

Microbial Induced Carbonate Precipitation (MICP) as Biotechnology in Applied Civil and Mining Engineering

Lead Guest Editor: Ling Fan

Guest Editors: Kang Peng, Shuquan Peng, Danial Jahed Armaghani, Ning-Jun Jiang, and Jian Zhou





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Review Article

Microbial-Induced Carbonate Precipitation: A Review on Influencing Factors and Applications

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Based on recent literary sources, this survey discusses the effects of main factors influencing the microbial-induced calcium carbonate precipitation (MICP), including the bacterial species, bacterial concentration, temperature, and pH value. While the MICP technology has been widely adopted to improve rock and soil characteristics, it has excellent development prospects in many other fields. The breakthrough solutions in the MICP technology are improving geotechnical and foundation sand properties, repairing cement-based materials, using mineralized film mulching to protect cultural relics, enhancing properties of tailings, desert control, and heavy metal environmental restoration, etc., are discussed. The experimental findings prove that MICP can improve the strength, stiffness, liquefaction resistance, erosion resistance, and permeability of geotechnical materials and maintain the good permeability and permeability of the soil and improve the growth environment of plants. It is an environment-friendly bioengineering technology. Because microbial mineralization involves a series of biochemical and ionic chemical reactions, there are many reaction steps in the solidification process and the solidification effect of MICP is restricted and affected by many factors. The comprehensive analysis and optimization strategy on MICP industrial implementation should account for micro- and macro-scale effects: the type of bacteria, the concentration of bacteria and cementation solutions, ambient temperature, pH value, and other factors directly affect the crystallization type, morphology, and size of calcium carbonate from the microscopic standpoint, while the macro-scale factors control the rock and soil mineralization. The limitations and prospects of the MICP technology are outlined.

1. Introduction

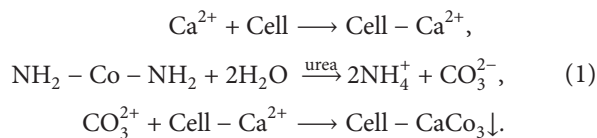
With the continuous development of the global economy, large-scale construction projects encounter geotechnical engineering problems such as weak subgrade, karst foundation collapse, soil slope landslide, embankment leakage erosion, and soil freeze-thaw cracking in alpine regions. Given these engineering diseases, traditional physical methods such as dynamic foundation compaction, soil cushion replacement, cement mixing pile setting, and other measures have a heavy workload, long construction period, and high cost. They need to support the development of large mechanical equipment, which cannot be used in some special cases. The chemical method is to pour the chemical

slurry into the target rock and soil. Because most grouting materials have strong alkalinity and biological toxicity, vegetation growth on the solidified rock and polluted soil becomes problematic, the environmental pollution is too large, and the application is limited in many cases.

Soil modification technology based on microbial-induced calcium carbonate precipitation (MICP) has attracted extensive attention in geotechnical engineering. The technology uses mineralizing bacteria in nature to induce mineral components (such as calcium carbonate, etc.) with a bonding effect to fill and repair cracks in stone materials and concrete materials, prevent building leakage, reduce the incidence of sandy soil liquefaction, and prevent soil erosion of sand/soil slope, sand dike erosion, piping, and other diseases [1, 2].

Compared with the traditional physical/chemical soil modification technology, MICP has more advantages in the transformation of rock and soil properties: its construction method is simple, it almost does not produce toxic and harmful substances, it has less chemical pollution to the surrounding soil water environment [3], the concentration of bacteria liquid and bonding liquid used for the modified object is low, compared with the traditional chemical slurry, it is easier to infiltrate in the rock and soil materials [4], and it can be used for large-scale treatment of rock and soil [5]. In addition, the durability of mechanical or hydraulic properties of rock and soil improved by MICP can be guaranteed while ensuring its strength [6]. Therefore, the soil modification technology based on MICP has good application scenarios and promotion prospects.

At present, the known microbial mineralizing bacteria mainly include urease-producing bacteria, sulfate-reducing bacteria, denitrifying bacteria, oxidizing bacteria, etc. Among them, urease-producing bacteria have been widely studied and applied due to their low cost, relatively easy extraction and cultivation, good mineralization and cementation effect, and easy control of the reaction process. The process of bacterial mineralization can be simplified as follows: in bacterial metabolism, the negative charge on the cell surface adsorbs Ca^{2+} from the environmental solution. After urea is added to the bacteria, the urease secreted by the cells decomposes urea to form CO_3^{2-} and NH_4^+ plasma, and Ca^{2+} combines with CO_3^{2-} to form calcium carbonate crystals on the cell surface (Figure 1). After a while, the whole cell was surrounded by calcium carbonate. After the bacteria became capsules, their living environment was limited, resulting in cell death. A large number of capsules formed a cemented structure, which played the role of bonding the microparticles in the geotechnical materials and filling the internal pores and cracks (Figure 2) [6, 8], and ultimately the performance of the geotechnical materials was improved. The main reaction equation is as follows [7]:



Among so many enzyme-decomposing bacteria types, *Bacillus* and *Sporosarcina* are the most selected bacteria in studies. These two kinds of bacteria have many advantages: strong adaptability to the environment, difficult aggregation between cells, the high specific surface area of cells, and they can use urea as energy and nitrogen source in metabolism. It can be transformed into CO_3^{2-} and rapidly deposit calcium carbonate under the condition of adding Ca^{2+} salt solution. Due to the fast and high yield of calcium carbonate produced by *Bacillus*, the advantages of bacterial mineralization and cementation process, these two kinds of bacteria are mainly used in MICP experiments and application projects [7, 8].

Because microbial mineralization involves a series of biochemical and ionic chemical reactions, the reaction steps in the curing process are complex. The curing effect of MICP is restricted and affected by many factors. Scholars

worldwide have experimentally derived qualitative and quantitative relationships between the crystallization process and effective calcium carbonate production. The roles of concentration and activity of bacterial solution, the concentration of cementation solution, pH value, soil particle size, and soil density in bacterial metabolism have been identified. These research results enabled the MICP reinforcement technology to safely, stably, and efficiently complete the reinforcement and transformation requirements of the target rock, soil, and geotechnical materials. This paper summarizes the most recent global research results on MICP concerning the key physical, chemical, biological, and abiotic factors influencing the microbial improvement of geotechnical materials. To provide some reference for the future theoretical research and engineering application of microbially reinforced geotechnical engineering materials, the account of these factors in the MICP optimization is discussed in detail.

2. Microbial-Induced Carbonate Precipitation (MICP)

Microbial-induced carbonate precipitation (MICP) technology has attracted global attention in the past two decades. Urease activity exists widely in bacteria, which is the most commonly used method to produce calcite by MICP. It is also one of the most important factors in research and application in the development of microbial cementitious materials. The importance of MICP and environmental protection has also been emphasized by Fujita et al. [9]. They studied microbial cement cementation of loose sand from three aspects: compressive strength, pore structure, and microstructure. In addition, the research on the repair of surface defects and cracks of cement-based materials by microbial cement featured two parameters of surface water absorption and compressive strength recovery coefficient. The research progress of microbial cement in repairing cemented loose particles, surface defects, and cracks of cement-based materials in China has been reviewed by Rong and Qian [10].

Calcium carbonate (CaCO_3) precipitation generally occurs through two different mechanisms: biological control and biological induction. In the process of biologically controlled mineralization, the nucleation and growth of mineral particles are mainly affected by organisms. Organisms synthesize minerals in their unique form, which has nothing to do with environmental conditions. Bio-induced mineralization refers to the production of calcium carbonate by bacteria. The type of minerals produced by this kind of mineralization process is mainly affected by environmental conditions. There is no special structure or specific molecular mechanism involved. Different types of bacteria and abiotic factors (salinity and medium composition) promote calcium carbonate precipitation in different environments in various interrelated ways [8, 11]. The schematic diagram in Figure 3 illustrates the relevant principles of the MICP technology.

Calcium carbonate can precipitate rapidly in all the bacterial culture experiments providing urea and calcium,

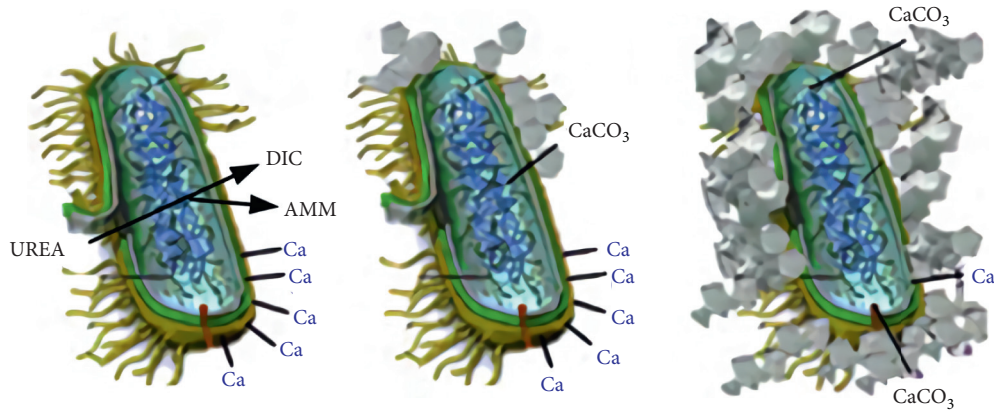


FIGURE 1: Schematic diagram of biomineralization of urease-producing bacteria [7].

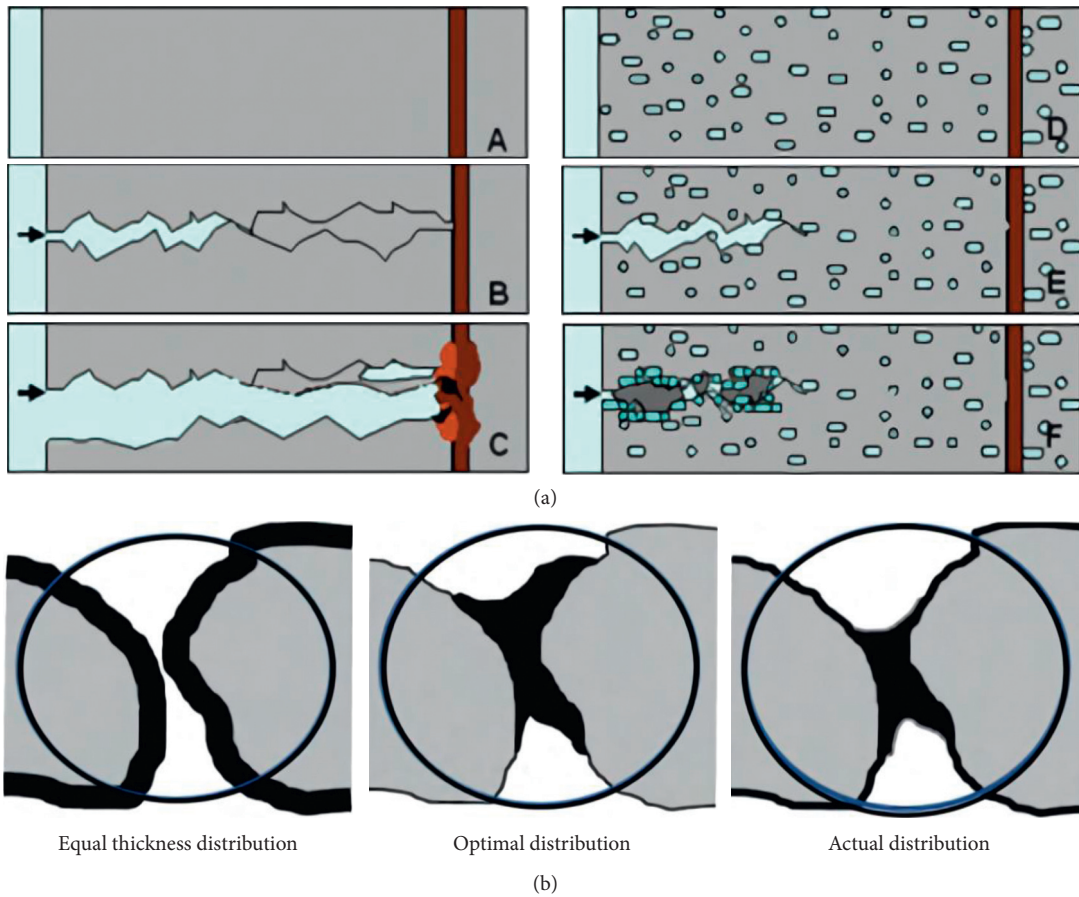


FIGURE 2: Schematic diagram of biomineralization principle of urease-producing bacteria. (a) Schematic diagram of fracture repair principle. (b) Schematic diagram of MICP solidification and cementation [6, 8].

and the polycrystalline carbonate calcite is always the main product of X-ray diffraction analysis. The relationship between calcium concentration and equilibrium prediction obtained by Fujita et al. [9] indicated that the precipitation rate of calcium carbonate was directly related to the hydrolysis rate of urea.

The effect of calcium sources on the MICP process is quite significant. Therefore, the effects of four different

calcium salts, calcium chloride (a), calcium acetate (b), calcium lactate (c), and calcium gluconate (d), on the formation of calcium carbonate crystals by *Pasteurella kctc3558* have been studied by Gorospe et al. [12]. While preliminary test results revealed that the addition of any calcium salt would harm the urease activity of *Pasteurella*, the difference in calcium carbonate precipitation morphology patterns was found to be related to the calcium salt used. Different

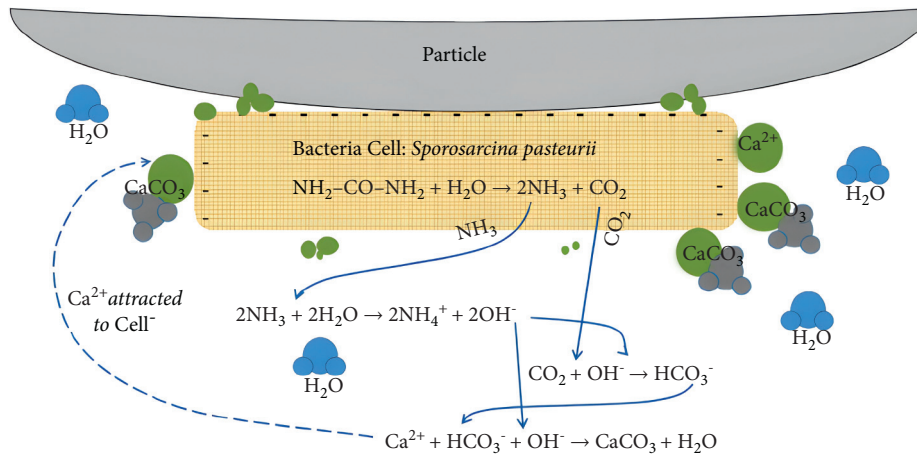


FIGURE 3: Schematic diagram of microbially induced calcium carbonate precipitation on the surface of particles.

calcium sources (calcium chloride, calcium oxide, calcium acetate, and calcium nitrate) were added, and *Bacillus CR2* was used for MICP. The experiment lasted for seven days, and the bacterial growth, urease activity, calcite yield, and pH value were measured. The results showed that calcium chloride has high urease activity and high calcite yield, being a good calcium source for the MICP process [13].

The effect of the environment on MICP was evaluated by soil column tests and intermittent tests [14]. Microbial growth and mineral precipitation were evaluated in fresh water and seawater. Environmental conditions that may affect bacterial urealytic activity, such as ammonium concentration and oxygen utilization, as well as the urealytic activity of living and lysed cells, were evaluated.

The treatment formula, injection rate, and soil particle characteristics are other factors to evaluate the influence of uniform cementation induction in the soil. The effect of ground conditions on MICP was analyzed in the study [15]. The effect of microbial cementation in treated sand and silt, silica sand with different relative densities, silt with different relative compaction, and weathered soil with different particle size distributions was tested. The microbial cementation degrees of sand, silt, and weathered soil samples were quantitatively analyzed by scanning electron microscope (SEM), X-ray diffraction (XRD), energy dispersive X-ray (EDX) spectroscopy, and mapping analysis. According to the test results, a considerable amount of microbial cementation is estimated according to the soil conditions. Therefore, implementing this new biological grouting on the soft foundation can improve the strength and stiffness of the soft foundation.

In the process of MICP reaction, Kitamura et al. [16] studied the controlling factors and mechanism of calcium carbonate polymorph crystallization by adding sodium carbonate solution to calcium hydroxide suspension. A series of research results showed that if the concentration of calcium ion was equal to or close to the solubility of calcium hydroxide, the low concentration of carbonate ion in the "diffusion field" around sodium carbonate solution droplets (low local supersaturation) was conducive to the crystallization of aragonite.

3. Key Influencing Factors of MICP

The conventional precipitation process of calcium carbonate is simple, being mainly affected by calcium ion concentration, pH value, and effective nucleation site [8]. However, MICP is a very complex biological and chemical process. MICP is mainly affected by the following key factors: (i) bacterial species, (ii) bacterial concentration, (iii) ambient temperature, (iv) pH value, (v) the ratio of cementation solution (calcium ion concentration), (vi) the nature of cemented materials: soil, sand, or others), and other factors. The precipitation of CaCO_3 needs enough calcium and carbonate ions to produce the required amount of precipitation for cementation.

3.1. Bacterial Species. The type of bacteria influences the crystal form, morphology, and deposition rate of calcium carbonate. The cementation effect of microorganisms on geotechnical materials is determined by different crystal types and the amount of calcium carbonate generated by MICP [17].

Therefore, it is of practical significance to study the mineralization characteristics of different types of bacteria for bacterial reinforcement and engineering applications. Different bacteria have different characteristics of calcium carbonate crystals. Mastering the mineralization characteristics of different bacteria can maximize the cementation effect of rock and soil in engineering applications. Scholars worldwide investigated the mineralization characteristics of different types of bacteria in recent years. Many valuable studies on the types and control of calcium carbonate crystals formed by mineralization, adaptability of different bacteria to the environment, and MICP expansion from laboratory conditions to the field have been performed to improve the urease production of bacteria. SEM images of carbonate crystals precipitated by different bacterial species in the research process have been obtained and analyzed by Dhami [18], as shown in Figure 4.

According to the production of urease, carbonic anhydrase, extracellular polymer, and biofilm, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus*

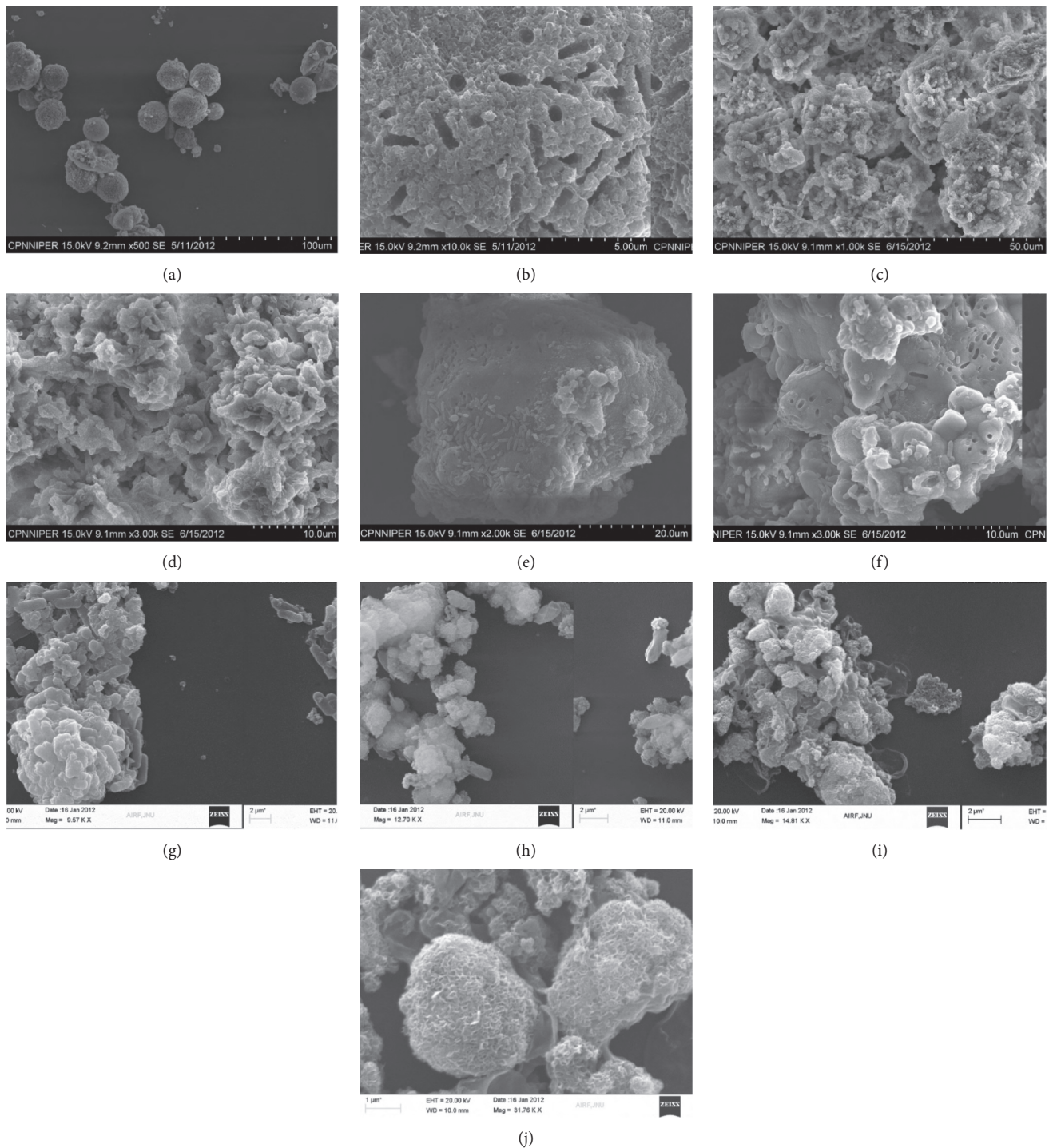


FIGURE 4: Scanning electron microscopic images of carbonate crystals precipitated by (a, b) *B. megaterium* (Bm), (c, d) *B. cereus* (Bc), (e, f) *B. thuringiensis* (Bt), (g, h) *B. subtilis* (Bs), and (i, j) *L. fusiformis* (Lf).

subtilis, and *Clostridium* were isolated from calcareous soil. All bacterial cultures were incubated in niobium medium with urea (a 2% concentration) and 25 mM calcium chloride (pH=8.0) at 37°C for three weeks. Among the isolates, *Bacillus megaterium* showed the highest urease activity (690 U/ml), followed by *Bacillus thuringiensis* (620 U/ml), *Bacillus cereus*, *Clostridium*, and *Bacillus subtilis*, which

produced 587, 525, and 515 U/ml on day 5, respectively [18]. *Bacillus megaterium* plays an important role in repairing building materials, soil reinforcement, heavy metal recovery, and surface protection of cement and concrete materials. At the same time, it can also degrade the insoluble phosphorus compounds in the soil, making them soluble substances that crops can absorb. When *Bacillus megaterium* and *Bacillus*

sphaericus were cocultured, they exhibited the effects of nitrogen fixation and synergism, which were very suitable for making microbial fertilizers.

