EICP technology on peat soil, the choice of urease solution should match the urease activity of Bacillus subtilis urease.



(a) Urease activity at different centrifugation times



(b) Urease activity at different bean flour concentrations

Fig. 4 Urease activity as a function of centrifugation time and concentration

Therefore, in this experiment, the centrifugation time for the urease solution is set to 20 minutes, and the concentration of soybean powder is 20 g/L.

3.2 Amount of calcium carbonate induced

According to Eq. (1), the results of calcium carbonate yield are calculated and presented in Table 5. By comparing the results of the test tube experiments, it can be observed that under three different calcium ion concentrations, the calcium carbonate yield of MICP technology is higher than that of EICP technology. In the MICP reaction, when the calcium ion concentration is 0.5 mol/L and the ratio of calcium chloride to urea is 1:1, the highest calcium carbonate yield is 99.08%. In the EICP reaction, when the calcium ion concentration is 0.5 mol/L and the cementing solution ratio is 1:1.25, the highest calcium carbonate yield is 94.81%. The main reason for this difference is that the bacterial culture medium itself contains nitrogen and carbon sources. The increase in urea concentration not only increases the theoretical yield of carbonate ions but also accelerates the rate of calcium carbonate formation. Additionally, the auxiliary proteins present in the culture medium play an important role in maintaining urease activity.

3.3 Changes in mechanical properties of soil before and after reinforcement

The changes in mechanical properties of the soil before and after reinforcement are shown in Figs. 5 and 6. Compared with the remolded soil, the cohesion of the samples after reinforcement with MICP, EICP, and EICP combined with lignin increased by 6.37 kPa, 8.31 kPa, and 10.29 kPa, respectively. The internal friction angle increased by 3.823°, 5.16°, and 8.151°, respectively. The unconfined compressive strength increased by 6.67 times, 19.75 times, and 25.74 times, respectively. The permeability improved by two, two, and three orders of magnitude, respectively, and the permeability coefficient was calculated using Eq. (2). The change in unconfined compressive strength was greater than the change in cohesion, primarily due to the size difference between the direct shear test mold (61.8×20 mm) and the unconfined compressive strength test mold (39.1×80 mm). On one hand, the volume of the unconfined compressive strength test sample

Table 5 Calcium carbonate yield statistics table

reinforcement methods	cementitious mixture proportioning	calcium ion concentration/(mol·L^{-1})		
		0.50	1.00	1.50
MICP	1:0.75	96.27	75.91	64.01
	1:1	99.08	72.43	68.32
	1:1.25	93.24	77.39	63.9
EICP	1:0.75	72.86	57.04	45.03
	1:1	90.43	61.86	39.35
	1:1.25	94.81	60.87	45.52

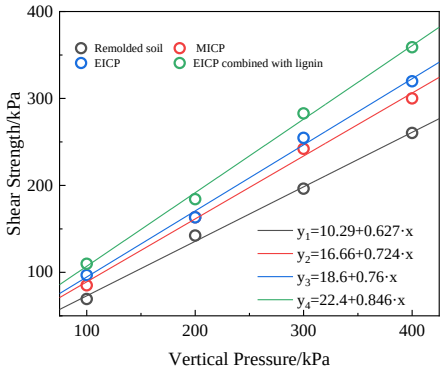


Fig. 5 Shear strength results

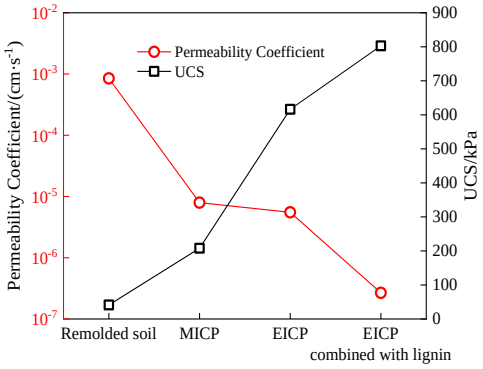


Fig. 6 Unconfined shear strength and permeability coefficient results

is relatively larger, requiring more slurry injection compared to the direct shear test sample, which consequently generates more calcium carbonate crystals. Calcium carbonate crystals have higher mechanical strength, significantly enhancing the mechanical properties of the sample. On the other hand, the shape of the unconfined compressive strength test sample is "slender," while the shape of the direct shear test sample is "stout," which introduces a size effect. Furthermore, the failure

