

Research Article

Investigation on the Shear Behavior and Mechanism of MICP-Treated Loess Soil

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Microbially induced carbonate precipitation (MICP) has been utilized as a new method to improve loess soil strength. In this study, we investigated the influence of the main parameters on the shear strength of MICP-treated loess specimens. Initially, culture media with different formulas and pH values were examined to identify the most efficient medium for loess soil. To explore the shear behavior of MICP-treated loess under general stress levels, unconfined compressive strength (UCS) tests and triaxial tests relevant to the compression strength and vertical loads were performed on MICP-treated loess with different calcium sources, cementation concentrations, and curing periods. Subsequently, calcium chloride was selected as the optimal calcium source based on the ultimate strength of the MICP-treated loess. The effective cementation concentration in the loess soil was between 1.0 and 1.25 M. The ultimate strength of the MICP-treated loess was 3.6 times of the untreated loess. The stress-strain curves indicate that a higher cementing effect can be expected with an increase in the curing period. The formation process of calcium carbonate and the micromorphology of the MICP-treated loess samples were examined using scanning electron microscopy. In this study, we present an environmentally friendly technique for improving loess soil strength.

1. Introduction

Owing to the limitations of traditional improvement methods, microorganisms have been explored for improving the soil-bearing capacity in an eco-friendly manner [1, 2]. *Bacillus pasteurii* is one of the commonly used in microbially induced carbonate precipitation (MICP) method. The hydrolysis of urea by *Bacillus pasteurii* is the most important process for carbonate precipitation where carbonate can be produced. Calcium carbonate crystals were generated in the presence of a calcium source, and the precipitation process continued with the aforementioned process. The chemical reactions in this process are described as follows [3].

$$\mathrm{CO(NH}_2)_2 + 2\mathrm{H}_2\mathrm{O} \longrightarrow 2\mathrm{NH}_4^+ + \mathrm{CO}_3^{2-} \tag{1}$$

$$\operatorname{Ca}_{2}^{+} + \operatorname{CO}_{3}^{2-} \longrightarrow \operatorname{CaCO}_{3}(\downarrow)$$
 (2)

The difference in the permeability profile modification between bacterially formed $CaCO_3$ and the enzymic method was also compared by Nemati et al. [4]. The cementation effect in the presence of bacteria is almost tripled compared to that in enzymatic conditions. Thus, the feasibility of MICP for soil reinforcement has been verified in many experiments, particularly in sand [5–7]. Subsequently, influencing factors, such as temperature, pH, grain size and distribution, cementation concentration, and curing periods have been explored by many researchers [8–10]. Additionally, researchers conducted various experiments on the compressive strength and permeability of MICP-treated soils by conducting direct shear or triaxial tests. The crystals induced by this process can fill the pore spaces between the soil particles, which in turn decreases soil porosity. The particle shape and degrees of saturation were further explained by Cheng et al. [11]. Owing to the improvement in the relative density of the biocementation effect, the cyclic response of calcareous sand was investigated by conducting extensive experiments. Cementing methods such as mixing or injecting bacteria, have been compared to reveal the nonuniform distribution of $CaCO_3$ [12, 13]. However, previous research focused on coarsegrained soils. In engineering, various types of soil must be reinforced using green techniques, especially in the present scenario of green development.

Laboratory-based tests were conducted to explore the mechanical behavior of MICP-treated fine-grained soils, such as silt and clay [14-16]. The effect of the MICP method on the strength behavior of fine-grained and residual soils was also studied considering various engineering scenarios [17–20]. Wang et al. [21] investigated the microstructure, unconfined compressive strength (UCS), and thermal conductivity of compacted CDG soil treated using the MICP method which indicated the feasibility of MICP treatment in weathered soil. Loess is a special soil widely distributed in western China. Despite the considerable attention paid to MICP on soil strength improvement, little research has been conducted on the strength of MICP-treated loess soils, especially on the influencing parameters of shear strength improvement and the calcium carbonate formation mechanism [22, 23]. Sun et al. [24] investigated the collapsibility of MICP-treated loess soil; however, the strength improvement of the loess soil requires further investigation.

