# Microbially Induced Carbonate Precipitation

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Microbially induced carbonate precipitation (MICP) is a promising technology for solidifying sandy soil, ground improvement, repairing concrete cracks, and remediation of polluted land. By solidifying sand into soil capable of growing shrubs, MICP can facilitate peak and neutralization of CO2 emissions because each square meter of shrub can absorb 253.1 grams of CO2 per year.

Keywords: micro-organism ; urease ; curing ; sandy soil ; desertification

## 1. Introduction

Microbially induced carbonate precipitation (MICP) is a promising technology applied to many civil and environmental engineering scenarios, especially combating desertification [1]. Desertification refers to the process of land degradation in arid, early semi-dry, and arid and subhumid areas under the action of various factors, including climate and human activities. The severe problem of global desertification is caused by natural and human factors, climate change, wind and rain erosion of the soil, the pursuit of economic benefits, destruction of vegetation, and unreasonable use of water resources, all of which aggravate the formation of desertification. Because the emergence of desertification has caused a significant impact on the environment and economical construction, it is highly urgent to control it [2]. Kimura et al. [3] classified and counted the global dry areas in 2017 according to the satellite-based aridity index (SbAI). Figure 1 [3] shows areas of global arid areas in 2017 and their proportion in the total land area. It can be seen from Figure 1 [3] that the total area of global arid areas accounts for 41% of the total land area. Figure 2 [4] shows the global desertification risk level distribution map from 2000 to 2014, estimated based on the global Desertification Vulnerability Index and the ratio of areas with different risk levels. The colors in Figure 1 represent different levels of global desertification risk.

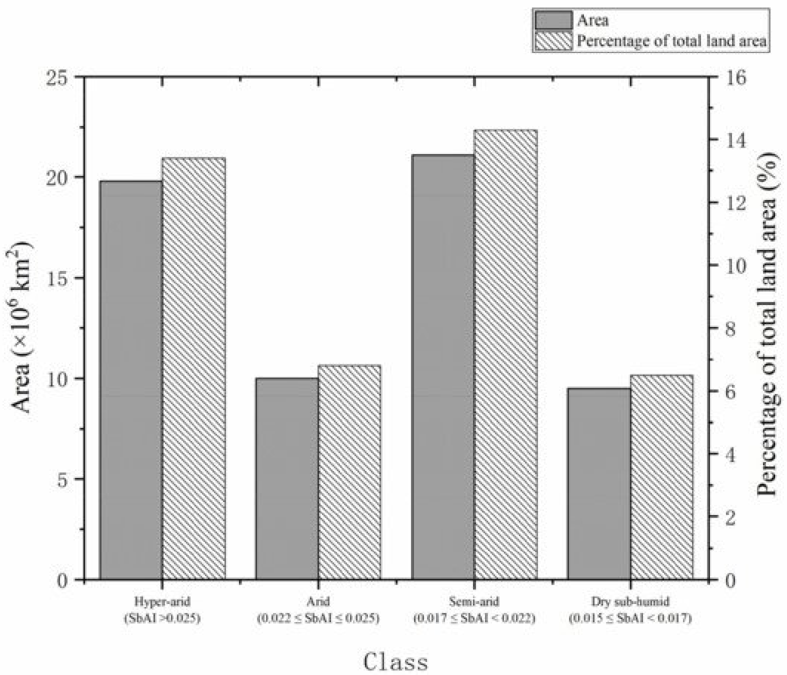


**Figure 1.** Schematic diagram of arid areas worldwide and their percentage of total land area in 2017 based on the Satellite Drought Index (SbAI) [3].