The culture experiment of Okwadha and Jin [19] on *Sporosarcina pasteurii* strain ATCC 11859 showed that the decomposition rate of urea positively correlated with the bacterial cell concentration. Within their respective economic advantages, urea concentration, calcium concentration, and bacterial cell number were directly proportional to the amount of CaCO_3 deposition. According to the experimental analysis, the main components of calcium carbonate were calcite crystals and a small number of aragonite crystals. The above study confirmed the quantitative effect of bacterial plugging on the hydraulic conductivity of porous media. The amount of CaCO_3 precipitation was directly proportional to the amount of bacterial cell deposition. The number of depositing cells determined the relative reduction rate of hydraulic conductivity.

The number of depositing cells was affected by the density of bacterial cells introduced into the bacterial suspension [20]. The phenotypic mutant of *Bacillus pasteurii* (MTCC 1761) was developed by ultraviolet irradiation. The calcite produced by this mutant through biomineralization was very active. The screening and cultivation of mutant strains could provide a more effective strategy for plugging the gaps in building structures [21]. The relevant experiments have been carried out to find microorganisms with satisfactory performance in various environmental conditions (not limited to laboratory temperature). Among all the tested ureolytics, *Bacillus sphaericus* was the most suitable for biodegradation in practice [22]. Urease activity is an essential factor in the process of MICP. Using immobilized enzymes instead of whole immobilized cells provided a better repair effect, according to Bachmeier et al. [23].

The screening and cultivation of mutant strains may provide a more effective strategy for plugging the gaps in building structures. This is also a typical case of cross-application of biotechnology, which has a good development prospect. It is also the key direction of cross research between geotechnical engineering and biological science.

3.2. Bacterial Solution Concentration and Cementation Solution Concentration. Bacteria are important for the formation of calcium carbonate crystals in the process of MICP. Tournay and Ngwenya [24] reported that bacterial extracellular polymeric substance (EPS) positively affected the crystal type and morphology of CaCO_3 precipitation, which was vital for the cementation of geotechnical materials. EPS could inhibit the precipitation of aragonite and promote the precipitation of calcite, which was beneficial to the MICP process.

The number of microorganisms in a mineralized environment strongly impacted the concentration and supersaturation degree of Ca^{2+} , CO_3^{2-} plasma [25, 26]. In the experiment of culturing *Bacillus Pasteurella* ATCC strain 11859 at 25°C performed by Okwadha and Jin [19], the ionic strength was proportional to the concentration of urea, calcium, and bacterial cells. Bacterial cell concentration on

urea decomposition rate exceeded the initial urea concentration.

The concentration of the bacterial solution was found to strongly influence the morphology of calcium carbonate crystals [27]. Most calcium carbonate crystals were spherical or nearly spherical at a 100% concentration of the bacterial solution. When the bacterial solution concentration was reduced to 50%, some crystals had irregular shapes, while others appeared as regular cuboids or aggregates. When the concentration of the bacterial solution was further reduced to 25%, the share of regular cuboids or aggregates in the crystals further increased; in a pure aqueous solution, the crystals were orthorhombic hexahedral single crystals, twins, and their aggregates. The crystal morphology at different bacterial concentrations is shown in Figure 5.

The comparative tests of two bacteria concentrations, two culture media, and six cement solution concentrations showed that the composition of culture medium had no direct effect on MICP efficiency, while the type and concentration of cement solution affect the strength of mortar [25].

In the design and implementation of MICP, the concentration of cementation fluid was found to play an important role [28, 29]. Compared with high-concentration cementation fluid, low-concentration one produced more uniform and stronger crystals. However, the latter operation process needed more steps, time, and costs than the former one. Given the engineering nature of soil improvement, more simple and effective methods need to be further explored.

Al-Thawadi and Cord-Ruwisch [30] revealed a positive correlation between the average particle size and the cell concentration, while the urease activity increased with the cell concentration. The precipitated crystal grew with the carbonate production rate, so the bacterial concentration was reported to be the key parameter controlling the crystal size. In practical application, it may be more advantageous to increase the concentration of bacteria in coarse sand. The average size of the sphere increased with the concentration of the cementation solution (urea and calcium ions). At a concentration of the cementation fluid of 10–250 mM, the spherical size increased sharply; when the concentration exceeded 10–250 mM, the spherical size growth became saturated. Therefore, the optimal concentration of bacteria liquid and cementation liquid was considered beneficial for improving the cementation strength and maintaining good air permeability.

3.3. Ambient Temperature. The change of environmental temperature will directly affect bacteria's growth and metabolic activities, thus changing the deposition rate and yield of calcium carbonate, changing the type and morphology of crystals. The change of temperature will affect the crystal size and the cementation mode of purpose calcium carbonate between soil particles, which will directly affect the effect of microbial mineralization and cementation. Therefore, it is necessary to study the effect of temperature on MICP activity [19, 31]. Studies have shown that the catalytic activity of

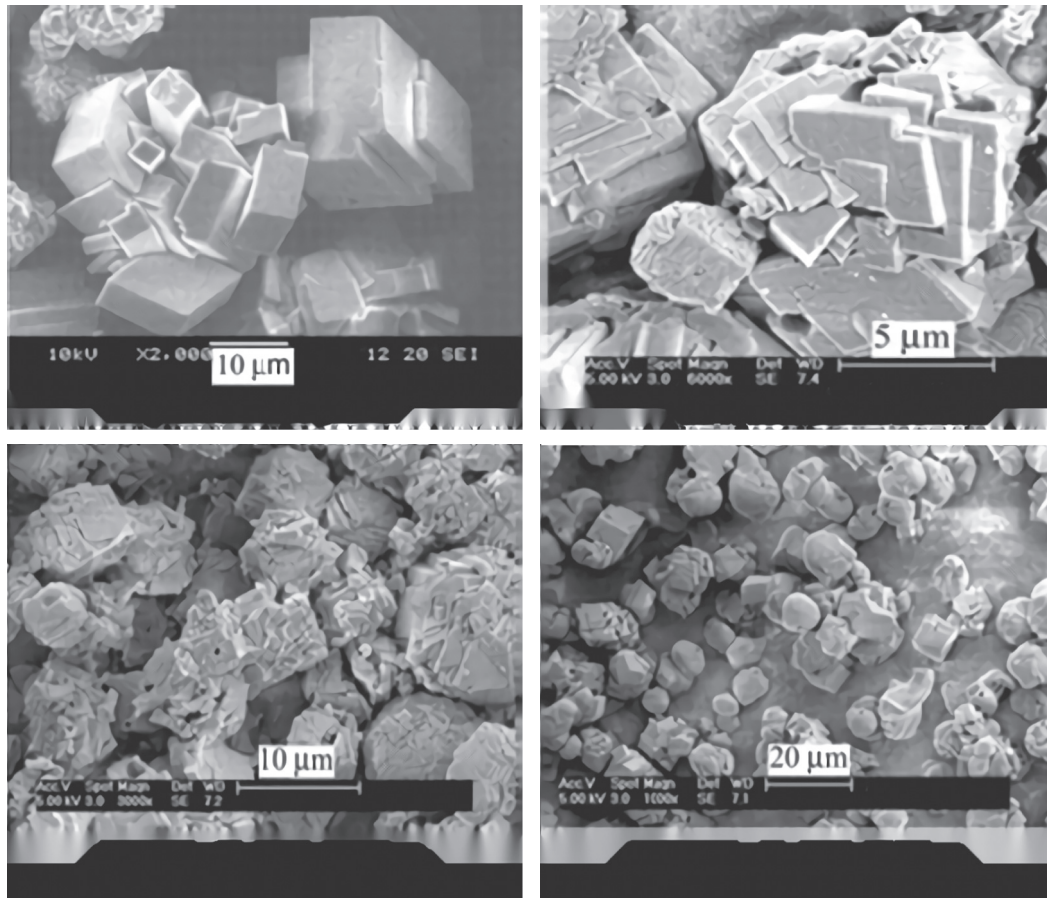


FIGURE 5: SEM of crystalline CaCO_3 at different bacterial concentrations (20°C) [27].

urease was the strongest at $20\sim 37^\circ\text{C}$ [19]. In this temperature range, intrinsic to most MICP studies, the urease activity increased with temperature. However, the successful practical application should envisage the bacteria activity adaptation within a wider temperature range.

In the MICP investigations [2, 32], the temperature effect on the biodegradation performance of different urealytics, including the reinforcement and protection effect of the treatment, the growth, and urealytics activity of urealytics at different temperatures (10 , 20 , 28 , and 37°C), was analyzed. The results showed that *Bacillus sphaericus* was the most suitable for biodegradation under various environmental conditions among all the tested microorganisms. The effect of temperature on the morphology and quantity of biocrystals produced on limestone with different urealytic microorganisms was investigated by scanning electron microscopy, as shown in Figure 6 [22].

3.4. pH Value. The pH value plays an important role in the metabolic activity of urease-producing bacteria, calcium carbonate deposition, and improvement of geotechnical properties. Most urease bacteria commonly used in microbial mineralization are suitable for growth in an alkaline environment. For example, the suitable pH value of *Bacillus* is about 9.0. An economic growth method for the large-scale culture of *Pasteurella* has been developed [33]. The organism

was moderately alkalophilic, with the optimum pH for growth of 9.25. Under nonsterile conditions, sufficient bioagglutination activity could be cultivated through minimal upstream and downstream processing. The production cost was reduced by 95%. In the new medium, a high level of urease activity (hydrolysis of 21 mmol urea for 1 minute) was produced at a low cost (0.20 USD per liter), which was vital for the field application of MICP.

Since many engineering applications need to be implemented in an acidic environment, it is of practical significance to study the bacterial activity in different pH ranges. Recently, a scientific research team [34] used the MICP technology to study the mechanical properties of iron tailings under low pH conditions. Compared with the conventional solution, the calcium carbonate content of the *Sporosarcina pasteurii* (DSM 33) strain increased first and then dropped when the fluid pH changed from 4.5 to 6.5 under the condition of 1×10^8 cells/ml bacterial solution, calcium carbonate content reached the maximum at pH 6. Compared with traditional cementation, the spray method using low pH bacteria solution could make the adhesive more uniform, with a better bio-coagulation effect. The results showed that this method was especially suitable for the solidification and dust suppression of iron tailings sand and the reinforcement of tailings pond, providing guidance for applying the MICP technology in acidic environments.

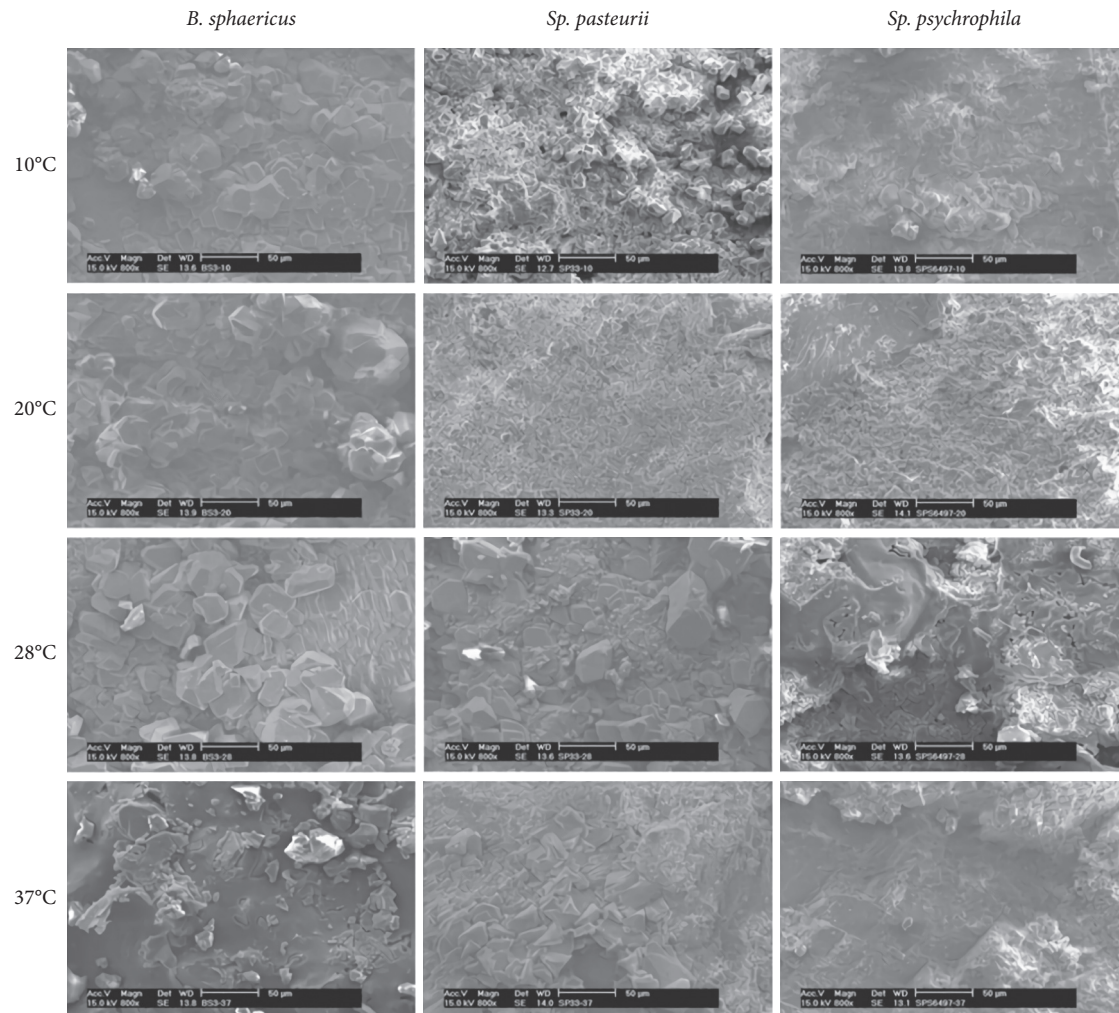


FIGURE 6: SEM images of different types of crystal precipitation of urealytics at different temperatures [22].

Other studies have shown that the increased pH leads to increased carbon dioxide demand of growth medium [35]. This phenomenon may be helpful for MICP technology to absorb carbon dioxide and reduce the greenhouse effect.

In the research process, some scholars adjusted the pH value of the environment from 9.0 to 8.0, and white precipitates were successfully generated with the introduction of calcium ions. The XRD analysis showed that the main component of precipitates was still calcite. Still, the microstructure and morphology of particles changed significantly, including petal shape and square shape, and the size of particles changed little, as shown in Figure 7 [36].

Studies on the MICP application at different pH values are conducive to promoting and implementing this environmental-friendly technology.

4. MICP Application Scenarios

4.1. Experimental Study. Microbial mineralization and cementation are complex and multidimensional processes that involve biological and nonbiological factors, organic-inorganic factors, and solid-liquid-gas coupling factors. At present, most of the research is still limited to laboratory

research. Large-scale and effective practical application research still needs more attention and effort.

Some studies have shown that the overall mechanical properties of concrete were improved by adding the *Bacillus subtilis* cell wall [37]. The traditional low strain rate indoor pressure test is difficult to study the threat of dynamic disturbance to the cemented backfill. Some scholars [38] studied the high strain rate compression behavior of the cemented backfill under different dynamic loads by using the split Hopkinson pressure bar (SHPB) and determined the failure mode of the specimen through macro and microanalyses. There is little research on the related aspects of microbial cemented backfill. In the process of bacterial mineralization, calcium carbonate was formed by Ca^{2+} ions and dissolved CO_2 . The addition of bacterial cell walls increased the carbonation of calcium and the formation of calcium carbonate in concrete. After experimental treatment, the compressive strength of concrete increases by 15% and the porosity decreases after 28 days of curing. The *in vitro* calcium carbonate precipitation tests showed that bacterial cell walls, rather than dead cells, accelerated the carbonation of calcium ions in calcium hydroxide solution. Calcium carbonate filled the microvoids in concrete, thus

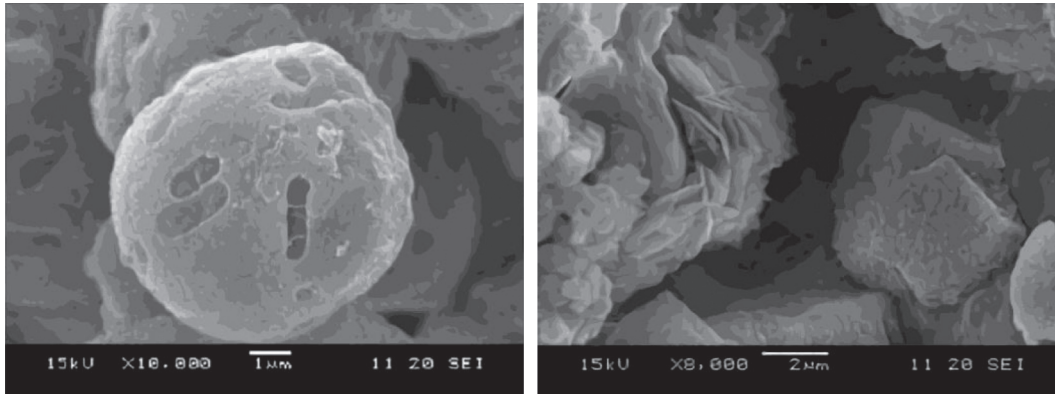


FIGURE 7: SEM images of CaCO_3 deposited by microorganism under different pH conditions [36].

optimizing the MICP process. Thus, the bacterial cell wall was a promising additive in improving concrete's mechanical and carbonation properties.

Erşan et al. [39] revealed that nitrate reduction improved the crack closure performance of microbial mortar. Through the experiment of nitrate-reducing bacteria and two different porous protective carriers, at the end of 56 days, the crack water tightness of the test block was restored to 85%, indicating the self-healing of concrete cracks. As long as there were nutrients in the cracks, the microbial activity continued and might heal larger cracks.

The research [40] proved that different repair methods and calcium source types have significant differences in the repair effect of concrete cracks. The microbial self-healing technology with calcium glutamate as a calcium source had more significant advantages. Thus, Yunus et al. [41] reported that after adding a bioremediation agent to the mortar mixture, the pore volume of mortar increased, and the bio-based self-healing mortar had no obvious effect on the development of compressive strength at the age of 28 days. In a series of healing ability tests, after incubation in a water bath for 28 days, the cracks of bacteria-based mortar were healed, which improved the impermeability of bacteria-based mortar.