In this study, the urease concentration in different culture media and the adaptability of bacterial growth in loess soil were investigated to obtain a favorable condition for bacterial growth. Influential factors, such as calcium sources, bacterial retention days, i.e., curing periods, and cementation concentrations, were explored. The optimal calcium source and cementation concentration were determined based on UCS test results. The relationship between the ultimate strength and calcium carbonate content is discussed at various concentrations. The curing age and shear-strength improvements were investigated using triaxial tests. Furthermore, the calcite formation process of MICP-treated loess at the meso-micro scale was also delineated.

2. Soil and Cementing Method

2.1. Bacteria Cultivation. The Bacillus pasteurii BNCC337394 adopted was in a lyophilized powder form and vacuum-sealed in ampoules that needed to be cultivated in a culture medium. It is an aerobic bacillus with strong environmental adaptability and can easily survive in alkaline environments. To eliminate the influence of pH on bacterial growth and urease activity, the initial pH of the medium was set to be roughly 7.0 [13]. The bacteria were continuously expanded for 36 h under suitable conditions. To prevent the influence of miscellaneous bacteria, the container was covered with a sterile film and placed in a constant temperature shaking box for the cultivation of *B. pasteurii*. The cultivation of the organism was conducted under sterile conditions by UV at 30°C. The activated bacteria were preserved using a slant preservation method for future use.

To explore a more efficient medium for bacteria, an inoculation ratio of 1:1 was used to cultivate the bacterial solution. Different types of culture media were compared based on the optical density of the bacteria at 600 nm (OD₆₀₀) using an ultraviolet-visible spectrophotometer [8]. Table 1 indicates five different sources of culture medium formulas, and a microplate reader was used to monitor the OD₆₀₀ value of the different culture media. The culture medium ingredient from the American Collection of Microorganisms, which consists of 20 g of yeast extract (YE), 10 g of ammonium sulfate (NH4)₂SO₄, and 24 mg of nickel chloride hexahydrate (NiCl₂·6H₂O), was selected for future cultivation owing to its high bacteria activity.

The growth process of the bacteria suspension can be divided into four stages, as shown in Figure 1. In the first 4 h, the growth curve is flat, and the bacteria have just been added to the medium and need to adapt to the new environment to reserve energy; Then, in the logarithmic phase, the bacteria multiply and proliferate rapidly, and the number of bacteria increases rapidly with a stable progression, where the OD_{600} reaches to 1.5 (OD_{600} is the absorbance value of a solution at a wavelength of 600 nm, implying the concentration of bacterial cells). Bacteria consume nutrients and produce unfavorable metabolites that reduce the bacterial reproduction rate. After this period, the number of dead bacteria increased significantly, and the metabolic activity of the bacteria tended to stagnate. As instructed by Wang et al. [8], the concentration of the bacterial suspension (cells/ml) at pH = 8 is approximately 1.2×10^8 cells/ml. Therefore, it is advisable to select bacteria cultured for 32-44 h for subsequent tests. The pH was varied from 6 to 10, and an appropriate pH of 8 was recommended.

2.2. Loess Sample Preparation. The loess soil used in this study was sampled from an engineering spot of a loess area in Xi'an Shaanxi Province, China (Figure 2). The dry density of the loess sample was 2.71 with a void ratio of 0.60. The liquid limit and plastic limits of the loess soil are 26.0% and 17.7%, respectively. The optimal water content is 16.3%, and the plastic index is 8.3, respectively. The soil sample was prepared using several layers according to the instructions of the experimental group according to the manual. To control the initial water content of the samples, the ratio of bacterial suspension and cementing reagent in the test was 1:1, and the samples with a water content of 20% were prepared with equal mol bacterial and cementing reagents instead of distilled water. UCS tests were conducted on an LSY30-type stress and strain-controlled triaxial apparatus to evaluate the strength improvement by the MICP biocementation method with a specimen of $39.1 \times 80 \text{ mm}$ (diameter × height). An axial load was applied at a rate of 0.5 mm/min according to the standard until brittle cracks appeared on the specimen. To explore the stress-strain behavior of the MICP-treated loess, the sample was also placed on the SJ-1A triaxial shear test apparatus for triaxial compressive tests. The shear rate of the sample was 0.15 mm/min. When the axial strain $\varepsilon > 15\%$ or deviatoric stress was stable, the specimen was damaged. The peak value achieved during the compression test is defined as the maximum deviator stress (q_u) .