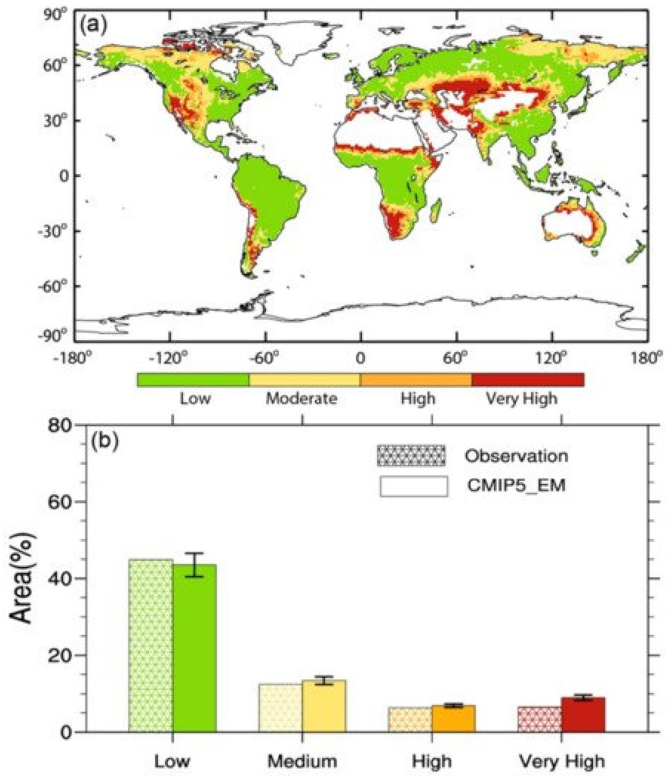


**Figure 2.** (**a**) The global desertification risk level distribution from 2000 to 2014, estimated based on the Global Desertification Vulnerability Index; (**b**) The proportion of areas with different desertification risk levels in the global land area [4].

Sand consolidation uses various ways to reduce the sand porosity and fix the sand particles [5]. As one of the common ways of consolidation, grouting consolidation is defined as applying pressurized grouting slurry to infiltrate within the void of sandy soil, followed by the compaction and solidification of sand along with the slurry [6]. The grouting method usually includes the chemical grouting method and the biological grouting method [7][8]. In the early stage, methods of sand consolidation in desertification were limited to chemical grouting with cement, lime, and other chemical materials. However, various sand consolidation methods emerged following extensive research, including sand fixation with the sand barrier, chemicals, and microbial grouting [7][8][9]. The grouting method can only be used for coarse sand with a particle size greater than 4.75 mm, and microbial sand consolidation can be used for fine or medium sand with less than 0.6 mm [10]. Chemical grouting methods cost less, but chemical grouting materials (e.g., cement, lime, or adhesive) are harmful to the environment. The microbial grouting method has a relatively high cost but is friendly to the environment and can effectively improve the properties of sand [7][8]. **Figure 3** [6] shows a schematic diagram of the grouting method. Microbial sand fixation refers to adding cementation solution to stimulate bacteria and then forming calcium carbonate crystals in the sand to consolidate the sand. Cementation solution generally refers to a mixture of calcium salts, nutrients and urea. **Figure 4** [11] shows a diagram of the experimental setup for MICP. Microbial sand fixation not only has the advantages of environmental protection, low pollution, effective maintenance of soil moisture in sandy deserts, improvement of soil fertility, and improvement of soil thermal conductivity, but can also turn the sandy desert into soil and increase the area of state-owned arable land, which is of practical significance for the curbing of desertification. Countries around the world put forward the strategic goal of carbon peak, and carbon neutrality due to global climate change leads to many extreme climate events. “Peak carbon” refers to when carbon dioxide emissions reach a peak and then stop rising and gradually fall back. Carbon neutrality means achieving zero carbon dioxide emissions by offsetting total greenhouse gas emissions through afforestation, energy conservation, and emission reduction. The United States and the European Union have announced carbon neutrality by 2050 and China by 2060. Microbial sand fixation can also facilitate carbon peaking and carbon neutralization because microbial sand solidification technology can enable the sand to grow shrubs. Li et al. [12] found that each square meter of shrub can absorb 253.1 grams of carbon dioxide per year. All in all, microbial sand fixation has gradually become an important research topic [13][14].