In the study [42], bacterial spores were first wrapped in hydrogels and then incorporated into specimens to form self-healing to study their healing efficiency. The hydrogel-encapsulated spore specimens showed obvious self-healing advantages: the maximum healing crack width was about 0.5 mm and the permeability decreased by 68% on average. The maximum healing crack width of other samples of nonbacterial series was 0–0.3 mm; the average permeability is only reduced by 15–55%. The efficiency of self-healing needs to be combined with the actual application environment.

Designing appropriate self-healing efficiency technology and evaluation method in various natural environments is very important. Biological composite cement (bacteria solution, urea/magnesium chloride mixture) was found to bind the loose sand into a biological sandstone, according to [43]. The injection times of bacterial solution had a strong effect on biological sandstone's mechanical properties and porosity. The compressive strength of biological sandstone is

positively correlated with the injection time. With an increase in the content of biological composite cement, the pores of the sand column were filled more intensively, while the final defect volume was reduced. The XRCT analysis revealed that the average hardness of solidified natural sandstone was $4.4 \text{ g/m}^2/\text{h}$, while the wind erosion rate was zero.

In addition to these experimental findings, other authors attempted to improve sand properties via the MICP approach. The research [25] confirmed the importance of properly selecting microbial species to enhance mortar's compressive strength. The growth medium had no obvious effect on bacteria, while the type of matrix solution and its molar concentration significantly affected the strength of mortar. The SEM analysis implied that due to the bacterial mineralization precipitation, the fiber material in the pores grew, enhancing the mortar strength.

With the participation of bacteria, the mortar strength was improved by increased porosity and more compact pore size distribution of the cement mortar. The cementation effect of MICP on the sand at different saturations was studied [44], who reported that high soil strength could be obtained at certain calcium carbonate contents. Rong and Qian [10] examined the microbial cementation of loose sand from three aspects: compressive strength, pore structure, and microstructure. Different experimental variables were explored, including soil type (residual tropical soil and sand), soil density (85, 90, and 95% of their respective maximum density), and treatment conditions (untreated, treated with cement only, treated with *Bacillus megaterium* only, and treated with *Bacillus megaterium* and cement). The results show that MICP effectively improved the shear strength of residual soil and sand, reducing the hydraulic conductivity.

On the other hand, the improvement effect varied with soil density, soil type, and treatment conditions, according to [45], who conducted unidirectional flow tests in a sand column to study the range and distribution of filled pore space under different injection strategies. Compared with the simultaneous injection of *Sporosarcina pasteurii* and cementation solution (parallel injection), when *Pasteurella* is injected first, and then cementation solution is injected (phased injection), the precipitation formation and

distribution were more uniform. In the study [46], MICP-treated sandstone was prepared for the first time. The X-CT technology revealed that the microstructure of natural sandstone became dense with age, the internal defects gradually decreased with time, and the MICP growth rate in sandstone gradually dropped with the decrease of pore size. Different biological agents and cations were used to treat the sand to improve the effect of MICP-based biological cementation. These data can be used as reference values for geotechnical applications, such as biological blocking to reduce sand permeability and biological cementation to improve soil shear strength.

Some studies have confirmed that denitrification-induced carbonate precipitation can also be used for soil improvement and foundation reinforcement [47]. However, because the precipitation rate of carbonate produced by the denitrification method was much lower than that by the urease method, the respective methods need to be further optimized [48].

The process of biological grouting was schematically presented by Yu et al. [49], as shown in Figure 8.

The eventual aim of biomineralization research is field application. Scale-up experiments have been conducted to demonstrate the feasibility of process application (VAN PAASSEN LA 2009). To evaluate the potential of bio-grout in field application, test volumes of 1 and 100 m³ were used to simulate a three-dimensional environment, which was easily controlled and monitored, under the conditions and injection techniques similar to those envisioned in practice, as shown in Figure 9.

In the 100 m³ experiment, the fluid was injected in batches through three water injection wells and pumped out by three pumping wells located at a 5 m distance from the other side. The flow rate was about 1 m³ H⁻¹, and the hydraulic gradient was 0.3 m M⁻¹. One day after fluid flushing, geophysical measurements (shear wave propagation from the top to the bottom of the cement) revealed a significant (almost tenfold) increase in the average stiffness around the injection point. The artificial cone penetration test proved that after several days of scouring, the cone resistance around the water table exceeded 5 MPa.

4.2. Technology Application. MICP technology has been instrumental in restoring and protecting historic buildings and sites [50, 51]. A 100 μm-thick calcium carbonate film was deposited on the surface of cement paste after seven days' treatment. The capillary suction coefficient of cement paste was reduced by 90% by brushing bacteria solution on the agar surface. Compared with the spraying method, the brushing method ensured higher urease activity for a long time. When the fine sand, urea, calcium ions, and concentrated bacterial solution were injected into the artificial crack of cement paste, the bacteria produced crystal precipitation under the continuous supplementation of nutrients. The compressive strength of the specimens was restored to 84% of the initial strength by precipitation crystals, indicating that the bioremediation of surface defects of cement-based materials was quite effective.

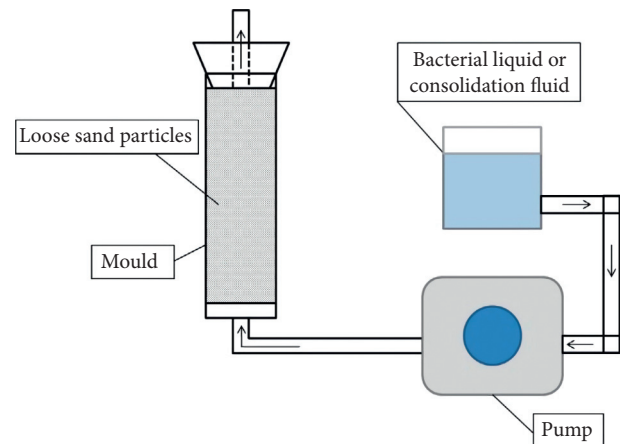


FIGURE 8: Schematic diagram of the bio-grouting process [49].

The research [52] focused on the durability of cementitious materials formed during the MICP action. The biological deposition treatment improved mortar samples' carbonation, chloride ion penetration, and freeze-thaw resistance values. The coating system could reduce the permeability of cementitious materials and improve the durability of the treated interface. Some scholars have proposed a microbial precipitation protection method for calcareous weathered stone carvings. This method did not introduce foreign substances into the internal porous network of the protection object, which was suitable for protecting marble statues and works of art [53]. Some experiments used noninoculated culture media to activate natural microbial colonies in quarry limestone and consolidate them in porous media without blocking pores. This may be more suitable and safe to protect ornamental stones. Jimenez-Lopez et al. [54] reported that the bacterial enzyme digestion method could greatly increase cement test block's surface impermeability and acid and alkali resistance. Adding the best concentration of calcium ions before adding urea and bacteria could play to the sedimentary layer's best protection effect, with a good application prospect in the surface protection of building materials [55].

The unconfined compressive strength of the two kinds of soil after being treated with MICP technology was significantly improved (by 1.5–2.9 times) and the soil strength increased with the treatment time. Compared with other soil improvement technologies in practice, the cost of the microbial agent was relatively low. As an additional advantage, MICP was a green, stable, sustainable, and eco-friendly emerging technology with broad prospects [56]. Some research methods have reduced the production cost by 95% and produced a high level of urease activity (hydrolysis of 21 mmol urea per minute) at a low cost (0.20 USD per liter) in the new medium, which was encouraging and guiding for MICP to go out of laboratory research and enter field application [32]). When the mixture of urea and seawater was used to treat the sandy soil through porous sand instead of a high concentration of calcium and urea solution, the UCS was increased twice under the condition of producing the same number of crystals. The test sample strength increased, while the permeability was maintained by a 30% level,



FIGURE 9: A wide range of experimental devices for biological cementation (1 m^3 and 100 m^3) [32].

indicating good drainage capacity. This new exploration of MICP technology provided great potentials for applying biocementation technology in the marine environment, such as slowing down the liquefaction of seabed sediments, preventing beach sand erosion and cliff erosion, etc. [57, 58].

In vivo soil engineering is a new interdisciplinary method, which regards soil as a living, active, and biochemical system capable of providing sustainable solutions. The proposed framework and method of this technology, as well as the relationship and interaction between these different components, are shown in Figure 10 [59].

Experiments and numerical simulations, including timely process monitoring, have made significant progress in understanding and controlling MICP at all length scales. Some researchers carried out 720 UCS tests under different levels of factors. They collected the strength evaluation database and verified the feasibility of the prediction model [60]. If a similar model can be established in the performance research of microbial cemented backfill, the experimental process will be greatly optimized, and the research efficiency will be improved. It is very promising that MICP can be applied to improve soil mechanical and hydraulic properties, immobilize heavy metals, and sequester atmospheric carbon in the future [59]. As early as 1987, some scholars put forward the possibility of bacteria as the nucleation sites of authigenic minerals to control metal pollution. Forming a gelatinous matrix around the granular matter in structure and chemical composition was found conducive to bacteria cementing and fixing metal ions [61]. A possible long-term remediation strategy for groundwater pollution in studying pollutant calcite coprecipitation with atcc11859 bacteria was outlined by [62]. The enrichment experiments of indigenous microorganisms capable of internal soil engineering hydrolyzing urea in the presence of calcium chloride were carried out under laboratory and field conditions. The results showed that when the soil was treated with nutrients, CaCl_2 , and urea, a large amount of calcite would be precipitated in the soil depth by indigenous microorganisms, which greatly enhanced their ability to resist earthquake liquefaction. The indigenous microbial enrichment process could be used in the field on a large scale because it required no cultivation of foreign bacteria, and the technology cost was lower. Using local bacteria to induce calcite precipitation to improve soil

was less invasive to owners and less toxic to the environment [63]. The local microbial colonies were activated to cement the porous media in the quarry limestone without introducing new inoculated bacteria while maintaining a certain permeability [54]. Burbank et al. [63] conducted cone penetration tests and cyclic triaxial shear tests using natural primary bacteria to induce calcite precipitation. The results showed that primary bacteria could significantly change soil engineering properties, improve sand liquefaction resistance, and have economic advantages over exogenous bacteria.

The bacteria were permanently adsorbed on the sand by injecting the bacteria solution into the sand body first and then the fixed solution (i.e., the solution with high salt content). This method could prevent the blockage in the process of injection, the reinforcement effect was more uniform, and the effect and efficiency of foundation reinforcement were improved [64]. Reservoir permeability was the key factor of water drive oil recovery. Bacterial precipitate crystal was a temporary plugging agent, which could permanently block and significantly reduce the permeability of porous media. The successful application of MICP technology in the water-driven oil recovery industry needed further screening and domestication [65]. As early as 1996, some scholars proposed using microbial precipitation as a plugging agent and cementing agent for porous media. This process could be used to exploit underground reservoirs and cement loose sand and control the flow of pollutants in aquifers [66]. By introducing the development opportunities of MICP in new interdisciplinary fields such as microbiology, geochemistry, and civil engineering, the publication [67] outlined the advantages of biological mediated soil improvement, evaluated the change of pore space volume and distribution characteristics in soil by calcite precipitation through microtechnology, and analyzed the advantages and restrictions of the MICP technology in terms of microbial and soil size compatibility. The mature application of MICP technology can largely cement and improve the structure of the loose sand layer. With the maturity of this “green” technology, new possibilities could be created in soil improvement, including the treatment of liquefiable sand layer, underground pretreatment before tunnel excavation, reduction of building settlement, and stability of the dam,

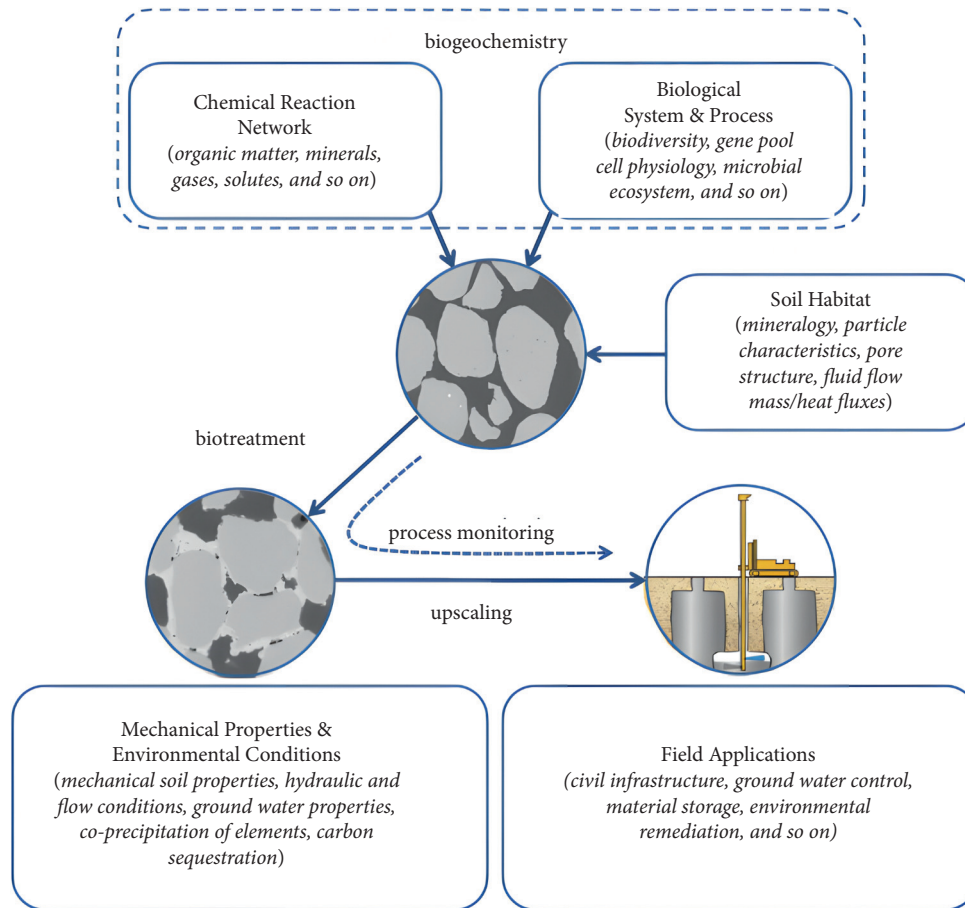


FIGURE 10: Schematic diagram of the method framework of "internal soil engineering." A more in-depth representation can be found elsewhere [59].

embankment, and slope [7]. Some scholars have studied that after the gas pipeline is corroded underground, it is more obviously affected by the vibration of blasting engineering [68]. According to the research results of the above authors, the quality of underground soil will be greatly improved after microbial modification. Whether this operation can have a good effect in resisting blasting vibration is also worth studying. The bacteria will be permanently adsorbed on the sand by injecting the bacteria solution into the sand body first and then the fixed solution (i.e., the solution with high salt content). This method can prevent blockage in the process of injection, ensure a more uniform reinforcement effect, and enhance the efficiency of foundation reinforcement [64].

Research efforts have also been focused on establishing a mathematical model to predict the simple biogeochemical reaction migration consistent with the experimental data. Thus, such a model elaborated for half-meter one-dimensional sand column quite accurately reflects the changes in the micropollutant treatment scheme and provide a fine-control idea for microbial soil improvement [69, 70]. The MICP technology has the advantage of green ecology in the aspect of calcium carbonate deposition. The scientific community speculates whether the MICP repair technology can be used based on the research on the mechanical

properties of rock mass, in the aspect of rock mass damage and strength recovery after damage, and then in the aspect of mine vibration reduction after rock mass damage repair.

Figure 11 shows the active process of MICP in self-repairing concrete, the process of dust suppression, the application scenario, and the feasibility study prospect of desert control.

The metabolic activities of bacterial cells can effectively improve the geo-mechanical properties of sand. Growing cells improve the properties of sand, while dead cells and resting cells usually lead to a small increase in friction angle and bearing strength. Using MICP technology in low saturation formation can obtain higher soil strength under similar calcium carbonate content. This important finding shows that this stabilization process can achieve the best performance at a lower cost. This process reduces the demand for water and chemicals, making it more economically viable. Therefore, the technology will likely become more environmentally friendly than previously thought sustainability [44, 71].

In the study of [72], *Bacillus megaterium* was used to trigger calcite precipitation. The results showed that the engineering properties of typical tropical residual soil treated by MICP were similar to those treated by fine sand; the shear strength and hydraulic conductivity increased by 69 and

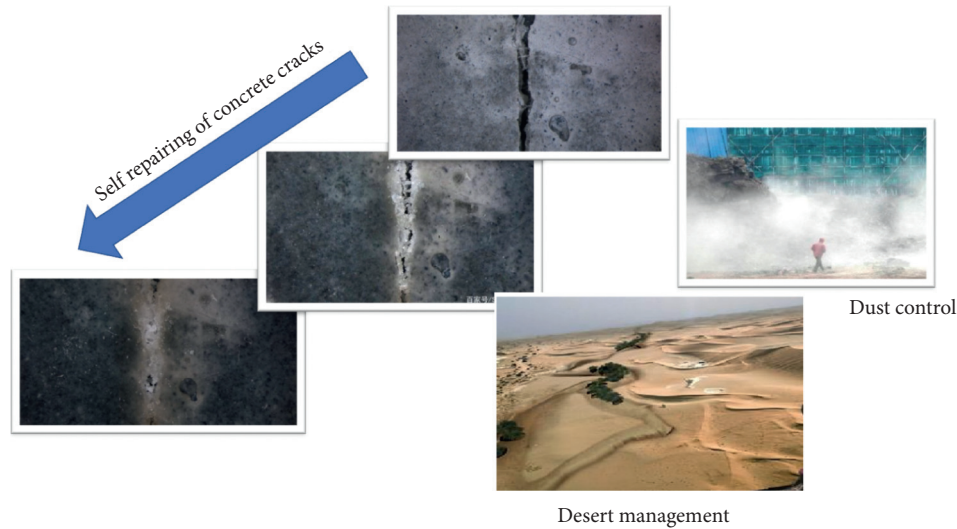


FIGURE 11: Application scenarios of MICP technology.

90%, respectively. The performance of sand changed from “soil-like” to “rock-like” with the improvement of treatment level. The dynamic test results [73] show confirmed that the antiliquefaction ability of MICP-treated sand significantly exceeded that of the untreated loose sand.

5. Discussion and Technology Prospects

This review covered the current development of MICP technology and the prospects of different application approaches. As a new interdisciplinary technology with good environmental adaptability, MICP technology has developed rapidly in the past two decades. Despite some field applications, the wide implementation of MICP was limited by several problematic issues. To mitigate them, the following tasks need to be properly formulated and resolved via innovative technologies:

- (1) Reducing the cost of technology is still a key factor for the large-scale application of biomineralization engineering. At present, the cost of MICP technology is relatively high, which hinders the large-scale application.
- (2) The treatment of by-products (such as ammonia) in the process of biomineralization has not been reasonably solved. The promotion of this technology should avoid generating harmful substances to the environment (e.g., ammonia). Meanwhile, some studies show that the biomineralization process may absorb carbon dioxide, reducing the greenhouse effect [35].
- (3) It is very difficult for geotechnical engineers to obtain strains that meet the actual needs of professional genetic transformation technology. It needs the strong support of biological process professionals. Interdisciplinary cooperation should be closer in the future.
- (4) There are huge differences in available application scenarios, which lead to the lack of a unified and

effective implementation strategy. The domestication of local bacteria is considered a breakthrough solution [63]. This approach can avoid polluting the local environment with foreign bacteria and reduce construction costs.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Damping Properties and Microstructure Analysis of Microbial Consolidated Rubber Sand

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Up to now, there are few reports on the application of microbial-induced calcium carbonate precipitation (MICP) consolidated rubber sand. By means of uniaxial or cyclic loading test and SEM test, the consolidation effect of rubber sand samples with different rubber particle content after MICP consolidation is tested and analyzed. The results show that MICP is not affected by the amount of rubber particles; rubber particles improve the compressive strength and deformation ability of consolidated rubber sand samples and significantly enhance the damping ratio, resistance to deformation, and energy dissipation ability of consolidated rubber sand samples. Rubber sand after MICP consolidation is a good shock damping material. The conclusion of this paper provides reference data for the application of microbial-induced calcium carbonate precipitation consolidated rubber sand.

1. Introduction

Earthquake, mechanical vibration may lead to the vibration destruction of construction [1, 2], and waste tyres will cause serious environmental pollution. It is one of the main ways to recycle waste tyres by crushing them into rubber particles and then used in civil engineering. Mixtures of small-sized rubber and sand have important applications in geotechnical engineering [3, 4], and a study has shown that rubber sand is a suitable and cheap damping material: Tsang [5] studied using rubber sand to replace foundation soil; when the maximum dynamic shear modulus of rubber sand cushion is 7.5 MPa and the thickness is 10 m, the peak value of horizontal acceleration of superstructure is 75% lower than that before replacement, and the peak value of vertical acceleration is 90% lower than that before replacement; Panah and Khoshay [6] filled the rubber sand into the pipe pile, and the lateral static load test, free vibration test, and forced vibration test are carried out, respectively. The test results show that the pipe pile has good deformation ability and damping characteristics.