Medium	Source	$OD_{600 \text{ nm}}$
YE (20 g), ammonium sulfate (10 g), and nickel chloride hexahydrate (24 mg)	American Collection of Microorganisms	1.60
Peptone (5 g), beef extract (3 g), and urea (20 g)	Beina Microorganism Collection	1.18
Tryptone (15 g), soy peptone (5 g), sodium chloride (5 g), and urea (5 g)	German Collection of Microorganisms	0.90
YE (20 g), ammonium sulfate (10 g), and 0.13 M Tris base American Collection of Microorganisms		0.21
Soy peptone (5 g), YE (3 g), manganese sulfate (0.01 g), and urea (20 g)	China Microbial Culture Collection Center	0.18

TABLE 1: Different types of liquid culture medium.



FIGURE 1: Variation of bacteria OD_{600} with cultivation time at different pH.

In previous research on sand, MICP treatment with a peristaltic pump was injected into the specimen; however, it is difficult to eliminate the inhomogeneity [11]. Natural loess soil is a type of special soil with obvious structural properties and fissures that allow the bacteria and cement solution to pass through; this indicates its feasibility for MICP treatment. As the first step of MICP treatment application into loess soil, a remolded loess sample was tested which indicated that the original structure had been damaged. Therefore, the bacterial liquid and cementation concentration were mixed with the loess samples to ensure homogeneity. Deionized water from the control group was mixed with untreated loess as a parallel specimen. The specimens prepared for the tests are shown in Figure 3.

2.3. Testing Program. To study the different factors on the reinforcement of loess by microbial grouting, different cementation concentrations (BC1-BC5) from 0.5 to 1.5 M were used; BC0 represents natural loess. Various calcium sources including calcium chloride (CS1), calcium lactate (CS2), and calcium acetate (CS3) were compared. The reten-

tion times, including 0, 3, 7, and 14 days, were numbered from RT1 to RT4 to investigate the curing period. For each test, treatment samples were tested in triplicate, and calcium chloride was used as the cement reagent for the subsequent tests, except for investigating the calcium source. The concentration ratio of calcium chloride and urea was 1:1. The test arrangements used in the study are listed in Table 2. To investigate the micromorphology of MICP-treated loess specimen, scanning electron microscopic (SEM) was conducted using a scanning electronic microscope (ZEISS Sigma 300, Germany). The specimens were sampled from the MICP-treated loess samples at different concentrations with a cubic dimension of 1 cm. The specimens were dried and coated with gold to prevent electrical charging. SEM images were captured at a magnitude of $10 \text{ k} \times$.

3. Results Analysis

3.1. Effect of Calcium Source on Loess Soil Improvement. To explore the influence of the calcium source type, i.e., inorganic or organic calcium, different calcium sources were considered. Various calcium sources, calcium chloride (CS1), calcium lactate (CS2), and calcium acetate (CS3), were selected. The stress-strain curves of the MICP-treated loess samples with different calcium source conditions are displayed in Figure 4. With an increase in the strain, the stress of the MICP-treated loess sample first increased and then decreased. The ultimate stress occurs within a strain range of 0.25-0.75%. When the calcium source was CS1 (calcium chloride), UCS was the highest, which is 1.26 MPa. CS2 (calcium lactate) reagent has a relatively lower UCS of 1.02 MPa. The UCS of the reinforced loess sample was the smallest when the calcium source was CS3 (calcium acetate). Thus, calcium chloride was selected as the calcium source for subsequent tests.