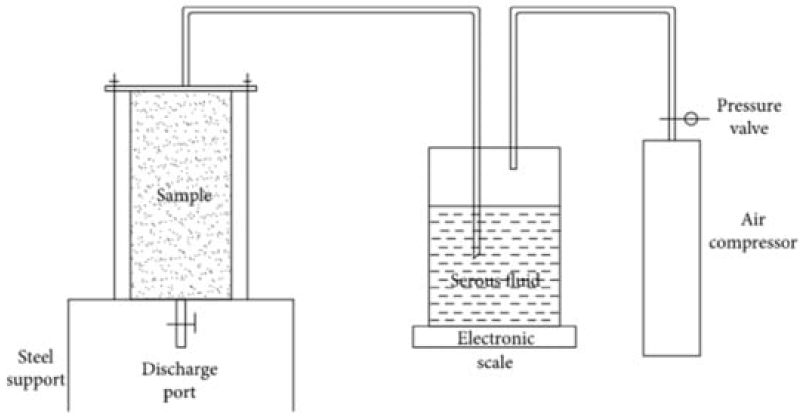


**Figure 3.** Schematic diagram of grouting consolidation device [6].

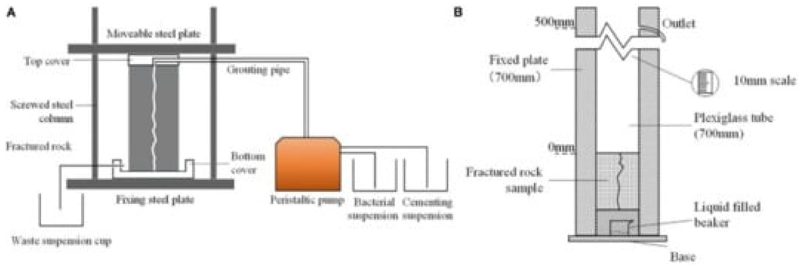


**Figure 4.** Schematic diagram of MICP experimental device. (**A**) Grouting device; (**B**) Seepage device [11].

The source of the urease-producing microbe of MICP can be indigenous or exogenous [15][16][17]. If the urease activity of the local (indigenous) urease-producing bacteria is high, the local bacteria can be directly used for the sand fixation. However, if the activity of local urease-producing bacteria is low, the cementation solution with nutrients should be added to stimulate the local bacteria to produce enough urease. In addition, exogenous urease-producing bacteria can be added to increase the effect of microbial sand consolidation. After treatment with MICP technology, the porosity and water conductivity of soil are reduced due to the combination of $CaCO_3$ precipitation and the medium, and the soil after solidification is not easily liquified by the action of earthquakes [18][19][20]. Due to the tiny pores in the clays, it is difficult for the bacteria to enter the clays, and therefore there are few studies on the microbial solidification of clays. Liu et al. [21] studied the effect of MICP on the repair of dry cracks in clays. Sun et al. [22] treated a sand-clay mixture with MICP and found that different amounts of clays need to be added to solidify sand with different particle sizes.

Microbial sand fixation technology is not limited to small-scale laboratory studies but is also applied to large-scale outdoor studies. **Figure 5** [23] and **Figure 6** [24] are photos of extensive outdoor experiments. **Figure 7** [25] is a schematic diagram of the test system. Meng et al. [26] conducted outdoor experiments in Ulan Buh Desert, and the results confirmed that the use of *Sporosarcina pasteurii* to consolidate desert soil could improve the wind erosion resistance of soil. Outdoor microbial sand fixation experiments need to overcome many difficulties and the cost is relatively high. According to the results of small sand fixation experiments in the laboratory, part of the results of large outdoor experiments can be predicted by using the model [27].

**Figure 5.** Photos of microbial sand fixation in the field [23].

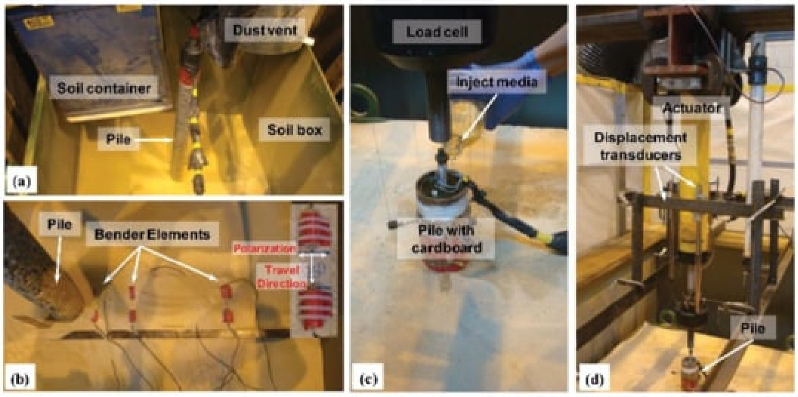


**Figure 6.** Detailed device drawing for large MICP grouting (**a**) soil raining; (**b**) bender element installation; (**c**) media injected from the top of the pile; (**d**) pull-out loading setup [24].