The microbial-induced calcite precipitation is a common biomineralization process widespread in nature, which has been widely used in geotechnical engineering [7]. Studies have shown that MICP is an effective biological consolidation technique to improve the deformation resistance and enhance the impermeability of consolidated sand samples: DeJong et al. [8] studied the shear strength of the MICP consolidated sand samples; the test results show that the shear strength of the sand samples is significantly improved; Li et al. [9] found that MICP can reduce the porosity and permeability of consolidation sand samples and increase the unconfined compressive strength; Liu et al. [10] studied the dynamic characteristics of calcareous sand after MICP consolidation, and it is found that the dynamic shear stress ratio and the ability to resist deformation of calcareous sand after MICP consolidation are obviously improved.

At present, there are few reports on the use of MICP consolidated rubber sand. A single-axis or cyclic loading test of MICP consolidated rubber sand samples with different rubber particle content was carried out in this paper. The test results and microscopic analysis provide reference data for the application of MICP consolidated rubber sand.

2. Testing of Raw Materials and Methods

2.1. Testing Raw Materials. The CASO⁺ Urea Medium was used to activate *Sporosarcina pasteurii*, and the pH value of the medium was adjusted between 7.3 and 7.8 with sodium hydroxide standard solution, and the growth curve of *Sporosarcina pasteurii* in the medium was measured (Figure 1). As shown in Figure 1, OD600 bacterial concentration increased and finally reached a plateau with the increase of duration. There were 15 g casein peptone, 5 g soybean peptone, 20 g urea, and 15 g sodium chloride contained in each liter liquid medium.

The particle size range of standard sand is 0.08 mm–2 mm (Figure 2) and the rubber particle size is 400 mesh (Figure 3). The PVC tube is perforated with built-in gauze as the mold for the sample, while the mold specification is $\Phi 50 \text{ mm} \times 110 \text{ mm}$ (diameter \times height) (Figure 4). The purpose of drilling PVC pipe is to ensure that the nutrient solution around the sample can flow freely into the sample and the gauze is to prevent the rubber sand from leaking out of the mold.

2.2. Test Methods. Taking standard sand 345 g, rubber particles were weighed according to 0%, 1%, and 3% of the standard sand quality in turn. The standard sand and rubber particles were mixed into three groups of rubber sand samples (Table 1). When mixing, the standard sand and rubber particles are evenly mixed by continuous mechanical stirring for 5 min. Each rubber sand sample was injected with 45 g of *Sporosarcina pasteurii* liquid and put into the $\Phi 50 \text{ mm} \times 110 \text{ mm}$ mold in three layers.

The rubber sand mold was immersed in the consolidation nutrient solution for microbial consolidation, and the consolidation time was 7 days, with the oxygen continuously pumped into the nutrient solution during consolidation. There were 15 g ammonium chloride, 2 g sodium bicarbonate, 73.5 g calcium chloride, 30 g urea, 15 g casein peptone, and 5 g soybean peptone contained in each liter consolidation nutrient solution. The flow rate of oxygen pumped into the consolidated nutrient solution should be controlled between 1.2 l/min and 1.5 l/min. After consolidation, the rubber sand sample is removed from the mold and dried to constant weight (Figure 5).

The consolidated rubber sand samples after drying were tested as follows: (1) physical properties test; (2) uniaxial, cyclic loading (Figure 6); (3) mechanical properties test.

3. Test Results and Analysis

3.1. Physical Performance Testing. The mass of calcium carbonate after consolidation of samples (1)~(3) (Table 1, note 4) is 29.20 g, 29.98 g, and 29.27 g, while dry density increment (Table 1, note 5) is 0.13 g/cm^3 , 0.14 g/cm^3 , and 0.11 g/cm^3 in turn. It can be seen that rubber particles do not significantly affect the induction process of microorganisms, and the consolidation effect is only related to the amount of *Sporosarcina pasteurii* liquid injected.

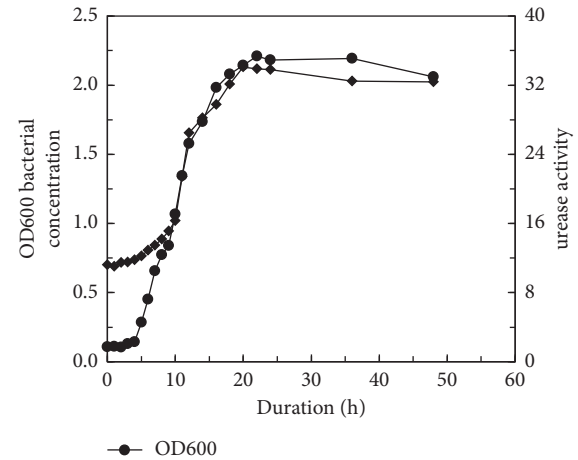


FIGURE 1: Growth curve of *Sporosarcina pasteurii*.

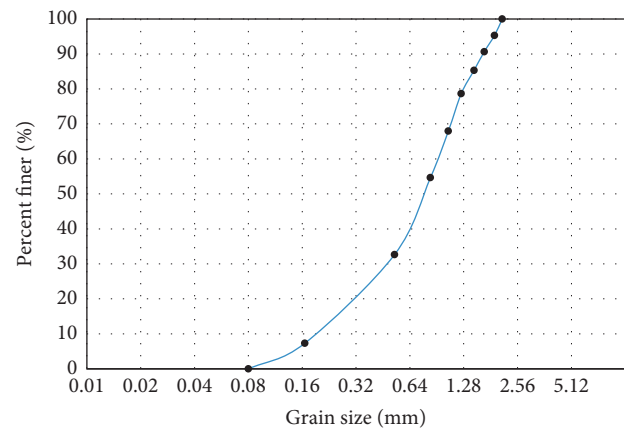


FIGURE 2: Standard sand gradation curve.



FIGURE 3: Rubber particle.

3.2. Uniaxial Compressive Strength Test. The uniaxial compressive strength of samples (1)~(3) after consolidation is 183.44 kPa, 223.17 kPa, and 216.34 kPa in turn, and the limiting compressive strain is 0.21, 0.20, and 0.29 in turn, as shown in Figures 7 and 8. It can be seen that the ultimate uniaxial compressive strength and ultimate compressive strain of rubber sand samples increase with the increase of rubber particle content after consolidation, which mean that



FIGURE 4: Test die.

TABLE 1: Sample parameters.

| Sample number | Standard sand (g) ① | Rubber particles (g) ② | Before consolidation | | | After consolidation | |
|---------------|------------------------|---------------------------|----------------------|--|-----------------------------------|----------------------|--|
| | | | Rubber sand (g) ③ | Dry density of rubber sand (g/cm^3) ④ | Bacterial liquid quality (g) ⑤ | Rubber sand (g) ⑥ | Dry density of rubber sand (g/cm^3) ⑦ |
| (1) | 345 | 0 | 345.00 | 1.60 | 45 | 374.20 | 1.73 |
| (2) | 345 | 3.45 | 348.45 | 1.61 | 45 | 378.43 | 1.75 |
| (3) | 345 | 10.35 | 355.35 | 1.65 | 45 | 384.62 | 1.76 |

Note. (1) The data in the table are the average values of the three samples in the same group. The content of rubber particles is 0%, 1%, and 3% of the standard sand. (3) $\text{④} = \text{③}/(\pi/4 \times 52 \times 11)$; $\text{⑦} = \text{⑥}/(\pi/4 \times 52 \times 11)$. (4) The quality of calcium carbonate after consolidation = $\text{⑥} - \text{③}$. (5) The dry density increment of rubber sand after consolidation = $\text{⑦} - \text{④}$.



FIGURE 5: Sample of consolidated rubber sand.

rubber particles improve the compressive strength and deformation ability of consolidated rubber sand samples.

3.3. Mechanical Performance Tests for Cyclic Loading

3.3.1. *Hysteresis Curves.* Cyclic loading tests were carried out on the demolded and dried consolidated rubber sand samples by using a servo press (Figure 6), with a stress amplitude of 20–100 kPa, loading frequency is 0.1 Hz, and

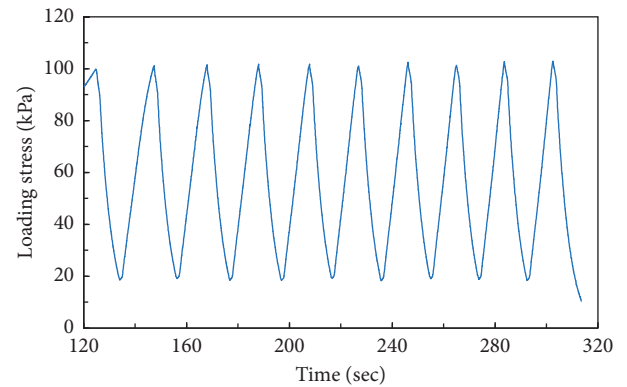


FIGURE 6: Cyclic loading system.

the number of loading and unloading cycles is 9. The hysteresis curve can be used to analyze the damping ratio, stiffness, and energy dissipation capacity of consolidated rubber sand samples with different rubber particle content. The schematic diagram of hysteresis loop analysis is shown in Figure 9; the damping ratio λ and the hysteresis loop slope κ are calculated by the following formulas:

$$\lambda = \frac{A}{(4\pi A_s)}, \quad (1)$$

$$\kappa = \frac{(\sigma_{d\max} - \sigma_{d\min})}{(\varepsilon_{d\max} - \varepsilon_{d\min})}, \quad (2)$$

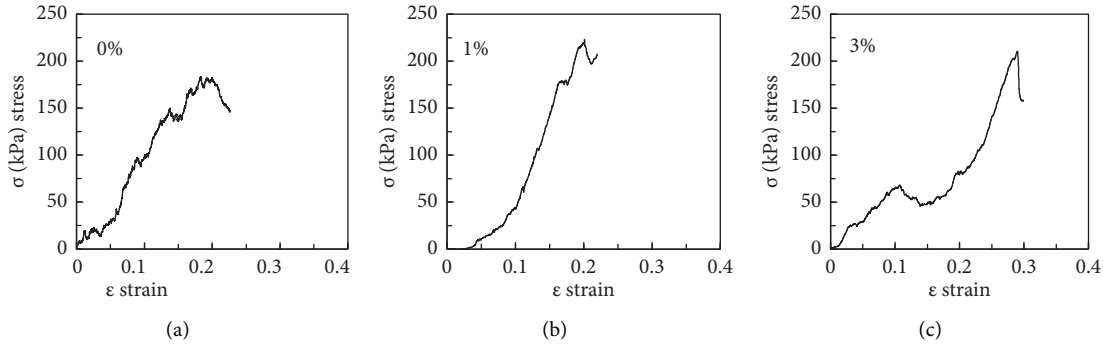


FIGURE 7: Stress-strain curves of bonded rubber sand samples.

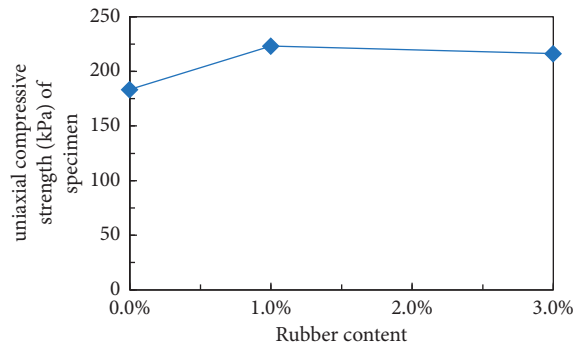


FIGURE 8: Effect of rubber particle content on uniaxial compressive strength of consolidated rubber sand samples.

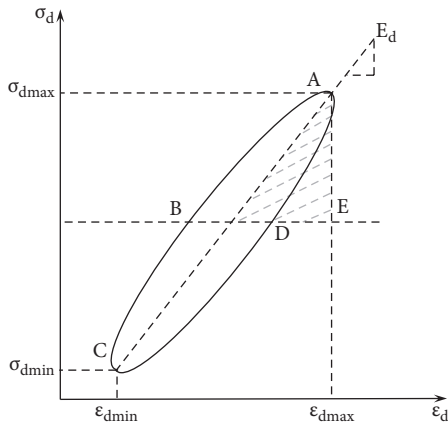


FIGURE 9: Calculation diagram of hysteresis loop.

where A is the area of the hysteresis loop, reflecting the magnitude of the energy dissipated in a cycle period; A_s is the area of the triangle region AEF (Figure 9). The maximum and minimum dynamic stresses are σ_{dmax} and σ_{dmin} , respectively; the maximum and minimum dynamic strains are ε_{dmax} and ε_{dmin} , respectively.

The hysteresis curves of consolidated rubber sand samples with different rubber particle content are obtained by the test. As shown in Figure 10, the dynamic strains of the sample increase with the increase of rubber particle content.

3.3.2. Damping Ratio. The damping ratio curve of consolidated rubber sand samples with different rubber particle content is shown in Figure 11: with the increase of loading and unloading cycle, the irreparable plastic deformation of the sample becomes larger and larger, which leads to the decrease of damping ratio. The damping ratio of rubber particles with 3% is significantly higher than that of other two groups, which shows that the effect of rubber particles on the damping ratio of consolidated rubber sand is remarkable.

3.3.3. Hysteresis Loop Slope. The hysteresis loop slope κ can reflect the stiffness of the consolidated rubber sand sample. The hysteresis loop slope of consolidated rubber sand samples with different rubber particle content is shown in Figure 12: with the increase of loading and unloading cycle times, the deformation ability of the sample becomes smaller and smaller, which leads to the increase of hysteresis loop slope. The slope of hysteresis loop increases gradually with the increase of rubber particle content, which indicates that the effect of rubber particles on the deformation resistance of consolidated rubber sand sample is significant.

3.3.4. Hysteresis Loop Area. The hysteresis loop area A can reflect the energy dissipation capacity of consolidated rubber sand samples. The greater the A , the stronger the energy dissipation capacity of the samples. The hysteresis loop area of consolidated rubber sand samples with different rubber particle content is shown in Figure 13. As the number of loading and unloading cycles increases, the irreparable plastic deformation of the sample increases and the energy dissipation capacity of the sample becomes smaller and smaller. The energy dissipation capacity of the sample with 3% rubber sand particles is significantly higher than that of the other two groups, indicating that the effect of rubber particles on the energy dissipation capacity of consolidated rubber sand sample is significant.

4. SEM Analysis of Bonded Rubber Sand Particles

The SEM photographs of the consolidated rubber sand specimens after consolidation and drying (Figure 14) show

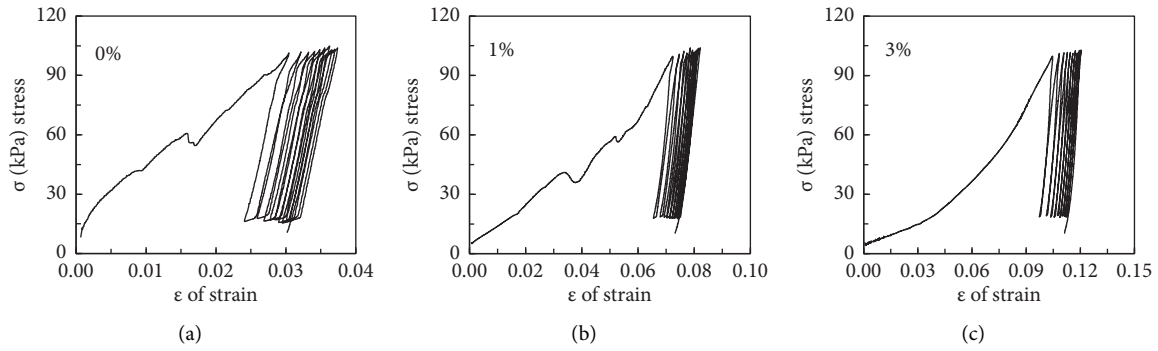


FIGURE 10: Effect of rubber particle content on hysteresis curve.

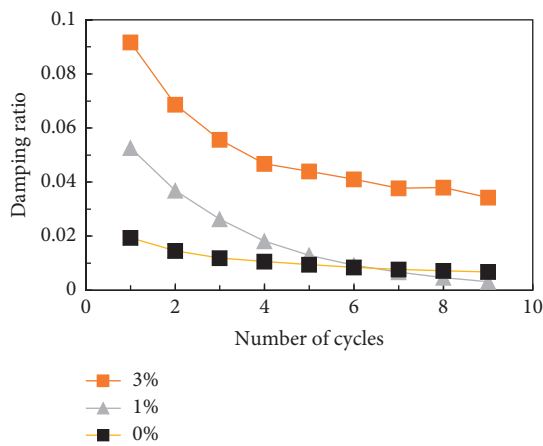


FIGURE 11: Effect of rubber particle content on damping ratio.

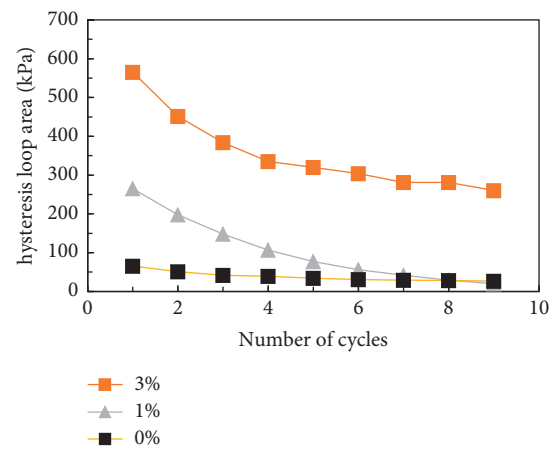


FIGURE 13: Effect of rubber particle content on hysteresis loop area.

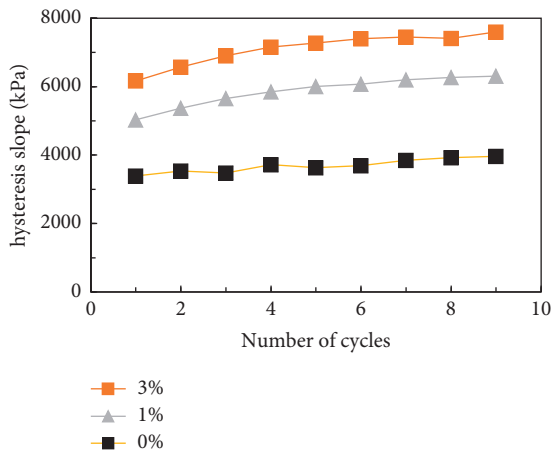


FIGURE 12: Effect of rubber particle content on the slope of hysteresis loop.

that the calcium carbonate crystals induced by *Sporosarcina pasteurii* adhere to the surface of sand particles and rubber particles, effectively filling the gap between sand particles and rubber particles, and solidifying sand particles and rubber particles into rubber sand consolidation body.

In the rubber sand consolidation body, the sand particles are the skeleton of the consolidation body, rubber particles with honeycomb structure are filled between sand particles, and honeycomb structure absorbs external input energy through deformation. Therefore, more rubber particles can make the consolidated rubber sand sample have the ability to bear larger deformation, and larger deformation capacity means that the sample can consume more external input energy. Therefore, consolidated rubber sand is a good damping material.

5. Discussion

Sporosarcina pasteurii will produce urease in the metabolic process; the enzyme can decompose urea, forming NH^+ and Ca^{2+} . When the solution contains a certain concentration of Ca^{2+} , Ca^{2+} will be adsorbed by the cells. Then the calcium carbonate crystals are nucleated by the cells and grow under gelling effect around the bacteria. The growing calcium carbonate crystals will fill the gaps among the particles of standard sand and embedded rubber and furthermore will cement these particles.