3.2. Effect of Cementation Concentration. The cementation concentration in the control group BC0 is zero, i.e., it represents untreated loess, and the other samples are numbered from BC1 to BC5, which represents the cementation concentration of 0.5, 0.75, 1.0, 1.25, and 1.5 M, respectively, as listed in Table 2. The measured unconfined compressive stress (q_u) is plotted against the strain values for different cementation concentrations, as shown in Figure 5. The stress-strain curve of the sample shows a nonlinear increase and then a dramatic decrease. The ultimate shear strength increased when the concentration of the cement reagent



FIGURE 2: Location of the loess sample in Shaanxi Province, China.



FIGURE 3: LOESS soil specimens.

TABLE 2: Test arrangements in this study.

Sample	Calcium source	Cementation concentration (M)	Retention time (day)
CS1	Calcium chloride		
CS2	Calcium lactate		
CS3	Calcium acetate		
BC0		0	
BC1		0.5	
BC2		0.75	
BC3		1.0	
BC4		1.25	
BC5		1.5	
RT1			0
RT2			3
RT3			7
RT4			14

was smaller than 1.0 M and showed a significant decrease when the cementation concentration was 1.5 M. Generally, the strength of the MICP-treated loess sample was obviously higher than that of the natural soil. As shown in Figure 5, larger q_u values were obtained from BC3 compared with the samples treated with 1.25 M and 1.5 M solutions, which were larger than those of the samples treated with 0.5 M and 0.75 M solutions. Biocementing can increase the peak stress value of naturally remolded loess by up to 3.6 times. The UCS of different specimens may vary because q_u is governed by the propagation of the cementation concentration in the specimens.

To explore the relationship between calcium carbonate precipitation and q_u , calcite value was calculated for different cementation concentrations. After completing the UCS test, the loess samples from each group were subjected to acid washing to determine the calcium carbonate content in the microbially solidified loess samples. Calcium carbonate in natural soil is very limited, especially in remolded loess, and can be neglected. The mass of the dried MICP-treated



FIGURE 4: Stress-strain curves of different calcium sources.

sample was M1. The sample was soaked in a 1.0 M hydrochloric acid solution until the mass remained constant (M2), and the mass of calcium carbonate produced by the MICP-treated loess was M1-M2. q_u and calcium carbonate contents of the samples under different cementation concentrations are shown in Figure 6. It can be concluded that when the cementation concentration is 1.0 M, the calcium carbonate content of the MICP-treated loess reaches its peak, whereas when the cementation concentration exceeds 1.0 M, the calcium carbonate content decreases. Particularly, when the cementation concentration is larger than 1.5 M, the formation of calcium carbonate is inhibited; thus, the reinforcement effect is reduced. The q_u of the MICP-treated sample increased significantly as the cementation concentration increased from 0.5 to 1.0 M. When the concentration was 1.5 M, the strength of the sample decreased significantly, indicating that the sample strength was closely related to the amount of calcium carbonate produced. The maximum shear strength was obtained when the calcium content reached its peak. The maximum produced calcium carbonate percentage was 3% with the largest q_{μ} at a cementation concentration of 1.0 M.

3.3. Effect of Retention Time. Consolidated undrained compression triaxial tests were conducted to investigate the shear strength of the MICP samples. The confining pressures were set as 100, 200, 300, and 400 kPa at a shear rate of 0.15 mm/ min. The tests were ceased when the axial strain was greater than 15% or the deviatoric stress was stable. Figure 7 shows the stress-strain curves of the MICP-treated loess samples for different curing periods. The deviatoric stress-strain relationship of the samples cured for 0 d (Figure 7(a)) exhibited a steady increase in the stress value with strain. The MICP-treated loess samples exhibit increased axial



FIGURE 5: Stress-strain curves of different concentrations.