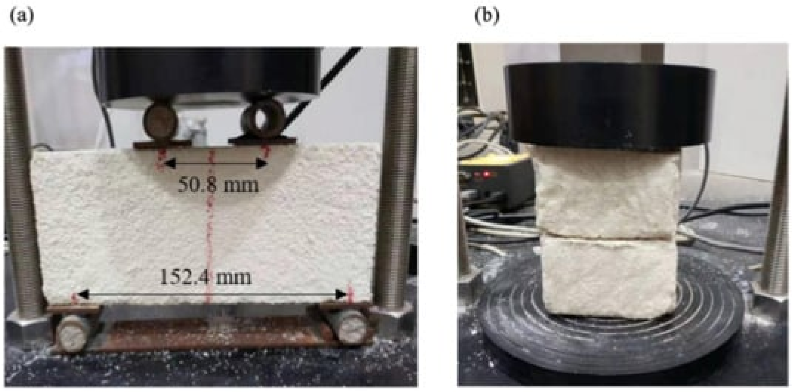


**Figure 7.** Schematic diagram of MICP test system. (**a**) Four-point bending test system; (**b**) Compressive strength testing system [25].

Microbial sand consolidation is affected by many factors, such as the concentration of the cementation solution, the concentration of culture liquid, temperature, calcium source and pH value. After optimizing various factors, the solidification effect of the sand body after microbial solidification can be effectively improved [28]. Micro-organisms can induce $CaCO_3$ to cement the curing medium, and the degree of curing needs to be measured or characterized by microscopic photographs taken by precision instruments. Scanning electron microscopy (SEM), light microscopy and other instruments are usually used to observe the generated calcium carbonate crystals, which can more intuitively reflect the effect of microbial sand consolidation. There are other methods to characterize the solidification effect of microbial cementation based on microscopic photographs, the determination of $CaCO_3$ content, permeability, shear wave velocity, Fourier transforms infrared spectrum analysis, and scanning electron microscopy [29][30][31].

MICP technology has dramatically developed in the past ten years in laboratory-scale solidified sand. However, there are still many challenges to overcome in applying MICP to field-scale practical engineering. Due to the long study cycle of MICP sand treatment and the high cost of large-scale field operations, few studies have been conducted on large-scale outdoor solidified sand [1]. In addition, the application of MICP to the harsh environment, including high temperatures, freeze–thaw cycles, wet–dry conditions, and acid rain, needs further study [32]. Ammonium ions produced during MICP can be hazardous to the environment if left untreated [33]. The removal method of the ammonium ion should also be further studied in the future.

## 2. Microbial Sources of Solidified Sand

There are two methods of microbially solidifying soil: one is the introduction of exogenous bacterially solidified soil, but as there are plenty of other microbes in the sand, the exogenous bacteria may compete with the existing microbes for nutrients. Therefore, it is necessary to add exogenous bacterial fluid constantly, which results in high cost. The other method is to use urea-producing bacteria existing in sandy soil, not by introducing exogenous bacteria, but by adding cementation solution and nutrients that facilitate the growth of indigenous bacteria. These two sand consolidation methods will affect the size and quantity of the calcium carbonate generated. Gomez et al. [34] found that compared with the calcium carbonate generated by exogenous bacteria, the size and quantity of calcium carbonate crystals generated by indigenous bacteria solidified sand soil were larger and fewer.

### 2.1. External Bacteria Solidifying the Sandy Soil

There have been many studies on introducing exogenous bacteria in order to solidify sandy soil. It is necessary to add cementing fluid to stimulate indigenous bacteria to produce calcium carbonate precipitation, and at the same time, introduce exogenous urease-producing bacteria that can enhance the effect of microbial sand consolidation. Bernardi et al. [35] solidified sand with *Sporosarcina paseurii*, and the minimum porosity ratio of sand was 0.5. When the concentration of urea was 200 mM, the concentration of calcium chloride was 100 mM, and the $OD_{600}$ of the bacterial solution was one, the porosity ratio of sand samples after 28 days of treatment decreased to about 0.33, because the generated calcium carbonate was blocked in the original gaps in the sandy soil. Nafisi et al. [36] compared the effect of curing silica sand with *Sporosarcina pasteurii* and urease powder. They found that compared with curing silica sand with urease powder, curing silica sand with *Sporosarcina pasteurii* generated more calcium carbonate, and the shear strength of the solidified sand sample was greater. However, Ahenkorah et al. [37] compared the mechanical properties of sand samples solidified by MICP and EICP, and found that the splitting tensile strength of sand treated by EICP is higher than that of MICP. Cheng et al. [38] solidified the sand by a single-phase injection of low pH integrated solution into the sand, and mixed $OD_{600}$ of 4.2 *Bacillus* sp. with 1 M urea-calcium chloride solution to form an integrated solution. The pH of the solution was adjusted to four, and the rate of solution transmission was 1 L/h. After six times of treatments, the compressive strength of the sand sample reached 2.5 MPa.