The MICP process fills the gaps between the standard sands and solidifies them as a structural base with the embedded rubber particles. The composite crystalline form

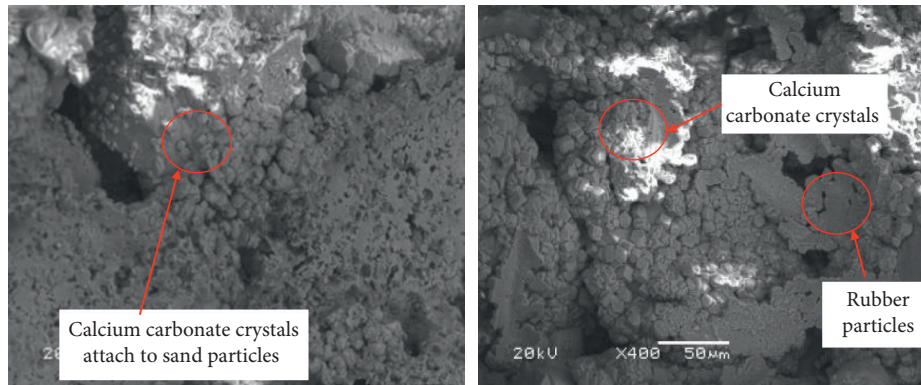


FIGURE 14: Photograph by electron microscope.

of calcium carbonate formed by the MICP process is very good at cementing the standard sand. The calcium carbonate in the form of composite crystals formed by the MICP process can well cement the pores between the standard sand and the foam particles, forming a homogeneous slab-like material with strength. The rubber is mixed into the standard sand. By using the consolidation of MICP, its filling effect and its own elastic behavior can improve the internal void structure of the material and effectively absorb the vibration energy. In Figure 14, the sand with strength formed by MICP is used as the base material. When vibration occurs, the macroscopically continuous vibration damping material will be microscopically affected by the stress or alternating stress to produce relative motion between molecules or lattices and plastic slip. When vibration occurs, the macroscopically continuous damping material will produce relative motion and plastic slip between molecules or lattices in the microscopic level due to stress or alternating stress, thus producing energy consumption. As a polymer, the relative motion between molecules can be easily generated. When rubber is added to the material, the relative motion and plastic slip between the rubber itself, rubber and other materials will be generated and can be greatly enhanced at the microscopic level, which increases the energy consumption of the material under vibration. The energy consumption of the material is increased under vibration, i.e., there is a large amount of damping, so as to obtain the vibration damping effect of the glued material.

6. Conclusion

In this paper, MICP was used to consolidate rubber sand with different rubber particle content. Based on the results of uniaxial and cyclic loading tests and SEM analysis, the conclusions are as follows:

- (1) The precipitation process of calcium carbonate induced by microorganism is not affected by the amount of rubber particles, and the consolidation effect is only related to the amount of *Sporosarcina pasteurii* solution mixed in.
- (2) Rubber particles improve the compressive strength and deformation ability of consolidated rubber sand samples and significantly enhance the damping ratio,

resistance to deformation, and energy dissipation ability of consolidated rubber sand samples.

- (3) MICP bonded the sand particles and rubber particles into rubber sand consolidation body. Because of the honeycomb structure characteristics of rubber particles, the consolidated rubber sand samples have the ability to withstand large deformation, which means that rubber sand is a good damping material.

Data Availability

None of the material in the paper has been published or is under consideration for publication elsewhere. The data used to support the findings of this study are included within the article.

Conflicts of Interest

The author declares no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

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Review Article

Geotechnical Engineering Properties of Soils Solidified by Microbially Induced CaCO_3 Precipitation (MICP)

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Microbially induced calcium carbonate precipitation (MICP) uses the metabolic function of microbes to carry out biochemical reactions with other substances in the environment. Through the controlled growth of inorganic minerals, soil particles are cemented and soil pores are filled to solidify the soil and reduce its permeability. Thus, the application of this technology was foreseen in geotechnical engineering and environment (building antiseepage, contaminated soil restoration, slope soil erosion, and sand liquefaction). In this review article, based on current research findings, the urea hydrolysis and the cementation mechanism of MICP are briefly described. The influences of factors such as enzyme activity, cementation solution concentration, pH, temperature, grouting method, and particle size on MICP-treated soil are discussed. The engineering properties of MICP-treated soils are evaluated, for instance, the strength, stiffness, liquefaction resistance, permeability, and durability. The applications of MICP technology in ground improvement, geotechnical seepage control, foundation erosion resistance, and fixation of heavy metals are summarized. Finally, future directions of the development of MICP technology are elucidated to provide a reference and guidance for the promotion of MICP technology in the geotechnical engineering field.

1. Introduction

With the rapid socioeconomic development and accelerated urbanization, urban infrastructure construction is facing unprecedented development worldwide. In this process, a series of engineering challenges exist, including improvement of weak ground, treatment of liquefied soil, remediation of contaminated soil, seepage and leakage control on dams, and dust and sand fixation. Conventional methods used to address these challenges have the disadvantages of high construction difficulty, long construction period, high energy consumption, single performance, and secondary pollution [1]. Microbially induced calcium carbonate precipitation (MICP) has emerged in recent years; it uses calcium carbonate (CaCO_3) induced by bacteria to cement loose soil particles, thereby improving the mechanical properties of soil. Compared with conventional materials, MICP technology exhibits greater potential for application and environmental sustainability in geotechnical

engineering area and is suggested as a feasible alternative by many researchers [2–4].

Since the 1960s, researchers have gradually realized that microbial metabolic activities are directly involved in redox processes in the environment, thereby altering the geological characteristics and affecting the material cycle and migration of the biosphere. In nature, there is a large number of microorganisms that continuously multiply, grow, and migrate in the soil; participate in mineralization reactions; produce secretions; and degrade organic matter [5]. Microbial mineralization is the process by which microorganisms use their metabolic reactions to produce the urease enzyme. This enzyme catalyzes the decomposition of urea in the surrounding environment to generate carbonate (CO_3^{2-}), which reacts with calcium ion (Ca^{2+}) to produce CaCO_3 precipitates. MICP technology was initially applied in the field of seepage and leakage control of porous media materials and was later extended to the repair of cracks in ancient buildings and rock materials. Studies on soil

improvement started relatively late [6]. Whiffin [7] was the first to propose the use of MICP technology for cement loose sand particles to improve soil mechanical properties such as the sand strength and stiffness of sand. Mitchell and Santamarina [8] identified the extensive application value and potential of microbially modified rocks and soils. The National Research Council of the United States established microbial geotechnical engineering technology as an important research topic of the 21st century [9]. Currently, MICP technology is mainly used in research on sand and is slowly being extended to other types of soil, such as silty soil, expansive soil, clayey purple soil, and red clay. Existing studies have shown that MICP technology can significantly improve the shear strength, liquefaction resistance, and erosion resistance of soils and substantially reduce the permeability of soils [10].

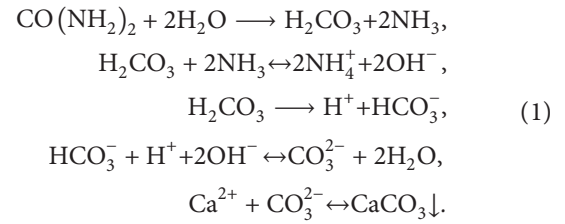
As a new research branch in geotechnical engineering, microbial environmental geotechnical engineering is an interdisciplinary subject of microbiology, chemistry, and geotechnical engineering that has been developed for over a decade. The biochemical reaction process of MICP is detailed in this review article, and the factors influencing MICP-treated soil are discussed. The relevant findings regarding MICP technology in applications such as ground improvement, rock-soil seepage control, ground erosion resistance, and contaminated soil remediation are summarized. The findings described in this review can provide a reference for the development and application of MICP technology in the geotechnical engineering field.

2. Principles of MICP Technology

2.1. Metabolic Process of MICP. MICP is a common phenomenon of microbial mineralization in nature [4, 6]. It involves a series of biochemical reactions with other substances in the environment through metabolic functions and absorbing, transforming, removing, and degrading these substances, and it induces the formation of mineral precipitates such as carbonate and sulfate through biological processes, thereby improving the mechanical properties of the soil. The production of CaCO_3 precipitates mainly depends on the presence of CO_3^{2-} and Ca^{2+} in an alkaline solution environment, which combine to reach a saturation state so that CaCO_3 precipitates. Different metabolic types of microorganisms can form different ways of bioinduced mineralization, such as urea hydrolysis, denitrification, ferric reduction, and sulfate reduction [5, 11]. Among them, urea hydrolysis to precipitate carbonate is the most simple, directly and easily controlled mechanism of MICP, which is widely used [12].

The urea hydrolysis is that urease enzyme produced by bacteria catalyzes hydrolysis of urea to NH_4^+ and CO_3^{2-} [13]. The production of NH_4^+ by ureolysis ultimately results in an increase in pH. The CO_3^{2-} content in solution increases with increasing pH. In urease catalysis, urease breaks the covalent bonds of urea, and urease-urea reaction intermediates are formed between the active center of urease and the substrate molecules of urea through short-range noncovalent forces, for instance, hydrogen bonds, ionic bonds, and hydrophobic

bonds. The main reactions of CO_3^{2-} crystallization induced by urease-producing bacteria are expressed as follows [14, 15]:



Notably, the equilibrium state of NH_4^+ or NH_3 in the solution is very important. The concentration of NH_4^+ or NH_3 is governed by Henry's law for gases dissolution, which makes it special in MICP because the bacteria represent a source of NH_4^+ , and could be manipulated by changing the temperature or pH, by introducing a sink for NH_3 or by releasing it out of the liquid by aeration. NH_3 that results from urea hydrolysis inside the cell diffuses out of the cell membrane due to the concentration gradient, and the pH of the cytoplasm equilibrates at 8.4 ($\text{NH}_4^+:\text{NH}_3 = 70:30$). NH_4^+ diffuses out of the cell, increasing the membrane potential, which occurs since NH_4^+ and NH_3 equilibrate when the surrounding solution reaches a pH of 9.25 [7, 16]. Lauchnor et al. [17] investigated the ureolysis rate with whole cells to determine the relationship between the ureolysis rate and the concentrations of NH_4^+ . Lee et al. [18] investigated the NH_4^+ concentration in the MICP process and used meter-scale experiments for the removal of NH_4^+ . Qin and Cabral [19] thought that NH_3 exists mainly in the form of NH_4^+ ions within the temperature (25°C) and pH values (7) by studying the kinetics of urea hydrolysis catalyzed by urease. Krajewska [20] also investigated NH_3 and considered that the role of urease in soil is to make urea available to plants by converting it to NH_3 ; if the hydrolysis rate is too rapid, it may cause NH_3 volatilization and then impact the environment [21]. Therefore, in the MICP process, the hydrolysis rate and ammonia and ammonium concentration should be of concern to investigators. In application, the method of inhibiting urease activity can be employed to avoid ammonia emission.

Based on the study of urea hydrolysis in solution and solid agar, realizing the metabolic process of MICP has some limitations at the microscale. Microfluidic chip technology can overcome this limitation and can be used for real-time observation of reactions, less reactant consumption, and excellent controllability of environmental factors. Wang et al. [22–24] utilized glass slides and microfluidic chips to carry out real-time in situ microscale experiments (Figure 1(a)) and realized the visualization of CaCO_3 precipitation process. By observing the behavior of bacteria and CaCO_3 crystals in this process, understanding MICP at the particle scale was improved. Based on microfluidic chip technology, He et al. [25] developed a visualization system for microbial reinforcement (Figure 1(b)) and carried out in situ microscopic research on the mechanism of CaCO_3 mineralization induced by microorganisms. The deposition of CaCO_3 crystals in the process of microbial reinforcement

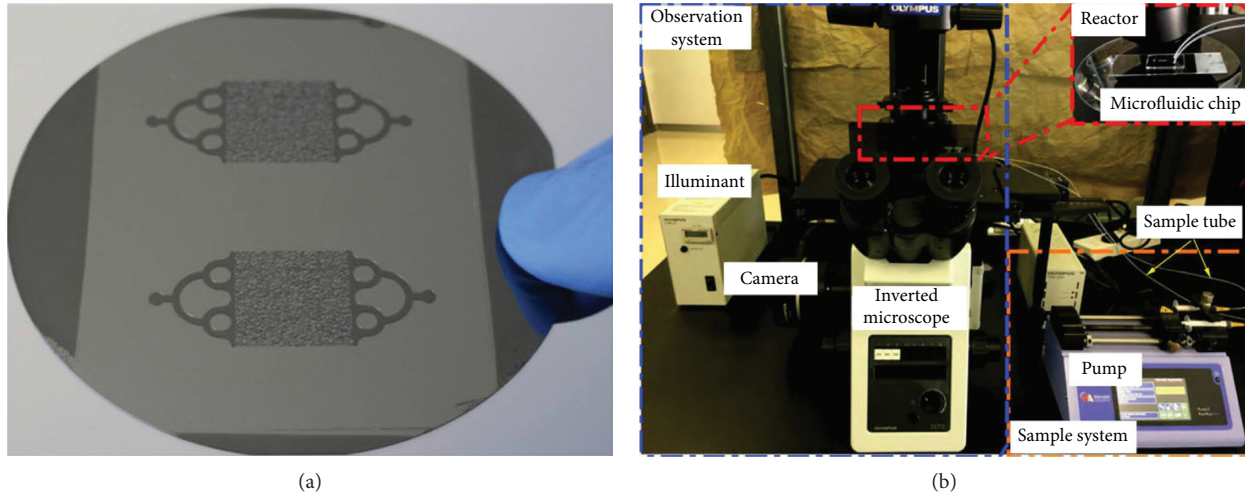


FIGURE 1: Microfluidic chip technology and its visualization: (a) microfluidic chip [23]; (b) schematic images of the microfluidics system [25].

was observed, and its temporal and spatial distribution, deposition mode, and growth rate were quantified.

2.2. Cementing Mechanism of MICP. The microbially induced formation of calcite may exhibit two extreme distribution states in the pores of soil particles. One is the formation of calcite of equal thickness around the soil particles. In this case, the cementation among soil particles is relatively weak, and there are no significant improvements in the soil properties. The other is the formation of calcite only at positions where soil particles are in contact with each other. This type of distribution allows calcites to be employed entirely for cementation among soil particles, which is beneficial to the improvement in soil properties [11]. Cui et al. [26] considered that the effective cementation of calcite crystals can be divided into two modes: (a) calcite-calcite cementation (caused by calcite crystal clusters between adjacent sand particles, Figure 2(a)); (b) particle-contact cementation (caused by calcite crystals precipitated at the particle-particle contacts, Figure 2(b)). Wang et al. [13, 23, 24, 27] used microfluidic chip technology to study the size, quantity, distribution, and morphology of CaCO_3 in the MICP process. The results showed that the formation of CaCO_3 crystals was distributed in both narrow and open pore throats. The higher the bacterial concentration was, the more the crystals formed in the same volume were. The shape and size of CaCO_3 precipitates changed during the MICP process. Irregular-shaped CaCO_3 precipitates formed during the initial stage but dissolved when new CaCO_3 crystals formed. In addition, with the dissolution of irregular-shaped CaCO_3 , spherical and rhombohedral CaCO_3 crystals formed. He et al. [25] carried out an in situ micromesoscopic study on the mechanism of microbially induced CaCO_3 mineralization using a visualization system for microbial cementation. They concluded that the convection and diffusion of solute molecules significantly influence the distribution of CaCO_3 crystals and that nonuniform spatial and temporal distributions of CaCO_3 are

present. The nonuniform temporal distribution was reduced as the reaction proceeded, while the nonuniform spatial distribution was maintained throughout the entire reaction process (0 to 2200 min). There were two patterns of CaCO_3 precipitation, i.e., at the pores and at the sand contacts, in microsized pipes, with the CaCO_3 at the pores growing uniformly and the CaCO_3 at the sand contacts exhibiting axes with different growth rates.

3. Analysis of the Influence Factors of Soil Solidification by MICP

Researchers have conducted in-depth and systematic studies on MICP-treated soils. The results showed that the main factors that affect the solidification results of MICP include enzyme activity, cementation solution concentration, pH, temperature, grouting method, and particle size [28].

3.1. Enzyme Activity. Urea hydrolysis mainly occurs through urease produced by microorganisms. Urease activity has a great influence on the formation of CaCO_3 precipitation. Miftah et al. [29] conducted tube experiments under different enzyme concentrations of 1.25, 2.5, 5, 10, 11, and 15 mL/L to determine the appropriate concentration of enzyme that yields a higher mass of precipitation. The results showed that the mass of precipitation and the precipitation ratio at the enzyme concentration of 10 mL/L, which is equivalent to 4650 U/L, were larger than those at other concentrations, so this concentration was selected for enzyme-induced carbonate precipitation (EICP) solution. Almajed et al. [30] obtained sand specimens with a high strength (1.8 MPa) treated with EICP by adding nonfat powdered milk to the treatment solution. The unconfined compressive strength increased as the concentration of enzyme (from 0.85 g/L to 3 g/L) increased. Martin et al. [31] successfully used a similar method to enhance the UCS of Ottawa 20/30 silica sand, F85 silica sand, Soda-lime glass beads, and local washed quarry sand with 12,600 U/l urease

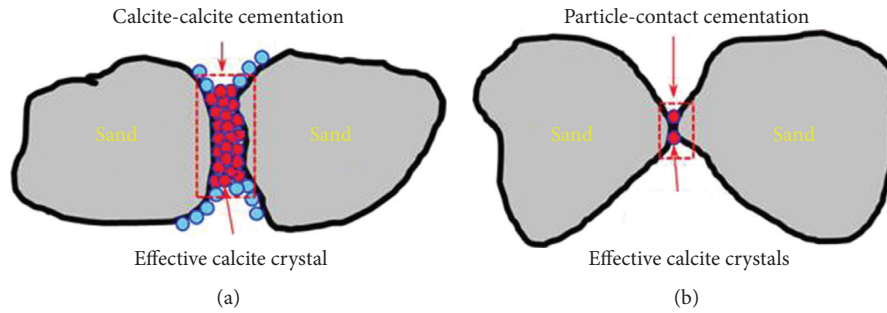


FIGURE 2: Effective calcite crystals in biocemented sand: (a) calcite-calcite cementation; (b) particle-contact cementation [26].

enzyme. Their experimental results indicated that the strength of Ottawa 20/30 sand was the largest, reaching 1.5 MPa. Konstantinou et al. [32] carried out a cementation test of very coarse sands (mean particle size is 1820 μm) by controlling the urease activity in bacterial populations used for biological cementation. The results showed that MICP on very coarse-grained materials was successful when urease activities less than 10 mmol/L/h were used with bacterial populations with optical densities equal to or greater than 2. Baziar et al. [33] utilized soybean enzyme to induce carbonate precipitation to stabilize loose soil-inducing fugitive dust and conducted wind tunnel tests to examine their erosion performances. The results indicated that soybean enzyme was easy to prepare and could help the control of dust emission caused by wind erosion. Chen et al. [34] investigated the effects of soybean urease-induced carbonate precipitation on the water retention ability of the ISO standard sand; the sands treated with urease (urease activity was obtained as 5.4 mM/min) and 1 mol/L urea-calcium chloride solutions had greater water retention ability. Instead of bacteria, Yang et al. [35] applied urease directly to fine sand with a premixing method. The results indicated that saturated specimens (urease activity was 5.77 mM urea/min) obtained better moisture stability than unsaturated specimens (urease activity was 4.55 mM urea/min). Some scholars [36, 37] have also used urease extracted from soybean to induce CaCO_3 deposition to solidify sand. In addition, urease can induce the formation of CaCO_3 crystals, which is effective in sand fixing. However, the application cost of using purified urease is expensive [36]. Some scholars extracted urease from plants for research. Because the cultivation conditions favorable for bacterial growth could not guarantee stable urease production [7, 32], most scholars discussed the different MICP processes with a focus on controlling the bacterial concentration [38–43].

3.2. Cementation Solution Concentration. Currently, the cementation solutions used in MICP tests are mostly a mixture of urea and Ca^{2+} solutions. Different concentrations and compositions of cementation solution significantly affect the CaCO_3 yield, mineralization efficiency, crystal structure, spatial distribution, and mechanical properties. Rebata-Landa [44] treated samples under the condition of circulating nutrient solution for 64 days using a 60 mL plastic syringe, showing that the CaCO_3 content in samples

gradually increased with increasing cementation solution concentration and reaction time. Nemati et al. [45] showed that the mineralization efficiency of CaCO_3 in solution exceeded 80% when the CaCl_2 concentration was less than 15 g/L, while the CaCO_3 production was essentially zero when the concentration exceeded 20 g/L; that the mineralization efficiency of CaCO_3 could reach 99% when the concentration ratio of CaCl_2 and urea was 2.5 or 3.0; and that too high or too low concentration ratio decreased the mineralization efficiency. Qabany et al. [46] carried out tests on the MICP cementation of quartz sand using *S. pasteurii* and concluded that within the concentration range of 0.25 to 1.00 mol/L, the higher the concentration of cementation solution was, the larger the size and the more nonuniform the distribution of the generated calcite crystals (Figure 3). Qabany and Soga [47] treated sand samples using 0.1, 0.25, 0.5, and 1.0 mol/L solutions of urea and CaCl_2 mixtures and found that the use of high concentrations rapidly reduced the permeability at an early stage. In addition, some scholars explored the CaCO_3 deposition amount, sample strength, permeability, and optimal conditions for CaCO_3 deposition for different concentrations of cementing solution (Table 1). The deposition of CaCO_3 increases as the concentration of cementation solution increases, but there are differences in the optimal cementation schemes, such as [41, 48, 50] (Figure 4). According to the research results of [49, 51], the maximum deposition of CaCO_3 occurs in cement solutions with high Ca^{2+} concentration. Therefore, the cementation solution significantly influences the bacterial activity, CaCO_3 precipitation, and soil cementation in MICP (Table 1). In applications, appropriate cementation solutions and concentration ratios should be selected according to the specific reinforcement requirements to meet the needs of actual engineering projects.