stress under undrained shearing conditions. This phenomenon may be attributed to an increase in the calcium carbonate content in the samples during the curing periods. In the initial stage of the treatment, the bacteria must survive and settle in the pores of the soil particles. With the urea hydrolysis process and injection of the calcium source, the mineralization reaction begins and calcite precipitation aggregates are generated to fill the pores. The pores between the particles began to bond from days 0 to 7 (Figure 7(b)). With a retention time of 14 d (Figure 7(c)), the loess samples considerably improved, indicating that the produced precipitation increased which cemented the loess particles and increased the compactness of natural loss, which is consistent with the existing literature [8]. The peak stress value increases by about 11%-22% when the retention time is 7 days, whereas the increasing rate is 7%-10% when the curing process lasts for the other 7 days. Considering the improvement in efficiency, the biocemented specimen can supply a desirable strength when cured for 7 days. The initial shear stiffness and ultimate shear stress of the MICP-treated loess were higher than those of the untreated specimens.

3.4. Improvement of the Shear Strength Parameters. Figure 8 shows the correlations between the shear strength parameter samples and cementation concentration of the MICP-treated loess soil. It can be concluded that the cohesion and internal friction angle reached their peak values when the concentration of the suspension was approximately 1.0 M. For example, the cohesion of the MICP-treated samples for BC1 to BC5 yielded values of 17.5, 43.0, 55.5, 32.5, and 23.0 kPa, respectively, which were much higher than the corresponding values (12.5 kPa) of the untreated samples. Improvement in internal friction angle was relatively observable, i.e., 35%, while the improvement in cohesion was considerable, i.e., 340% compared to untreated loess soil. It is expected that



FIGURE 6: Relationship between q_u and calcium carbonate content.

an improvement in the internal friction angle will lead to a more promising increase in the soil-bearing capacity. Thus, the applicability of the current study to loess soil is limited. More attempts on MICP-treated loess or in combination with other methods to improve loess strength should be conducted to broaden the application of the MICP method to loess soil. After treating the loess samples with relatively high cohesiveness, significant improvement in permeability was observed. In contrast, for the MICP-treated loess at all concentrations, the internal friction angle increased slightly and was also higher than that of the untreated loess.

3.5. Calcite Formation Process of MICP in Mesoscale. Calcium carbonate, which is a solid material, is the most common mineral produced during microbial-induced carbonate precipitation. Figure 9 shows the formation of MICP calcite in the loess samples under natural conditions. The surface of the sample was wetted only by bacterial and cement reagents on the initial curing day. The surface of the sample remained unchanged during the first five days, and calcium carbonate was distributed as white spots on the sample surface. From the 5th day of curing, slight calcification occurred on the surface of the sample, and calcium carbonate was locally generated. From the 9th day of curing, the distribution area of calcium carbonate crystals on the sample surface increased, indicating the increase in calcium carbonate content, the coverage rate was approximately 70%. From the 11th to the 14th day, the surface of the sample was almost 90% covered with white calcium carbonate precipitate. This may have been the main formation process of the MICPtreated loess. Although sand can provide a larger volume of pore passages for the bacteria and calcium reagent, bonding with the induced calcite wall is difficult. For loess soil, the relative sizes of bacterial cells and soil particles are small which provides sufficient cementation force. The microstructure of the MICP-treated loess investigation using scanning electron microscopy is presented in the following section.