Exogenous bacteria are also used in curing sand in the sea; Cheng et al. [39] proposed an innovative method of biological sand fixation method, which, without introducing $Ca^{2+}$, the $Ca^{2+}$ contained in seawater was used as the sole source of calcium, and then *Bacillus* sp. was introduced to solidify the sand in the seawater environment. The experimental results showed the feasibility of using $Ca^{2+}$ from seawater to solidify sandy soil, including that seawater can be used many times and is beneficial to improving the mechanical performance of sandy soil. The use of MICP technology for biocementation in the seawater environment provides a potential method for land reclamation. Xu et al. [40] proposed an experimental scheme similar to that of Cheng, using no additional introduced exogenous $Ca^{2+}$, and only using $Ca^{2+}$ from the fly ash of municipal incineration waste. The ratio of the fly ash to *S. pasteurii* bacterial solution was 1 kg:0.3 L. At 20 °C, humidity is not less than 95% for the 7 days curing experiment environment. The results show that the leaching rate of heavy metals decreases obviously after the solidification of fly ash, and the compressive strength increases by nearly 40% compared with that before the solidification. Wang et al. [41] used MICP technology to reduce the wind erosion rate of sandy soil. Their results showed that the wind erosion rate of untreated sandy soil was 10.23%, but when MICP treatment times were more than three times, the wind erosion rate of sandy soil dropped below 0.4%. Wind erosion rate is the ratio of the mass of the remaining sand that has been blown by the wind to the mass of the original sand that has not been blown.

Using exogenous bacteria to solidify sand requires the addition of bacterial liquid and cementation solution. The newly added exogenous bacteria will compete with the bacteria inside the sandy soil, so the bacterial solution needs to be added at intervals to ensure that the exogenous bacteria survive. Many studies have shown that exogenous bacteria can solidify soil, but by adding exogenous bacteria to the sand, it can be found that there is a lot of precipitation generated at the filling mouth, and the sediment distribution in the sand is not uniform. Moreover, the introduction of exogenous bacteria may not be conducive to the protection of ancient buildings, such as the reinforcement of the surface of ancient buildings and the repair of cracks, etc., the introduction of exogenous bacteria may destroy the dynamic balance of the bacterial community inside the original ancient buildings, and may cause secondary damage to the ancient buildings [42]. Therefore, solving these problems can be the direction of future research.

### 2.2. Solidification of Sandy Soil by Indigenous Bacteria

The indigenous bacteria themselves exist in the sandy soil and have strong adaptability to the environment. There are two ways to solidify sandy soil with indigenous bacteria. The first is to screen out indigenous bacteria from the soil for culture and then add the bacterial culture solution and cementation solution in the sand; the other is to directly add nutrients for the in-situ culture of bacteria and then add the cementation solution to the sand. The utilization of indigenous bacteria is economical and effective, causes less environmental pollution, and may lead to the uniform distribution of induced $CaCO_3$ precipitation [43]. The introduction of indigenous bacteria can be used for the conservation of ancient buildings. It can be seen from **Figure 8** that the untreated surface of ancient buildings in the $MgSO_4$ solution has a fast dissolving rate, and after dealing with the indigenous bacteria on the ancient building surface, the surface of the ancient building was not obviously dissolved under $MgSO_4$ solution erosion, indicating that indigenous bacteria carried out on the ancient surface treatment can reduce the salt chemical weathering and thus protect the surface of ancient buildings [42].