3.3. pH. Most urease-producing bacteria commonly used in MICP are heterotrophic facultative aerobic bacteria, which are suitable for growth in slightly alkaline environments. Therefore, the pH value has an important influence on growth [28]. Henze and Randall [52] revealed that *S. pasteurii* survived in a solution of pH 11.2, which allows MICP to create durable building materials. Stocks-Fischer et al. [14] examined the effect of pH on the activity of urease extracted from *B. pasteurii* and found that the urease activity gradually increased with increasing pH and peaked at pH of

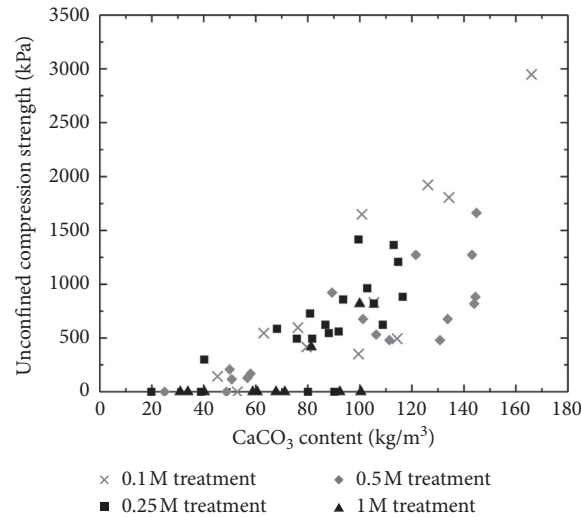


FIGURE 3: Unconfined compression strength plotted against CaCO₃ for different treatments [46].

TABLE 1: Previous studies on different cementation solution concentrations.

| Reference | Bacteria | Cementation solution concentration | Conclusion |
|-----------|----------------------|---|---|
| [41] | <i>B. megaterium</i> | 0.25, 0.5, 1.0 mol/L urea-calcium chloride | The shear strength of the specimens treated with 0.25 mol/L cementation reagent improved by 26–57%, and the hydraulic conductivity reduction ranged from 16 to 73% |
| [48] | <i>S. pasteurii</i> | 0.25, 0.5, 1.0 mol/L urea; 0.25, 0.5, 1.0, 2.0 mol/L calcium chloride | The UCS of sand column treated with 1 mol/L urea and CaCl ₂ solution is the largest |
| [49] | <i>S. pasteurii</i> | 2.5, 25, 250 mM Ca ²⁺ ; 333, 666 mM urea | Increasing urea and Ca ²⁺ concentrations increase the amount of carbonate precipitated. The CaCO ₃ precipitated depend more on the Ca ²⁺ concentration than the amount of urea |
| [50] | <i>S. pasteurii</i> | 0.5, 1.0, 1.5 mol/L urea and calcium chloride, concentration ratio: 1:1, 1:2, and 2:1 | The CaCO ₃ content and peak strength of the samples increase with the increasing of the cementation solution concentration, with the maximum being at concentration ratio 2:1 |
| [51] | <i>S. pasteurii</i> | 0.25, 0.75, 1, 2 mol/L urea and calcium chloride | An increase in urea and calcium concentrations increased the CaCO ₃ precipitation. The greatest amount of CaCO ₃ was achieved at the condition of 1 mol/L urea and 2 mol/L calcium chloride |

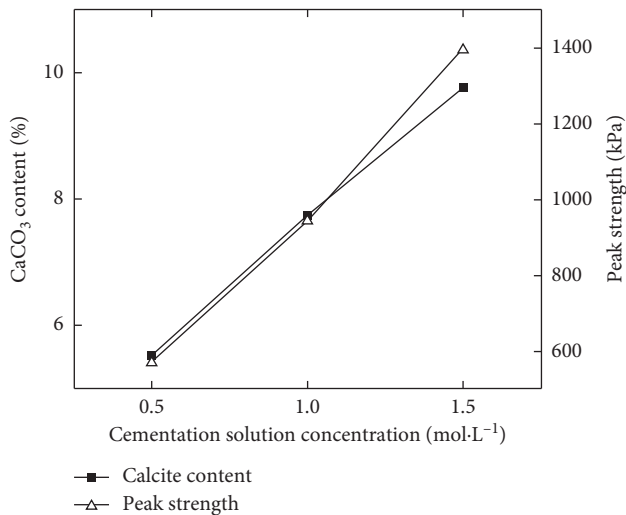


FIGURE 4: Relationship between CaCO₃ content and peak strength with different cementation solution concentrations [50].

7.5–8.0. Whiffin [7] inoculated bacteria grown under different pH conditions into a standard test solution with a pH of 7 and urea concentration of 25 mM/L and then measured the urea decomposition rate after incubation at 25°C for 5 h. The unit urea decomposition rate of the bacteria was the highest between pH of 7 and 8, which is slightly lower than the optimum growth pH of *S. pasteurii*. pH values have an important role not only in the growth of bacteria but also in metabolism, CaCO₃ deposition, and soil property improvement. Different pH values will change the concentrations of NH₃, NH₄⁺, CO₃²⁻, and HCO₃⁻ in the solution, thus changing the formation rate and yield of CaCO₃ [53]. Li et al. [54] investigated the precipitation kinetics and crystal morphology of MICP at initial pH values of 6.0, 6.5, 7.0, and 8.0 and concluded that high pH favored CaCO₃ precipitation (Figure 5). The CaCO₃ precipitates were mainly calcite crystals, which gradually changed from prism to pyramid-like or irregular polyhedral shapes with increasing deposition time. Cheng et al. [55] carried out biocemented sand

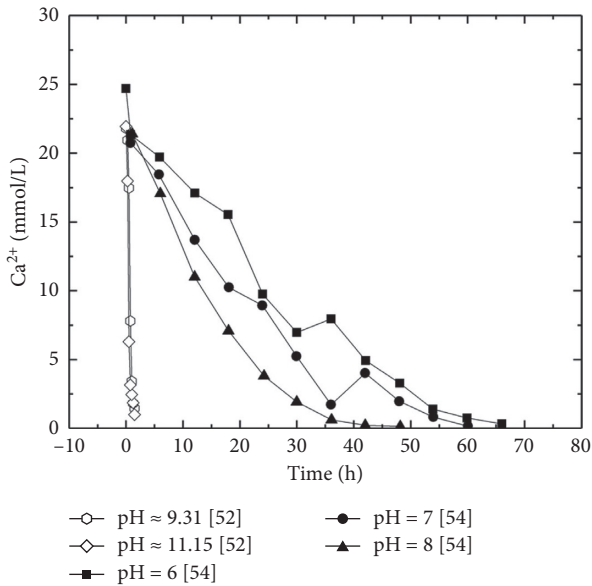


FIGURE 5: Changes in Ca^{2+} concentration at different pH values.

column tests using *B. sphaericus* under initial neutral, acidic, and alkaline pH conditions. They found that the CaCO_3 content in the samples increased with increasing pH, whereas the cemented sand column had the highest strength under neutral conditions. Keykha et al. [56] employed *S. pasteurii* for the biocementation of silty clay soil at pH values of 5, 6, 8, and 9 and showed that both the CaCO_3 content and the UCS of the sample increased with increasing pH. Ferris et al. [57] found that hydroxide ions improve the pH around the cells and that the appropriate pH for the MICP reaction ranges from 6.5 to 9.3. Urease still maintains good activity at pH of 9.0, and high pH can promote the conversion of bicarbonate ions to carbonate ions and the precipitation of CaCO_3 [58]. In summary, pH has an important influence on the growth and metabolism of urease-producing bacteria as well as on CaCO_3 precipitation and soil property improvement. High pH is conducive to the precipitation of CaCO_3 and improvement in the solidified soil strength. In actual engineering projects, a higher pH should be selected for solidification by MICP while considering the specific engineering needs. The impact of pH on the growth of bacteria, bacterial metabolic activity, urease activity, CaCO_3 morphology, CaCO_3 precipitation, and strength is summarized in Table 2.

3.4. Temperature. Changes in the ambient temperature affect the growth, reproduction, and functional metabolism of bacteria, thereby altering CaCO_3 production, precipitation rate, crystal type, crystal morphology, particle size, and cementation pattern of CaCO_3 between soil particles, which impacts the effectiveness of MICP-treated soil (Figure 6). Ferris et al. [57] investigated the effect of urease activity on the urea hydrolysis reaction in a groundwater environment of 10–20°C and found that urease activity depends on the temperature to a certain extent. A study by Nemati and Voordouw [61] showed that as the temperature increased

from 20°C to 50°C, the urease activity and CaCO_3 formation rate increased, which further affected the crystal morphology of the CaCO_3 precipitates (Figure 7). Cheng et al. [55] found that the amount of precipitated CaCO_3 at 50°C was more than three times that at 25°C, while the strength dropped by 60% as the temperature decreased from 50°C to 25°C. The main reason for these results is that when the temperature is high, the generated CaCO_3 particles are relatively small and cover the surface of the sand particles, whereas when the temperature is low, less precipitation of CaCO_3 occurs. However, the particles are relatively large, which can effectively bind the sand particles and thus increase the strength. Gillman et al. [62] pointed out that as the ambient temperature increased from 5°C to 20°C, the rate of urea decomposition by urease-producing bacteria increased nearly 18-fold. Kralj et al. [63] experimentally discovered that a change in the temperature of the inorganic salt solution influenced the precipitation rate of CaCO_3 without affecting its crystal type. By comparing the effect of temperature on the improvement in the strength and water absorption of limestone with *S. psychrophila* and *B. sphaericus*, Muynck et al. [60] found that the bacteria more effectively reduced the water absorption of limestone at low temperature (37°C) and achieved the best improvement in the strength of samples at moderate temperatures (20°C and 28°C), while both bacteria exhibited poor performance in improving the strength and impermeability at low temperature (10°C). Keykha et al. [56] applied an equal amount of *S. pasteurii* solution to cement silty sand columns at pH of 9 and temperatures of 30, 40, and 50°C and found that the cemented sand column at 40°C had the highest UCS. Bang et al. [64] investigated the wind erosion resistance of *S. pasteurii*-cemented samples at 20, 35, and 45°C and concluded that the wind erosion resistance of the samples gradually increased as the curing temperature increased. Peng et al. [65] found that the lower the soil temperature was, the lower the strength and the higher the permeability of *S. pasteurii*-treated soil were. For example, the UCS of the sand column at 10°C was one-third of that at 25°C, while the permeability at 10°C was three orders of magnitude higher than that at 25°C (Figure 8). In summary, the appropriate temperature for the MICP process is similar to the optimum temperature for the growth and metabolism of urease-producing microorganisms. At low temperatures, the crystal particles of CaCO_3 are large, CaCO_3 is uniformly distributed between the particles, and overall strength of the cemented samples is high. In contrast, at high temperatures, the crystal particles of CaCO_3 are small and the strength of the cemented samples is low, but the CaCO_3 yield is high and the erosion resistance is enhanced.

3.5. Grouting Methods. The homogeneity of MICP-treated soil is currently one of the important factors restricting the development of MICP technology. Grouting methods determine the CaCO_3 content and distribution homogeneity of the treated soil and further affect the strength and permeability of the soil. Common grouting methods include injection, soaking, and spraying (Table 3). Whiffin et al. [66]

TABLE 2: Impact of pH on some factors.

| Factors | pH | Conclusion | Reference |
|---------------------------------|---------------------------------------|---|-----------|
| Growth of bacteria | pH = 7, 8, 9 | The bacterial concentration is highest at pH of 9 | [59] |
| Bacterial metabolic activity | pH = 9.31, 11.15, 11.95, 12.24, 12.48 | The pH is kept above 12; the urease-producing bacteria are inactivated | [52] |
| Urease activity | pH = 6, 7, 8, 9, 10 | The enzyme activity increased at fast rate, peaking at pH of 8.0 and then decreasing slowly at higher pHs | [14] |
| | pH = 7, 8, 9 | The highest urease activity occurred at pH = 9 | [59] |
| CaCO ₃ morphology | pH = 6.0, 6.5, 7.0, 8.0 | High pH promoted CaCO ₃ precipitation, and precipitates were mainly calcite crystals, gradually changing from prism to pyramid-like or irregular polyhedral shapes | [54] |
| CaCO ₃ precipitation | pH = 3.5, 7, 9.5 | The maximum CaCO ₃ deposition at pH = 9.5 | [55] |
| | pH = 9.31, 11.15, 11.95, 12.24, 12.48 | The initial pH decreased to below 11.15, and the Ca ²⁺ concentration begins to decrease, hence CaCO ₃ precipitation | [52] |
| | pH = 5, 6, 8, 9 | The increase in pH increased the CaCO ₃ precipitation | [56] |
| Strength | pH = 5, 6, 8, 9 | The compressive strength of silty clay soil samples increased steadily as pH increased from 5 to 9 | [56] |

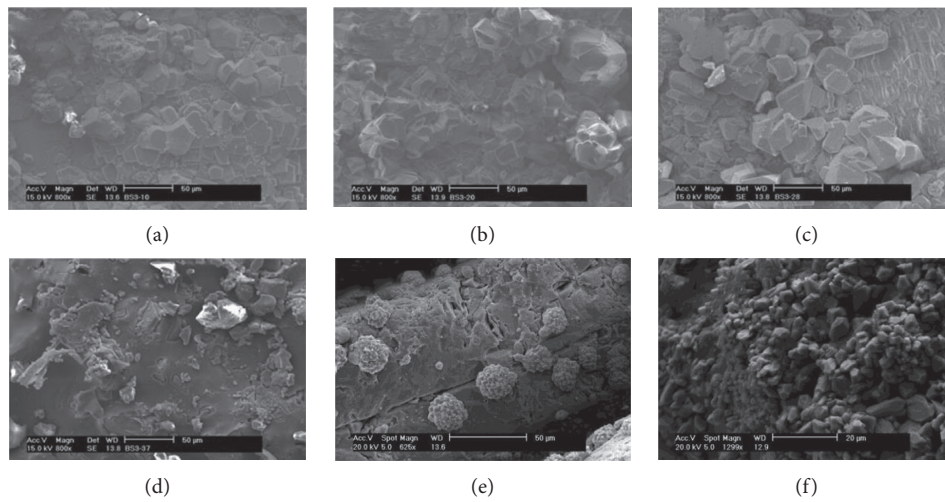


FIGURE 6: CaCO₃ crystals formed at different temperatures, (a)–(d) [60], (e)–(f) [55]: (a) 10°C; (b) 20°C; (c) 28°C; (d) 37°C; (e) 25°C; (f) 50°C.

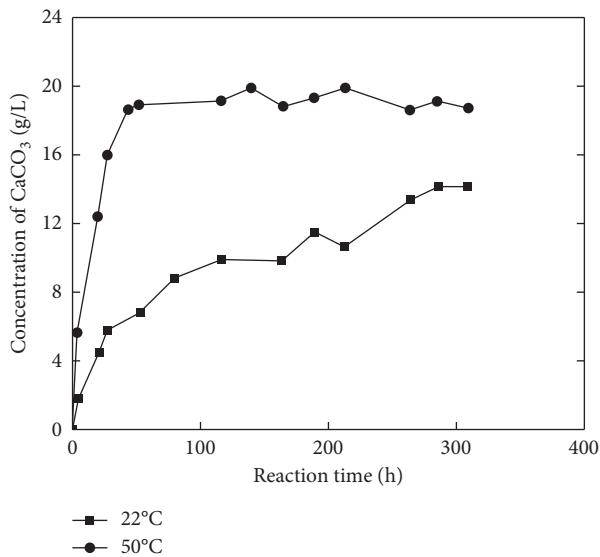


FIGURE 7: Effect of temperature on enzymatic CaCO₃ production [61].

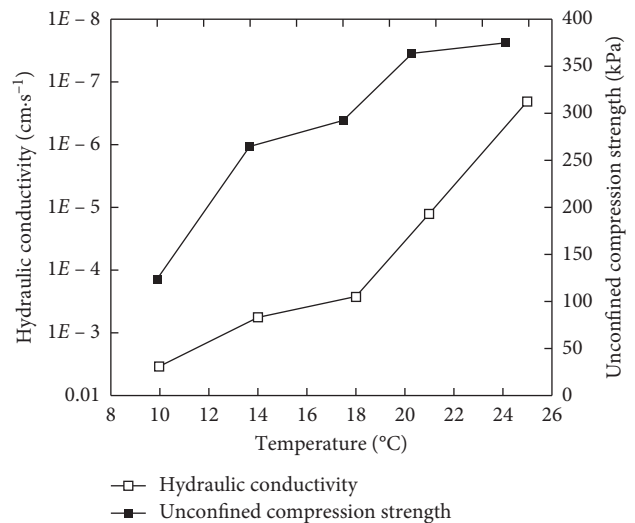


FIGURE 8: Unconfined compression strength and hydraulic conductivity of treated samples at different temperatures [65].

TABLE 3: Advantages and disadvantages of grouting methods.

| Method | Procedure | Advantage/disadvantage | Reference |
|----------------------------------|---|---|-----------|
| Two-phase grouting method | Placement: bacterial injection, CaCl ₂ injection | Avoiding clogging | [66] |
| Parallel injection method | The bacteria and the cementation fluid were injected at the same time | Most calcite precipitating close to the inlet area | [67] |
| Repeated staged injection method | Bacterial injection, static period for 2 h, cementation fluid injection | Reducing porosity | [67] |
| Soaking method | Bacterial injection, soaking in the cementation fluid | The pores becoming smaller or even blocked, the cementation fluid being difficult to penetrate into | [68] |
| Electro-biogrouting method | Imposing electric field with a certain voltage gradient | Promoting the diffusion of biomass in soil pore, more uniform | [69] |
| Spraying method | Spraying bacteria solution evenly on the surface of sand practices | Improving the average hardness and compressive strength | [70] |
| Unsaturated percolation method | Using peristaltic pump to control the infiltration rate (the saturation is about 75%) | Having better curing effect, with the average compression strength being 19.7 MPa | [71] |

proposed a 2-phase grouting method, in which a bacterial suspension is injected into the soil and allowed to stand for a period of time. After the bacteria adhere to the surface of the soil particles, the cementation solution is injected to start solidification. This grouting method can prevent the clogging of the injection nozzle to a certain extent and improve the homogeneity of the CaCO₃ that is generated inside the soil. Compared to the mixed injection method, this method can reduce the porosity of the solidified soil by an additional 20% using the same cementation solution and bacterial suspension [67]. Liang et al. [68] noted that the solidification result using the step-by-step grouting method on sand was superior to that using the soaking method. When the mixture of 0.5 mol urea and 0.5 mol calcium chloride was poured at 50 mL/h, the average UCS with the former method increased by 15.58% compared to that with the soaking method. Cui et al. [72] introduced 0.05 mol/L CaCl₂ solution (referring to a mixed bacterial suspension) into a pure bacterial suspension for artificial intervention in the bacterial distribution and comparatively analyzed the effects of the injection of pure/mixed bacterial suspension, mixed bacterial suspension solution, and traditional pure bacterial suspension on the dynamic characteristics of biocemented sand. The test results showed that the injection of a pure/mixed bacterial suspension could effectively increase the uniformity of the distribution of CaCO₃ crystals in the biocemented soil, thereby obtaining biocemented soil with a higher CaCO₃ content, larger dynamic elastic modulus, and greater energy dissipation capacity. Xu et al. [69] used the electro-biogrouting method (EBM) to solidify silt and concluded that compared with the traditional gravity grouting method and the low-pressure grouting method, the EBM promoted the diffusion of microorganisms in soil pores by applying an electric field with a certain voltage gradient in the silt, resulting in more uniform dispersion of CaCO₃. Zhan and Qian [70] treated sand with biocement using a spraying method; after seven spraying applications, the average hardness and compressive strength of the samples reached 31.5 GPa and 0.67 MPa, respectively. Andres et al. [71] compared the solidification results using the percolation method and reverse injection method to

treat unsaturated soil samples and found that the percolation method achieved a better solidification result, with an average UCS of 19.7 MPa. In summary, the distributed grouting method, multiconcentration grouting method, and electroosmotic grouting method are more valuable than the single grouting method in terms of improving the uniformity of the distribution of CaCO₃ and thereby increasing the overall strength of the solidified soil.