4. Discussion

Based on the results of the shear tests, large differences existed between the MICP-treated and untreated loess in terms of shear behavior. Previous studies have shown that MICP treatment can effectively improve the shear strength of sand, and these differences have been delineated in previous studies [3, 6]. In this study, we found that the MICP treatment is also applicable to loess soil owing to its unique structural properties. It was also observed that there was slight particle crushing that strengthened with increasing external load under a given cementation concentration and curing period. However, as expected, the stress-strain relationship showed the same trend as that of the untreated loess. The morphology of CaCO₃ crystals, which can be influenced by numerous factors, was reported by Al Qabany et al. [10]. To explain the specific mechanism in the MICPtreated loess, its structural characteristics were studied by observing the microstructure using SEM. As a reference, the original loess particles and CaCO₃ crystals were observed to illustrate the skeleton of the MICP-treated loess. Scanning electron microscopic tests (SEM) were conducted to illustrate the skeleton of the MICP-treated loess as well as to observe the micromorphology of the loess samples enhanced by MICP treatment at various concentrations. Loess specimens treated with cementation concentrations of 0, 0.5, 1.0, and 1.5 M curing for 14 days were examined under a magnification of 10 k×, as shown in Figure 10. CaCO₃ crystal precipitation on the loess particles is indicated by the yellow lines. The SEM images in Figure 10(a) show smooth loess particles of the untreated loess sample (M_0) without any crystals on the surface. With the MICP treatment, with the participation of the cement reagent solution, CaCO₃ crystals precipitated on the loess particle surface, acting as a new material to enhance natural loess structures. CaCO₃ crystals accumulated around the loess particles and partially covered the loess grains surface which might fill the pores between



FIGURE 7: Stress-strain relationship of MICP-treated loess under various curing times.



FIGURE 8: Strength parameter variation in direct shear tests.

the loess particles (Figure 10(b)). When the cementation concentration was 1.0 M, $CaCO_3$ crystals gathered into clusters, which coated the loess particles and filled the pores between them (Figure 10(c)). For a larger cementation concentration of 1.5 M, the size of the $CaCO_3$ aggregate was sufficiently large to connect the pores and exhibited an obvious bridge effect (Figure 10(d)), whereas the amount of $CaCO_3$ crystals did not increase owing to the depletion of the mineralization reaction.

In summary, the $CaCO_3$ crystals generated during the MICP process can act as a solid material that can effectively fill the pores between the loess particles, thus improving the strength of natural loess. Moreover, this process can change the connecting mode of natural loess particles. These calcium carbonate bonds between the loess particles can prevent particle sliding during compression which means that a greater shearing resistance force can be provided. With an increase in the cementation concentration, the connection between



FIGURE 9: Calcium carbonate on the loess sample surface.



FIGURE 10: SEM images of the natural and MICP-treated loess samples.

soil particles transfers from point connection to surface contact, especially for a cement reagent concentration of 1.0 M. The produced CaCO₃ aggregate can coat and bridge the loess grains strengthening the loess skeletons to prevent external loading.

5. Conclusions

The effectiveness of the MICP treatment in improving the shear resistance and shear strength of loess soil was investigated under various conditions. The effects of the bacterial culture medium, pH, and calcium source were evaluated at the initial stage. Subsequently, the influence of the cementation concentration and curing period on the shear behavior of the untreated and MICP-treated loess soils was examined. The following conclusions were drawn.

- (1) A culture medium ingredient from the American Collection of Microorganisms with a pH of 8 was recommended for future cultivation because of its high bacterial activity. Calcium chloride has been proven to be the most suitable one for improving the strength of loess soil
- (2) The ultimate strengths of the MICP-treated loess increased as the cementation concentration increased from 0 to 1.0 M and showed a slight decrease with increasing concentration, as did the calcite content. The ultimate strength is 3.6 times compared to untreated loess specimens with a cementation concentration of 1.0 M
- (3) The deviatoric stress of MICP-treated loess cured for 14 days increased by 40% compared to untreated

loess. The cohesion showed a considerable increase of 340% and an internal friction angle of 35% compared to that of untreated loess

(4) SEM images indicate that CaCO₃ crystals were generated which can bridge and bind the loess particles. With the participation of CaCO₃ aggregates, the shear resistance of MICP-treated loess effectively improved

The abovementioned results were obtained in laboratory condition. When it comes to field application, various environmental factors can affect the test results. Subsequently, other complex variables must be considered for large-scale field applications.

Data Availability

All data and models generated or used during the study appear in the submitted article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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