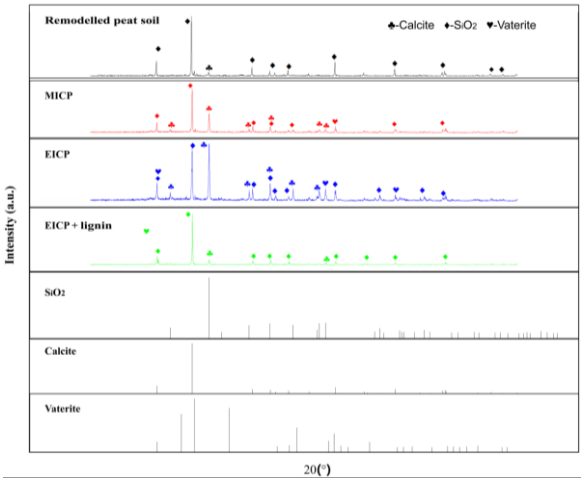


Fig. 7 XRD results graph

mechanism of unconfined compression is to some extent overcoming the tensile strength between soil particles, while shear strength overcomes the friction and bonding forces between soil particles. Existing research indicates that tensile strength is approximately 1/20 of shear strength (Sun *et al.* 2023).

Therefore, under the influence of different factors such as calcium carbonate crystal yield, size effect, and mode of action, the change in unconfined compressive strength is significantly greater than the change in shear strength indicators.

$$k = \frac{2.3al}{A(t_2 - t_1)} \lg \frac{h_1}{h_2} \tag{2}$$

Where a is the cross-sectional area of the variable-head permeameter (cm^2); l is the height of the sample(cm); t is the time(s); h is the head height of the water column (cm).

After comparison, it was found that all three reinforcement techniques enhanced the mechanical properties of peat soil, with the EICP combined with lignin technique showing the most significant improvement. Several factors may contribute to this result: Firstly, the EICP combined with lignin method utilizes soybean urease, which has a smaller size compared to other ureases. This allows it to freely move within the soil pores, facilitating the precipitation of calcium carbonate crystals in a more uniform distribution throughout the pores. As a result, some pores become either closed or semi-closed. Additionally, the crude extract of soybean urease used in this method contains plant protein components that can adsorb onto the surface of soil particles, thereby improving the permeability of the samples. Secondly, Bacillus subtilis is an alkaliphilic bacterium, and the acidic nature of peat soil inhibits the growth and reproduction of bacteria. This inhibition reduces the urease activity produced by bacterial metabolism, weakening the ability of Bacillus subtilis to induce calcium carbonate precipitation.

3.4 Effect of mineral composition

The XRD results, as shown in Fig. 7, indicate that compared to the standard diffraction patterns,