3.6. Particle Size. The soil particle size is an important factor affecting the MICP solidification results (Table 4), mainly because it affects the retention, adsorption, and transport of microorganisms between sand particles. Generally, bacterial cells have a size of approximately 0.5–3.0 μm; in particular, the sizes of *Sporosarcina* and *Bacillus* species are mostly 1–5 μm. Too small soil particles hinder the flow of bacteria and cementation solution in soil, meaning that CaCO₃ cannot be formed or is unevenly distributed, thereby resulting in poor solidification [8]. In addition, only the CaCO₃ deposited at the contact points between the soil particles can effectively improve the mechanical properties of the soil; excessively large particles reduce the number of contact points between soil particles, causing CaCO₃ to be mostly distributed on the surface of coarse particles, which causes poor solidification. Regarding the size compatibility between soil particles and microorganisms, Li et al. [75] proposed a formula for judging the suitability of soil characterized by the permeability coefficient for MICP technology and verified the formula through tests on the mechanical properties of nine types of soil. Cui et al. [73] investigated the effect of particle size on biocementation and concluded that the interparticle pores of sand of smaller particle size are more easily densely filled by CaCO₃ crystals, obtaining a larger proportion of effective CaCO₃ crystals and thus a biocemented sample that is “structurally” stronger and has a higher UCS. Rebata-Landa [44] tested cemented soil columns composed of 11 types of soil including kaolin, silt, fine sand, coarse sand, and gravel, from which the particle size ranges for effective cementation were obtained, and proposed a formula for calculating the CaCO₃ content

TABLE 4: Effect of soil particle size on consolidation.

| Material | Particle size | Conclusion | Reference |
|----------|--|---|-----------|
| Sand | A: 1.25~2.5 mm B: 0.5~1.25 mm C: 0.04~0.5 mm | Group C has the highest unconfined compression; the intergranular pores are easily filled by CaCO ₃ crystals | [73] |
| Sand | Mikawa $D_{50} = 600 \mu\text{m}$ Toyoura $D_{50} = 200 \mu\text{m}$ | The UCS of Mikawa sand is higher than that of Toyoura sand | [74] |
| Sand | Ottawa silica $D_{50} = 0.46 \text{ mm}$ Mississippi $D_{50} = 0.33 \text{ mm}$ | The solidification strength of Ottawa silica sand is higher than that of Mississippi sand | [42] |

suitable for two particle size ranges. Amarakoon and Kawasaki [74] compared the UCS of cemented silica sands with two particle size ranges (Mikawa sand $D_{50} = 600 \mu\text{m}$ and Toyoura sand $D_{50} = 200 \mu\text{m}$) and found that the Mikawa sand, with a larger mean particle size, exhibited a better solidification result, with a UCS of 3 MPa, while the Toyoura sand, with a smaller mean particle size, showed a fair solidification result, with a UCS of approximately 1 MPa. Similar to that of Amarakoon and Kawasaki, a study by Zhao et al. [42] concluded that the solidification strength of Ottawa silica sand with $D_{50} = 0.46 \text{ mm}$ was more than two times that of Mississippi sand with $D_{50} = 0.33 \text{ mm}$. In summary, particle size is the main factor affecting the MICP solidification results. Soil cementation and strength can be improved by enhancing the particle size gradation of soil. Solidification by MICP should consider the size of the soil particles. However, in practical engineering, the grades of soil particles vary, and different consolidation processes (concentration of the bacterial suspension, cementation solution concentration, grouting methods, etc.) could be used to consolidate the soil.

4. Engineering Properties of Soil Solidified by MICP

4.1. Strength. UCS and shear strength are important indicators for characterizing soil strength. By comparing the strengths of intermediate compressible and highly compressible clays before and after solidification by MICP, Animesh and Ramkrishnan [76] found that the UCS values of the two types of soils increased 1.5 times and 2.9 times due to solidification and determined the optimum mixing ratio of the bacterial suspension and cementation solution. Van Paassen et al. [77] noted that CaCO₃ crystals produced by MICP form bridges between sand particles, thereby increasing the strength and stiffness of the sand, and evaluated the feasibility of ground improvement by MICP through the analysis of the effects of the substrate solubility, CaCO₃ yield, reaction rate, and side-products on MICP. Gowthaman et al. [78] found that the UCS of soil reached 420 kPa after 10 days of treatment of indigenous microorganisms with pure chemicals. In comparison, after treatment of the indigenous microorganisms with inexpensive low-grade chemicals, the UCS of the soil was significantly improved, reaching 820 kPa, and the treatment cost was reduced by 96%. Rong and Qian [79] presented the feasibility of the solidification of

loose sandstone particles by MICP. The cemented bio-sandstone had a satisfactory compressive strength at certain ages, and as the magnesium carbonate content increased, the compressive strength and porosity both increased. Zhao et al. [42] compared the mechanical properties of soil treated with *S. pasteurii* and urease and found that the UCS of the biotreated soil was approximately five times that of the urease-treated soil, indicating that bacteria are more effective for soil solidification. Liu et al. [80] investigated the effect of ultrasound on MICP-treated sand and showed that after treatment by optimal ultrasonic irradiation, the production of CaCO₃ in aqueous solution and in sand columns increased by 28.5% and 35.6%, respectively, and the UCS of the treated sand samples reached 1.25 MPa, which was 91.6% higher than that of the control group.

Although the MICP treatment method can significantly improve the strength of the soil, it can also cause significant brittle failure of the treated soil, which is a problem that can be effectively addressed using the fiber reinforcement method. Choi et al. [81] found that the use of polyvinyl alcohol (PVA) fiber reinforcement can increase the UCS and splitting tensile strength of sand by 138% and 186%, respectively, while decreasing the permeability by 126% and reducing the brittleness (expressed as the ratio of UCS to splitting tensile strength) to half that of plain sand. Fiber reinforcement can increase the precipitation rate and yield of microbially induced CaCO₃, and the cementation effect of CaCO₃ has a facilitating role in fiber reinforcement. Hence, fiber reinforcement technology and MICP technology can be combined to achieve complementary advantages (Figure 9). Fang et al. [82] pointed out that the addition of fibers not only reduced the permeability of MICP-treated coral sand but also improved the dry density and uniaxial tensile strength as well as the ductility, failure strain, and tensile strength. The fiber content had a greater effect on the mechanical properties of the treated coral sand than the fiber length, with an optimum added fiber content of 0.2% and optimum fiber lengths of 9 mm and 12 mm. Xie et al. [83] pointed out that the incorporation of fibers into bio-cemented sand could greatly improve the UCS and residual strength of the soil samples and significantly improve the toughness of the soil samples upon failure (Figure 10). When a sample is damaged, the fibers act as "bridging" (Figure 11) that crosses the fracture and can bear a certain tensile stress, thus effectively inhibiting the further development of the fracture and delaying the total failure of the sample. Zheng

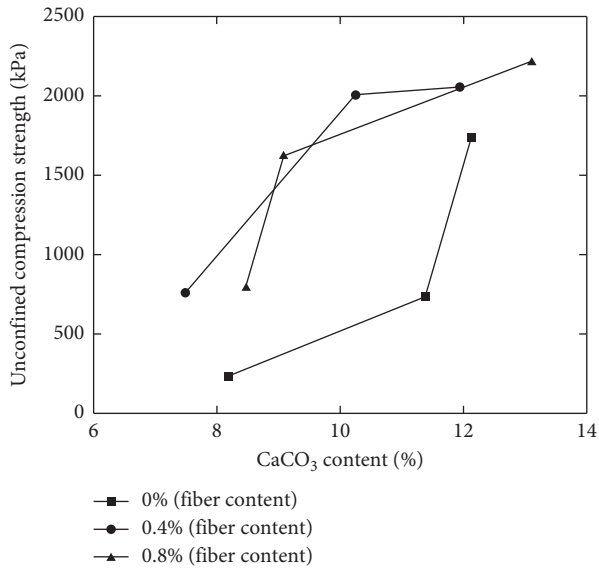


FIGURE 9: Unconfined compression strength plotted against CaCO₃ content for different fiber contents [81].

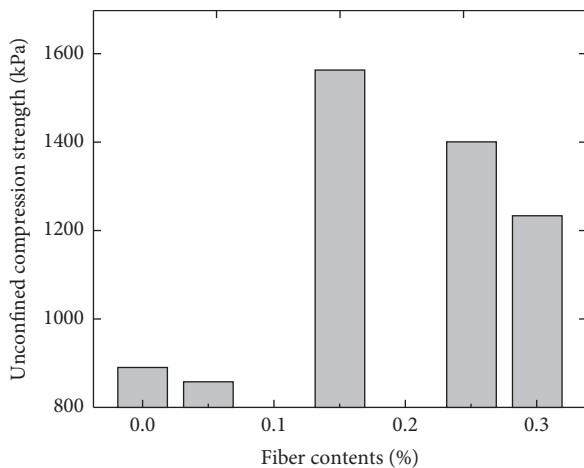


FIGURE 10: Unconfined compression strength of samples with different fiber contents [83].



FIGURE 11: "Bridging" effect of fiber impedes the further development of crack [83].

et al. [84] pointed out that, in the MICP process, CaCO₃ crystals can be effectively deposited onto the surface of the fibers, improving their surface roughness, and that a mixture of CaCO₃ and sand can provide anchorage to the fibers, thereby improving the shear strength and strain-softening properties of the biocemented sand to a certain extent. In summary, MICP technology can significantly improve the strength of soil, and a combination of MICP and fiber reinforcement can effectively solve the brittle failure problem of the solidified soil while improving the soil strength. In actual engineering projects, suitable solidification methods can be selected in response to engineering needs.

4.2. Stiffness. Stiffness is an important indicator for characterizing the ability of soil to resist elastic deformation and can be measured by the shear wave velocity obtained from shear modulus or bender element tests. DeJong et al. [11, 85, 86] used bender elements to monitor the variation in the shear wave velocity of microbially induced cemented sand and obtained the relationship between the shear wave velocity and the duration of injection and treatment. The shear wave velocity reached a maximum of 540 m/s at 1700 min, and the shear stiffness was significantly improved. In addition, grouting and treatment tests were carried out on a shallow foundation model, and static load test results showed that the bearing capacity and stiffness of the foundation were significantly improved, as demonstrated by a nearly fivefold decrease in the settlement under the same load. Van Paassen et al. [87, 88] carried out in situ grouting and improvement of a 100 m³ sand foundation. After the completion of treatment, shear wave velocities of different regions were detected. The shear wave velocities in different parts of the sand foundation increased significantly with an increase in cementation time, with an average shear wave velocity of 300 m/s, indicating that stiffness of the sand foundation increased effectively. In addition, it was suggested that the increase in stiffness should be quantified as a function of the volume of the injected solution and the distance from the injection points. Liu et al. [89] concluded that the role of CaCO₃ in the MICP process is to strengthen the connection between adjacent soil particles and increase the stiffness of the soil so that it can bear higher stress under the same strain. In summary, MICP technology can significantly improve the soil stiffness and is effective in improving soils that are mainly subjected to compressive stress.

4.3. Liquefaction Resistance. Saturated loose sand is prone to liquefaction under seismic or wave loads, causing severe damage to buildings or transportation infrastructure. Therefore, it is necessary to reinforce soil against liquefaction. As a new foundation treatment method, MICP reinforcement technology could effectively improve the antiliquefaction performance of liquefied sand foundation (Figure 12). After MICP treatment, the compactness of sand is increased, and the cement could improve the cementation between particles, which can improve the strength of soil, reduce the internal pore water pressure, inhibit the occurrence of large strain of sand, and protect against the

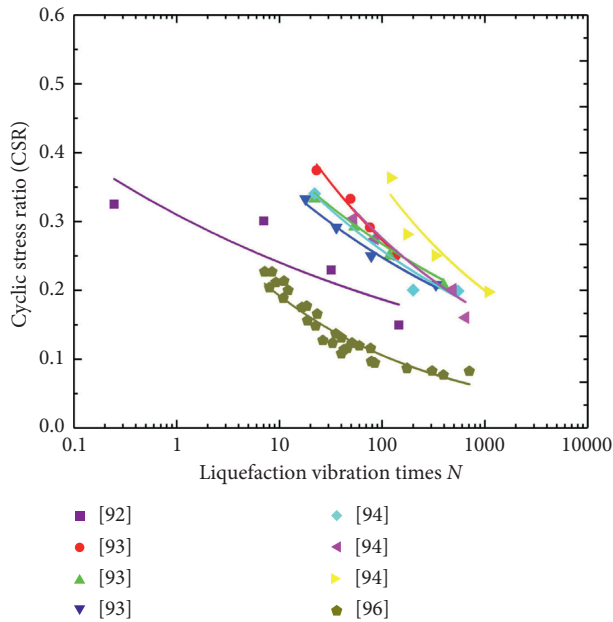


FIGURE 12: Relationship between CSR and N .

liquefaction effect. Liu et al. [90] divided the development of the pore pressure in MICP-cemented calcareous sand into four stages: the initial stage, stable development stage, rapid development stage, and complete liquefaction stage. The dynamic shear stress ratio and deformation resistance of the cemented calcareous sand were markedly improved, indicating that MICP cementation can significantly improve the liquefaction resistance of calcareous sand. Zhang et al. [91] carried out shake table tests on MICP-treated calcareous sandy foundations in the South China Sea. Compared to those of untreated foundations, the excess pore water pressure and ground settlement of the MICP-treated calcareous sandy foundation decreased, indicating that the liquefaction resistance of the treated calcareous sandy foundation was considerably improved. Zamani and Montoya [92] investigated the liquefaction characteristics of MICP-treated fine silty sand and found that the liquefaction resistance of fine silty sand was significantly improved after MICP treatment, the efficiency of which depended on the fines content (dictating the relative density) and the fabric governing the structure, indicating that MICP is a potential method for silty sand improvement. Xiao et al. [93] argued that the cyclic shear stress and confining pressure are important factors affecting the liquefaction of calcareous sand; an increase in the confining pressure causes a decrease in the liquefaction resistance of plain sand and MICP-treated sand samples, and an increase in cyclic shear stress causes a decrease in the cyclic strength. The number of liquefaction cycles increased with an increase in CaCO_3 content, indicating that MICP treatment can significantly improve the liquefaction resistance of calcareous sand. Han et al. [94] conducted dynamic triaxial tests of MICP-treated sand and concluded that solidified sands with different strengths can meet different engineering requirements, the solidification time can be shortened to 1 to 2 days by reducing the use of

bacterial suspensions and nutrients, and MICP is effective in improving the liquefaction resistance of sand. Han and Cheng [95] characterized the effect of different calcium salts on the MICP solidification efficiency using ammonium and found that calcium acetate was the most effective in improving the mechanical properties of liquefiable sand and that the hydraulic permeability of the solidified sample was significantly reduced. Cheng et al. [96] explored the liquefaction resistance of solidified sand using dynamic triaxial tests and found that when the amount of CaCO_3 that precipitated in the solidified sample was higher, the axial deformation of the sand sample was smaller, and the increase in the liquefaction resistance was more pronounced. The use of soil desaturation induced by microorganisms to alleviate soil liquefaction is also a method for preventing foundation liquefaction. The desaturation effect of soil is realized by the gas produced by the microbial denitrification process, which can effectively reduce the saturation of sand.

4.4. Permeability. The calcite formed by microbial mineralization fills the pores of soil particles, reducing the porosity and permeability of the soil (Figure 13). Lai et al. [97] tested the permeability of two kinds of sand using MICP technology at low pH; the results showed that the sample with a large decrease in permeability contained more CaCO_3 , while the sample with a small decrease in permeability contained less CaCO_3 . Therefore, the content of CaCO_3 was the key factor that affected permeability. Chu et al. [98] used MICP technology to form a cementation layer of a certain thickness on the sand surface. When an average of 2.1 kg of calcium (Ca) per m^2 of sand surface was precipitated, the permeability coefficient of the sand ranged from 10^{-4} m/s to 10^{-7} m/s. Qabany and Soga [47] investigated the permeability of British standard grade *D* silica sand with different dry densities and cementation concentrations. The results demonstrated that both loose samples and dense samples showed a reduction in permeability with an increase in the amount of CaCO_3 precipitation. Shen et al. [99] used *Bacillus megaterium* to solidify sandy clayey purple soil. The results showed that, with the formation of CaCO_3 , its permeability initially decreased with an increase in CaCO_3 content and then decreased by two orders of magnitude. Jawad and Zheng [100] pointed out that MICP technology has greater strength improvement and better solidification efficiency for dry sand and a better reduction in permeability for saturated sand. From the above-mentioned analysis, it can be concluded that, with CaCO_3 precipitation, the soil permeability decreased significantly, and the permeability decreased rapidly as the CaCO_3 content increased. Therefore, MICP technology has great prospects for the anti-seepage of dams, embankments, and reservoirs. Notably, MICP has been utilized to improve soil properties for different purposes [101], but these properties are not independent. The permeability, strength, shear wave, and uniformity of precipitation are correlated. Dawoud et al. [102, 103] examined the relationship between hydraulic conductivity, *s*-wave velocity, and calcite content with different treatment solutions, confining pressure and back

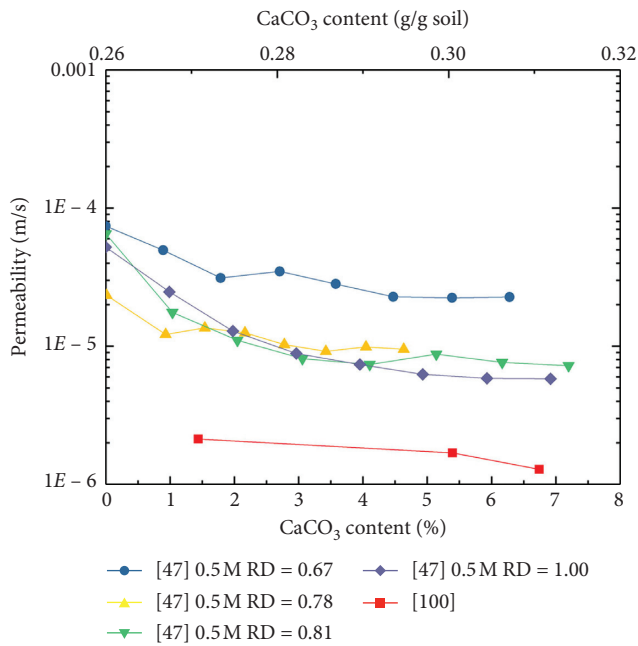


FIGURE 13: Relationship between permeability and CaCO_3 content.

pressure. The results indicated that the s -wave velocity could be considered a direct indicator of the calcite content. All samples had similar characteristic, and the hydraulic conductivity at early stages of treatment decreased slightly and then decreased quickly. The lack of uniform precipitates caused earlier clogging of the pores and a dramatic decrease in hydraulic conductivity. The researchers suggested that less uniformity was attributed to the common distribution of the injected bacteria and examined the effect of surfactants on the transport and distribution of the bacteria and on MICP efficiency and distribution. The results indicated that less than 2% of the bacterial cells were retained in sand columns when surfactant was applied, and had a better distribution of calcite precipitation.

4.5. Durability. Durability is an important indicator for evaluating the long-term performance of MICP-treated sand under environmental parameters (such as wet-dry cycles, freeze-thaw cycles, acid rain, wind erosion, etc.). Cheng et al. [104] investigated the mechanical properties of MICP-cemented biobricks; showed that the compressive strength of biobricks prepared under partially saturated conditions was 9 MPa, which is twice that under fully saturated conditions; and evaluated their water absorption, resistance to salt attack, and fire resistance. The results showed that biobricks are suitable for use as a building material and could also be a green alternative to traditional fired clay or cement bricks. Liu et al. [105] employed MICP to treat tiles to form an erosion-resistant layer and found that MICP could significantly improve the water resistance of ceramics, with higher concentrations of bacteria and cementation reagents enabling the sediment layer to provide better protection. The concentration threshold reduced the positive effect of the bacterial concentration on the water resistance of the surface

of the sample, and the protection layer provided satisfactory durability and effectively alleviated the weathering of the ancient clay roofing tiles. Chae et al. [106] found that, with a wind speed of 15 m/s, the amount of wind erosion of medium sand or fine sand after solidification using 0.5 M MICP solution was relatively small, so appropriate injection methods should be chosen to reduce soil erosion, considering the soil properties. According to the wind tunnel test results, Nikseresht et al. [107] argued that an increase in penetration resistance decreased the soil loss of soil MICP solidified with vinasse and molasse to approximately one-third of that of the blank sample, indicating that the soil treated by molasse and vinasse had a high resistance to wind erosion. Huang et al. [108] pointed out that due to the poor cementation between particles within the MICP-grouted shale residual soil, when the frost heave force generated by the pore water during the freeze-thaw cycle is greater than the MICP cementation strength, the cementation fails, which causes cracking of the sample, and the shedding area increases with an increase in water content and the number of freeze-thaw cycles. Gomez et al. [109] applied a bacterial suspension and cementation solution to cement and stabilize the surface of loose tailings sand, eventually forming a 2.5 cm thick hard cementation layer. The test results showed that the effective improvement depth reached 28 cm, significantly increasing the erosion resistance of the loose tailings sand. Jiang et al. [110] conducted a series of seepage erosion tests on mixed soil samples (sand:kaolin = 5:1) after bio-cementation and solidification. The results showed that the critical hydraulic gradient and shear stress of the solidified soil were significantly improved, while the amount of internal erosion under the same hydraulic conditions was significantly reduced. Cheng et al. [111] continuously injected artificial acid rain (pH = 3.5) into cemented sand columns. He found that mass was continuously lost and that the strength was reduced by up to 40%, indicating that biocemented sand has a low resistance to acid rain erosion, mainly because the H^+ in acid rain reacts with the CaCO_3 between the sand particles, destroying the cementation of CaCO_3 . Liu et al. [112] conducted a series of experiments on the long-term engineering performance of MICP-treated sandy soil under wet-dry cycles, freeze-thaw cycles, and acid rain conditions. The results showed that the durability of MICP-treated soil was weak under these conditions. The drop in UCS was nearly 80% after one wet-dry cycle, 58% after 15 freeze-thaw cycles, and 83% after 15 days of immersion in acid rain solution. In conclusion, the resistance to wind and water erosion of MICP-treated sand is significantly improved; freeze-thaw and wet-dry erosion are weak; and the resistance to acid rain erosion is poor because of the dissolution of CaCO_3 in acid.

5. Engineering Applications of Soil Solidification Using MICP

5.1. Ground Improvement. MICP technology can significantly improve the strength, stiffness, and liquefaction resistance of soil and is suitable for ground improvements such as improving the permeability of sand. Van Paassen et al.

[87, 88, 113] carried out a large-scale in situ sand foundation grouting and improvement experiment (Figure 14). First, a 1 m^3 sand pile experiment was conducted. With a single-point injection method, the bacterial suspension and cementation solution were sequentially injected from the center of the cubic sand pile at a constant flow rate. After 40 days of continuous treatment, the average content of CaCO_3 precipitates in the sand body was 100 kg/m^3 , and the highest uniaxial compressive strength of the sand sample was 9 MPa. On this basis, a 100 m^3 large-scale grouting experiment was conducted. Three injection wells and three pumping wells were arranged, and treatment was carried out continuously for 16 days using grout of the same composition to obtain a 40 m^3 clearly visible cemented sand body with a wedge shape. The average amount of CaCO_3 precipitation was 110 kg/m^3 , the uniaxial compressive strength of the cored samples ranged from 0.7 to 12.4 MPa, and the average shear wave velocity of the sand foundation was 300 m/s, indicating that the strength of the foundation after MICP treatment was significantly improved. Liu et al. [114] carried out an in situ experimental study of the MICP treatment of calcareous foundations on an artificially reclaimed island. The results showed that after three to four MICP treatments, a gradual increase in ground surface strength was detected. After nine MICP treatments, the surface strength was greater than 10 MPa and reached 20 MPa; the treated depth of the foundation reached 70 cm; and the UCS reached 821 kPa. Montoya et al. [115] conducted MICP treatment tests on the liquefiable sand free field using a centrifuge shake table and found that, after treatment, the pore pressures at different depths of the site decreased to varying degrees with different seismic intensities and that the postshaking surface settlement of the MICP-treated site was significantly smaller than that of untreated loose sand but that the surface acceleration was enhanced to some extent compared to that of untreated loose sand. Darby et al. [116] conducted 80 g centrifuge shake table tests on three different sets of treatment models with CaCO_3 contents of 0.8%, 1.4%, and 2.2%; the results showed that, after MICP treatment, the cone penetration resistance of the sand increased from 2 MPa to 5, 10, and 18 MPa and that the shear wave velocity increased from 140 m/s to 200, 325, and 660 m/s. As the level of cementation increased, the liquefaction resistance increased, no further liquefaction occurred, and the mechanical properties of the model sample gradually changed from those of soil to those of rock. He et al. [117] conducted shake table tests using microbial denitrification to produce gas bubbles and found that, at an acceleration of 0.5 m/s^2 , the untreated saturated loose sand was completely liquefied, with an excess pore pressure ratio near 1; the surface settled markedly; and the volumetric change reached 5%. After different levels of microbial treatment, the saturation of the sand decreased within the range 95%–80%. For the model with 80% saturation and at an acceleration of 0.5 m/s^2 , the excess water pressure ratio was only 0.1, and almost no settlement occurred at the surface.



FIGURE 14: Field test of MICP foundation reinforcement [113].

5.2. Foundation Seepage Control. The application of microorganisms in the field of soil antiseepage mainly uses the mineralization of microorganisms and microbial membranes. Compounds produced by microbial mineralization have the effect of filling and cementing in materials. Extracellular polymeric substances (EPS) secreted by microorganisms can be attached to the surface and interior of porous materials to form microbial membranes, which reduces the permeability of porous materials. Chu et al. [98] used microbial technology to construct a reservoir using 2.1 kg of CaCl_2 per m^2 of the sand surface, and after MICP surface treatment, the permeability of the sand could be reduced from an order of 10^{-4} m/s to an order of 10^{-7} m/s . Subsequently, samples were taken from the pool bottom for a four-point flexural test, the flexural strengths were found to be 90 to 256 kPa, and the UCSs of the sidewalls and pool bottom were found to be 215 to 932 kPa. Subsequently, samples were taken from the base of the pond for a four-point bending test; their flexural strengths were found to fall into the range of 90 to 256 kPa; and the UCSs of the samples taken from the walls and the base of the pond bottom were found to fall between 215 and 932 kPa; that is, different parts of the pond had strengths of varying degrees (Figure 15(a)). Liu et al. [119] reinforced the levee model by spraying microbial cells and nutrient salts and performed flume tests on the treated model. After multiple days of scouring, no erosion damage occurred in the overall model, except for a small amount of fine sand on both sides of the model sample, which was produced by the water flow. The permeability coefficient of the surface soil decreased from $4 \times 10^{-4} \text{ m/s}$ to $7.2 \times 10^{-7} \text{ m/s}$, and the maximum UCS was 9 MPa. Tan et al. [118] carried out field tests of MICP seepage control in three sections of the clay banks of the Dawa Reservoir by utilizing the mineralization of microorganisms and monitored both internal water heads with the pressure measuring pipe inside the dam and the leakage amount at the leakage site. The results showed that this technology could rapidly reduce the permeability coefficient of the clay bank by two orders of magnitude (Figure 15(b)). Gao et al. [120] proposed a

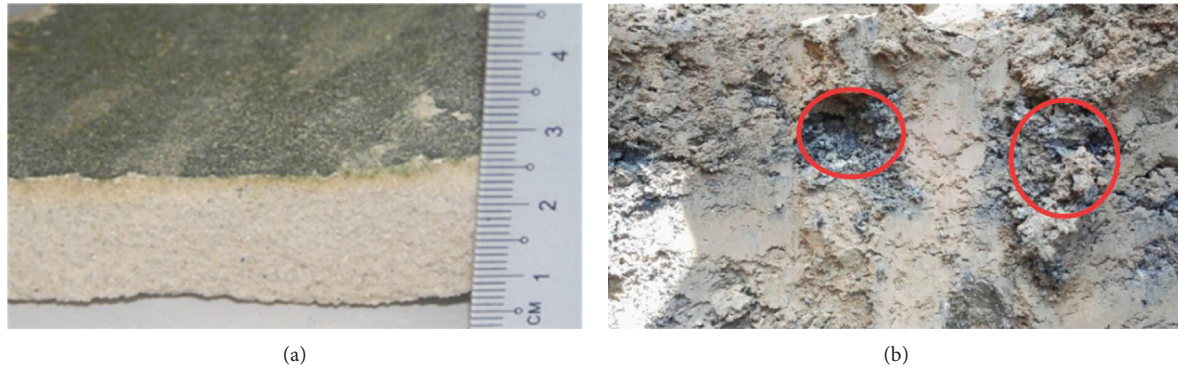


FIGURE 15: Permeability reduction effect: (a) a low-permeability layer surrounding the boundary of the pond [98]; (b) filled pores in the dam [118].

construction process for seepage control channels by MICP treatment: the channel site to be constructed was treated using the injection pipe process, followed by excavation, and then the channel surface was treated using spray and soaking techniques (with treatment liquid consisting of ureolytic bacteria, 0.5 mol/L calcium chloride, and 0.5 mol/L urea). The test results showed that the construction process can effectively reduce the permeability coefficient of the sand surface and thus meet the engineering requirements. Cheng et al. [121] applied CaCO_3 precipitation techniques combined with the reaction of sodium alginate with Ca^{2+} ions to form a gel-like calcium alginate for sand seepage control treatment. The test results showed that the permeability of the treated sand decreased from 5.0×10^{-4} m/s to 2.2×10^{-9} m/s and that the seepage control efficiency increased by one to two orders of magnitude compared to that treated with pure MICP technology. Blauw et al. [122] were among the earliest to apply biofilm technology to seal leakages in clay-core embankments along the Danube River, Austria, using in situ microbial growth for 23 days of nutrient injection. After 10–14 weeks, the discharge of the dam per unit time significantly decreased from 17.33 m^3 per day before repair to 2.35 m^3 per day after repair. The leakage at the grout inlet was tested again after 5 months and was found to be only 10% to 20% of that prior to treatment, indicating the feasibility of biofilm technology in the restoration of Earth structures. Therefore, microbial technology can achieve soil seepage prevention. For different engineering problems, different types of microbial reaction processes and construction techniques can be adopted. However, the natural environment of an actual project is relatively complex, and its durability needs further study.

5.3. Erosion Resistance of Foundations. MICP-treated soil has good resistance to hydraulic and wind erosion. Bang et al. [64] conducted six biocement surface spray treatments of a sandy embankment model. The flume scour test of the hydraulic model showed that the treated model experienced continuous erosion from the overtopping water with different flow rates for 30 days without collapse. The anatomical test of the model revealed that CaCO_3 aggregated within

3 cm of the surface and formed a crust-like layer. In addition, biocement can be utilized to control piping erosion within the soil. Jiang et al. [110] showed that, after the sand-kaolin mixture was treated with biocement, the critical hydraulic gradient increased significantly, and the mass of solid particles carried away by water erosion was greatly reduced. To prevent and control the erosion damage to embankments caused by overtopping flow, Liu et al. [119] improved the mechanical properties of embankment surface sand by spraying microbial cells and nutrient salts into the embankment surface layer so that the gelation of CaCO_3 could rapidly precipitate in the sand pores. The results showed that the maximum UCS could be 9 MPa and that the permeability coefficient was reduced from 4×10^{-4} m/s to 7.2×10^{-7} m/s. MICP technology can effectively improve the erosion resistance of the surface of the embankment model to prevent and control damage caused by overtopping flow (Figure 16). Naeimi and Chu [123] performed wind tunnel tests to verify the ability of microorganisms to treat sand surfaces for dust suppression and wind erosion resistance and estimated the amount of biological dust suppressants needed, which was less than the amount of conventional dust suppressant currently used for road and airport field applications. To study the feasibility, stability, and vegetation recoverability of the field stabilization of surface sand, Gomez et al. [109] conducted field tests of MICP-treated surfaces at a Canadian mine site. The test area consisted of four treated plots of different concentrations of MICP reaction solution, where each plot had an area of $2.4 \text{ m} \times 4.9 \text{ m}$ and a design treatment depth of 0.3 m. After surface treatment, dynamic penetration tests showed that the ground penetration resistance significantly increased, with the crust thickness ranging from 0.64 to 2.5 cm. The CaCO_3 content on the surface of the crust was approximately 2.1%, and the formed CaCO_3 decreased with an increase in depth and was only 0.5% at a depth of approximately 10 cm. The results from the standard penetration test showed no significant degradation of the ground strength on day 64. Zhan et al. [124] used the enzymatic action of *Paenibacillus mucilaginosus* to absorb and transform CO_2 to produce carbonate ions, which then react with calcium ions present in the environment to mineralize and form a calcite cementation layer with certain mechanical

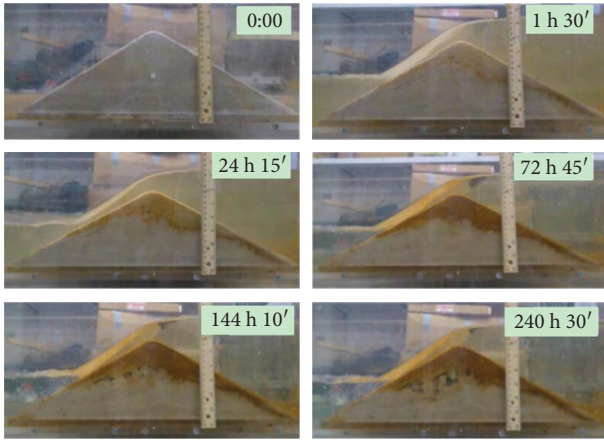


FIGURE 16: Flume test of treated model by spray method [119].

properties for dust suppression, and carried out a 900 m² field application using this technique. After biotreatment, the treated field area had an average solidification thickness of 13.2 mm and Shore hardness of 24.6. In a wind erosion test with a wind speed of 12 m/s, the mass loss of the treated soil was 30 g/(m²·h), representing a decrease from 2600 g/(m²·h) of the original untreated soil. In the rainfall erosion test, the mass loss of the biotreated soil was approximately 60 g/(m²·h), showing a decrease in the mass loss of 750 g/(m²·h) of the original untreated soil, and the residual Shore hardness remained more than 90% of that prior to rainfall erosion. In addition, the hard structure of the calcite surface layer formed by microbial mineralization can also improve soil moisture retention, which is beneficial to soil water retention and plant growth. Li et al. [125] combined straw checkerboard barrier (SCB) technology and MICP technology to study desertification prevention and control, for which 16 key factors were summarized and their parameters and effects were analyzed in detail. The results showed that MICP technology can effectively compensate for the deficiencies of SCB technology, and a combination of the two technologies could achieve satisfactory results in mitigating desertification and accelerating the process of sand fixation, vegetation recovery, and ecological restoration.

5.4. Fixation of Heavy Metals. Microbial mineralization converts ionic heavy metals into solid-phase minerals and precipitates heavy metal ions, resulting in a decrease in their bioavailability. The metabolites (sulfur ions and phosphate ions) of some microorganisms undergo precipitation reactions with metal ions, converting toxic and harmful metal elements to nontoxic or low-toxicity metal precipitates. Fujita et al. [126] conducted tests on the remediation of ⁹⁰Sr contamination at a site in the state of Washington in the United States, testing the addition of urea and molasses to promote the growth of native ureolytic microbes and the solidification and precipitation of heavy metals in the field and circulating the treatment using injection piping several meters apart during treatment and pumping. The test results showed that MICP technology can be employed to treat ⁹⁰Sr contaminants in the field. Xu et al. [127] also carried out a

field experimental study on the removal of heavy metal ions by MICP. Gram-positive bacteria were utilized as the strain for mineralization to treat an abandoned iron ore mine, with a field remediation depth of 20 cm and an area of 1000 m². The exchangeable concentrations of As, Pb, Cd, Zn, and Cu in the contaminated soil were 14.01, 4.95, 0.64, 33.46, and 12.95 mg/kg before treatments. The parameters for field MICP spray treatment included an ambient temperature of 30°C, pH of 5.5, urea dosage of 12.65 kg, and bacterial dosage of 160 L. After remediation, the exchangeable concentrations of the abovementioned heavy metals were reduced to 2.37, 1.25, 0.311, 16.67, and 3.42 mg/kg, respectively. Tests have shown that treatment of heavy metal-contaminated soil by spraying with salt mineralization microbes is effective, with the highest heavy metal removal rate being 83%, thereby significantly lowering the risk that heavy metal ions will be absorbed by crops. Cheng et al. [128] selected soil *Bacillus* bacteria as carbonate mineralization bacteria and used their substrate-induced enzymatic decomposition to produce CO₃²⁻ and thus mineralize and solidify the available heavy metals in the soil, such as the precipitation of Cd²⁺ to a stable state of carbonate, obtained in an available heavy metal removal rate that exceeds 50%. Ganesh et al. [129] used iron-reducing bacteria (*Shewanella alga*) and sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) to reduce hexavalent uranium to tetravalent uranium and then precipitated tetravalent uranium to form uraninite (UO₂ (s)), which was then removed from aqueous solution. Macaskie et al. [130] showed that the Gram-negative bacterium *Citrobacter* secretes large amounts of hydrogen phosphate ions via phosphatases to form minerals with heavy metals on the bacterial surface. Sondi et al. [131] successfully precipitated heavy metal ions from SrCl₂ and BaCl₂ solutions using urease to obtain SrCO₃ and BaCO₃ and investigated the effect of urease on the crystal growth process and final crystal shape during the precipitation process. Uniform nanoscale spherical particles were formed at the initial stage of the reaction, and the spherical particles were transformed into rod-like clusters of alkaline minerals in the subsequent stage of the reaction. Deng et al. [132] isolated a strain of *Penicillium chrysogenum* from soil contaminated by smelter slag and applied it to the remediation of contaminated soil, leaching out 62.8% of the Cd, 55.5% of the Cu, 53.9% of the Zn, and 14.4% of the Pb. Ren et al. [133] explored the leaching efficiency of *Aspergillus niger* for heavy metal-contaminated soils in Shenyang Smeltery and discovered that the leaching efficiency was the highest with a soil-solution ratio of 5%, reaching 25.2%, 98.3%, 2%, and 15.7% for Cu, Cd, Pb, and Zn, respectively. Banerjee et al. [134] isolated a strain of *Pseudomonas brenneri* from coal mine wastewater, which was able to remove 96.3% of 60 mg·L⁻¹ Cr (VI) within six days under optimized conditions (pH = 6.0, temperature: 30°C). Zheng et al. [135] verified that, in addition to effectively adsorbing Cr (VI), *Bacillus subtilis* has the ability to reduce Cr(VI) to Cr(III) under aerobic conditions, indicating that the *nfrA* gene is directly involved in the synthesis of reductase. In summary, microbial remediation technology, which is based on biomineralization, remediates heavy metal-contaminated soil through the

solidification or leaching of heavy metal ions. This technology is simple and easy to operate, does not cause secondary pollution, and can effectively reduce the impact of metal ions on the environment, showing favorable application prospects.

6. Conclusion and Prospects

As a new research field in geotechnical engineering, microbial geotechnology is an interdisciplinary subject involving microbiology, chemistry, and geotechnical engineering. In this review article, the hydrolysis reaction and cementation mechanism of MICP were briefly described; the influences of factors such as the bacterial concentration, cementation solution concentration, pH, temperature, grouting method, and particle size on MICP-treated soil were discussed; engineering properties such as the strength, stiffness, liquefaction resistance, permeability, and durability of MICP-treated soil were evaluated; and the applications of MICP technology in the areas of soil reinforcement, geotechnical seepage control, sand liquefaction control, fixation of heavy metals, wind erosion control, and sand fixation were summarized. Hence, this review article provides a reference for the development of MICP technology in the field of geotechnical engineering. The main conclusions are as follows:

- (1) MICP is a common microbial mineralization phenomenon in nature. Through metabolic reactions, MICP absorbs, transforms, removes, and degrades substances in the environment. Researchers utilized different metabolic processes to attain MICP, which include urea hydrolysis, denitrification, ferric reduction, and sulfate reduction.
- (2) The solidification efficiency of MICP is affected by factors such as the bacterial concentration, cementation solution concentration, pH, temperature, grouting method, and particle size. The selection of a reasonable optimization scheme is especially important for improving the mechanical properties of solidified soil.
- (3) MICP technology can significantly improve the strength, stiffness, liquefaction resistance, and durability of soil and reduce the permeability of the soil, which has notable advantages for embankment and dam seepage control projects.
- (4) MICP technology is applicable to soil improvement, geotechnical seepage control, sand liquefaction control, contaminated soil remediation, wind erosion prevention, and sand fixation. This method is expected to be implemented in CO₂ sequestration, desert greening, marine land reclamation, concrete repair, and ancient building restoration.
- (5) Currently, most of the MICP research has been conducted under laboratory conditions, while field-scale geotechnical engineering projects have been rarely conducted. The application of this technology in geotechnical engineering faces certain practical

challenges, such as technology optimization, operability, cost, equipment, and environmental factors.

Based on current research results, we believe that future MICP research should focus on the following aspects:

- (1) For different types of geotechnical materials and performance improvement requirements, bacteria with high enzyme-producing ability, high environmental adaptability, and high efficiency in solidification should be cultivated, taking into account the related costs
- (2) The engineering properties of the rock and soil should be improved by stimulating the production, reproduction, and metabolism of native microorganisms in the soil, which will in turn promote the homogeneity of MICP-treated soil by optimizing the grouting method
- (3) By means of meso/microscopic test methods, the meso/microscopic structural changes in the solidified soil caused by microbial cementation filling, ion transport, and fiber bending and interweaving should be investigated to reveal the underlying synergistic solidification mechanisms of MICP and fiber reinforcement
- (4) Based on studies of the erosion resistance of MICP-treated soils with single factors (wind erosion, water erosion, salt erosion, freeze-thaw erosion, and ultraviolet erosion), the durability of MICP-treated soils considering combinations of various factors should be evaluated

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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