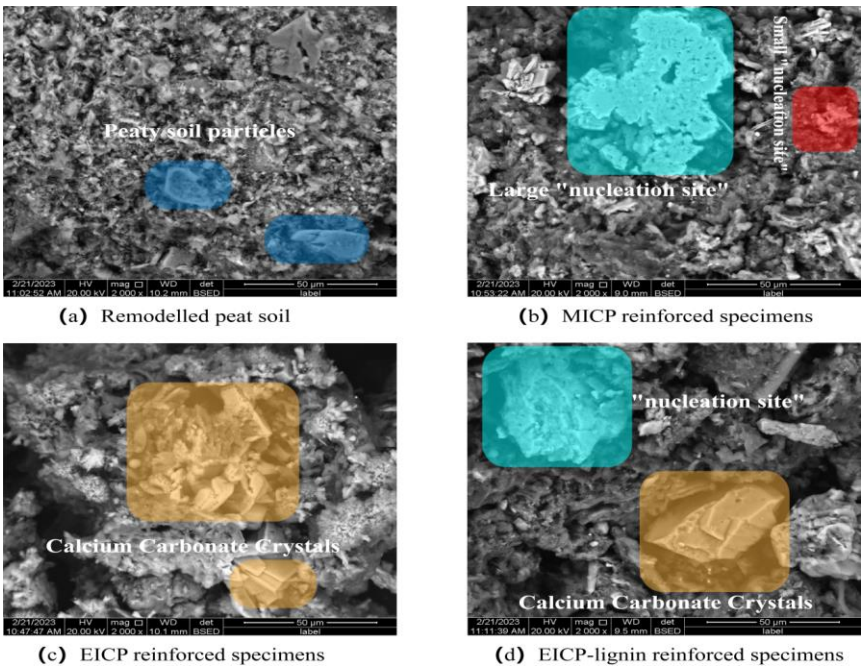
the peaks of SiO_2 are prominent in both the remolded soil and the samples reinforced by the three techniques. This suggests that the main component of the peat soil used in the experiment is SiO_2. There are small amounts of vaterite present in the remolded soil, while the content of vaterite increases after reinforcement by MICP and EICP techniques. However, the content of vaterite remains almost unchanged after reinforcement by the EICP combined with lignin technique. It is worth noting that vaterite is the least stable crystal structure in thermodynamic terms, with a loose and unstable structure. When vaterite is present between soil pores, it tends to transform into calcite due to its instability. However, different crystal forms of calcium carbonate may still cause variations in the physical and mechanical properties in the short term, thereby weakening the reinforcement effect (Radha *et al.* 2010).

3.5 Influence of microstructure

Through SEM image analysis of typical samples, it is evident that calcium carbonate crystals are formed on the surfaces and particle contacts of the remolded peat soil after being reinforced by the three methods, albeit with different crystal types and distributions, as shown in Fig. 8. Bacillus subtilis provides nucleation sites for calcium carbonate precipitation through its cell nucleus and extracellular polymers. These nucleation sites, distributed internally or on the surface of mineral matrices, preferentially bind ions from the solution, thereby providing growth points for mineral formation. Consequently, the calcium carbonate crystals produced by MICP technology primarily exhibit agglomerated crystals, with larger sizes and concentrated distributions. However, as Bacillus subtilis is an alkaliphilic bacterium, its growth and reproduction are inhibited in the acidic peat soil, resulting in reduced metabolism and hence less urease activity, thereby leading to a lower production of calcium carbonate. In contrast, the calcium carbonate crystals generated by EICP technology appear mainly as dispersed bodies, with a higher quantity and more uniform distribution. This is because EICP lacks nucleation sites, leading to the formation of disordered calcium carbonate crystals. The addition of lignin in the EICP reaction mitigates this deficiency, resulting in larger-sized calcium carbonate crystals. Consequently, the physical and mechanical performance enhancement of samples reinforced by EICP combined with lignin is most effective.

3.6 The mechanism of influence

The peat soil used in the experiment has poor particle size distribution and large pores after remolding, with weak inter-particle bonding, as shown in the initial state of the peat soil particles in Fig. 9. After reinforcement with MICP technology, the bacterial solution and cementing solution fill the voids between the particles, with bacteria adhering to the particle surfaces. The urease produced by bacterial metabolism hydrolyzes the urea in the cementing solution to produce calcium carbonate precipitation. With the presence of "nucleation sites", the calcium carbonate precipitate aggregates on the cell surfaces, resulting in localized strengthening, as shown in Fig. 9(a). After reinforcement with EICP technology, soybean urease solution and cementing solution fill the voids between the particles. The calcium carbonate precipitate produced by the hydrolysis of urea adsorbs on the particle surfaces. As the hydrolysis proceeds further, the resulting calcium carbonate precipitate is widely distributed within the particle voids. Since the free state of urease is less influenced by the soil environment, a larger quantity of calcium carbonate is produced, resulting in a higher proportion of non-pore area, as shown in Fig. 9(b). With the addition of lignin



(a) Remodelled peat soil (b) MICP reinforced specimens

(c) EICP reinforced specimens (d) EICP-lignin reinforced specimens

Fig. 8 Comparison chart of SEM results

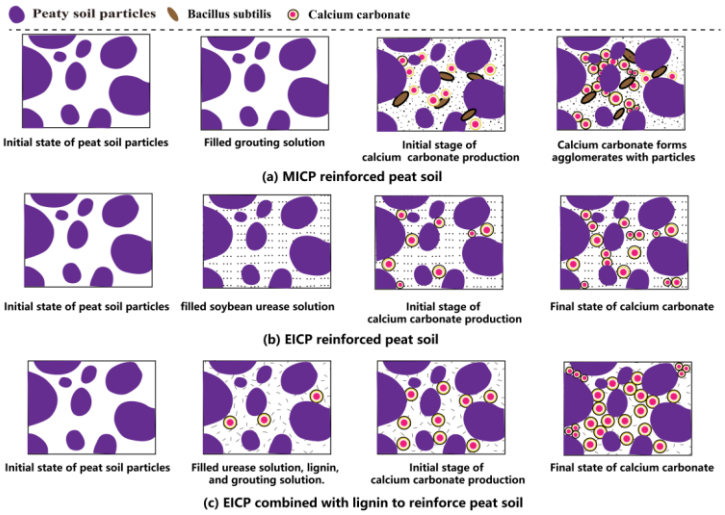


Fig. 9 Schematic diagram of micro-mechanism

in the EICP technology, the problem of lacking "nucleation sites" is resolved. The resulting calcium carbonate precipitates mutually adsorb, and the weak areas between particles are effectively filled, as shown in Fig. 9(c). The calcium carbonate precipitates produced by the above three reinforcement techniques fill the voids between the particles, acting to bond the soil particles

together, enhance inter-particle frictional resistance, and strengthen weak areas. This is manifested as an improvement in macroscopic physical mechanical properties and an increase in non-pore area ratio at the microscopic level.

4. Conclusions

The study compares the reinforcement effects of MICP, EICP, and EICP combined with lignin techniques on peat soil. The main research conclusions are as follows:

(1) In MICP reactions, the urease activity of Bacillus subtilis reaches its maximum after 20 hours of cultivation. The optimal precipitation rate of calcium carbonate occurs when the calcium ion concentration in the cementitious solution is 0.5 mol/L, and the ratio of calcium chloride to urea is 1:1. The activity of soybean urease increases with centrifugation time and concentration. The optimal precipitation rate of calcium carbonate occurs when the calcium ion concentration in the cementitious solution is 0.5 mol/L, and the ratio of calcium chloride to urea is 1:1.25.

(2) The results of physical and mechanical performance tests indicate that all three reinforcement techniques have a positive effect on peat soil, with the EICP combined with lignin technique showing the best enhancement.

(3) Microscopic experiments reveal that the calcium carbonate formed by the EICP combined with lignin technique exists in the most stable form of calcite, while the forms generated by MICP and EICP techniques are a mixture of calcite and vaterite. Mechanistic studies suggest that the calcium carbonate precipitates produced by microbial and plant urease fill the intergranular voids, connecting the soil particles and strengthening the weak areas of the soil.

(4) All three reinforcement techniques effectively improve the strength of soil and enhance the bearing capacity of foundations. They also provide valuable guidance for repairing cracks in concrete structures.

MICP and EICP technologies are currently primarily applied in soil reinforcement and concrete crack repair, showing broad prospects in structural engineering applications. As low-carbon and environmentally friendly repair techniques, they hold significant research value, particularly in the restoration of historic buildings and concrete structures. Further investigation into their reinforcement mechanisms is warranted.

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