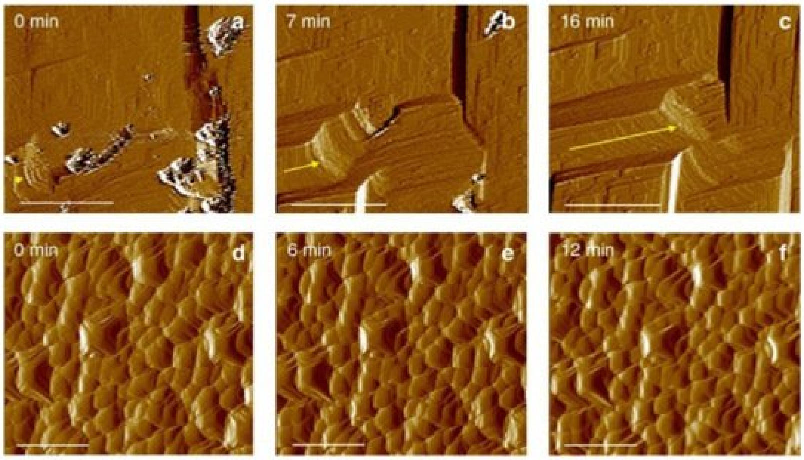


**Figure 8.** AFM image of the calcite surface over time after exposing the ancient building surface to MgSO4 solution. (**a**–**c**) Untreated; (**d**–**f**) Indigenous bacteria-treated [42].

Cheng et al. [44] enriched urease bacteria in soil and added the cementation solution to the soil for in-situ curing experiments. The results showed that in-situ curing does not cause surface blockage on the 1 m-high soil column, but the method of injecting the cementation solution should be constantly optimized in order to achieve deeper curing. Burbank et al. [45] found that urease-producing micro-organisms can be isolated in soils lacking urea or a high concentration of ammonia, and these microorganisms could be applied to mineralize the soil and repair the existing cracks in the soil. Kumari et al. [46] reported that MICP could fix Cd in the soil at low temperatures. *E. undae* YR10 isolated from the Yangtze River basin near Chongming Island fixed Cd in farmland soil near Chongming Island at 10 °C and 25 °C, and then converted Cd into components in carbonate. Burbank et al. [47] selected the soil with high urease content for microscopic tests and cyclic triaxial shear tests and found that not introducing exogenous bacteria and directly adding mineral solution can induce calcite precipitation, so as to improve the anti-liquefaction ability of sand; this method can achieve greater economic benefits than the addition of exogenous bacteria. Chahal et al. [48] screened and isolated urease-producing bacteria from alkaline soil to repair cracks formed in concrete and improve the life of the concrete. Gowthaman et al. [49] successfully isolated *Lysinibacillus xylanilyticus*, a bacterium from the sub-arctic region, which can produce urease at low temperatures, and successfully applied this bacterium to a slope-improvement project. The greater the urease activity of urease-producing bacteria, the better its effect in solidifying sand. Wang et al. [50] screened urea-producing bacteria from beach sand and studied the effects of different media and urea concentrations on bacterial urease activity. The experiment results showed that nitrogen-rich composite media such as YE and NB increase bacterial urease activity, and urease activity at 100 mM concentration is highly efficient. Khan et al. [51] isolated a urease bacterium named *Parahodobacter* sp. from a beach of coral sand. *Parahodobacter* sp. was used to cure the coral sand for 28 days, and the compressive strength of the solidified coral sand reached 20 MPa. Oualha et al. [52] screened and isolated two strains of indigenous bacteria *B. cereus* from Qatari soil, namely QBB4 and QBB5. Both of these strains can solidify Qatari soil with a high pH and in a poor environment, and in a field experiment, the $CaCO_3$ content generated by soil solidified with QBB4 increased by 16.2% compared with the original soil. Song et al. [53] isolated *Staphylococcus succinus*J3 with high urease activity from the soil in a mining area and showed that the application of coal ash in a bacterially mineralized mining area could play a positive role. After solidification, the maximum wind speed of coal ash reached 45.5 m/s, and the maximum wind pressure reached 912 kPa. For the first time, Imran et al. [54] isolated indigenous urea-lytic bacteria from coastal erosion areas in Greece and showed that $CaCO_3$ could be generated, effectively protecting the coast from erosion. Chu et al. [55] isolated urease-producing bacterium (UPB) VS1 from tropical beaches and found that the solution of urea and calcium chloride added was lower than the sand surface, and calcium carbonate is evenly distributed in the sand. The solution of urea and calcium chloride added was higher than the sand surface, and the resulting calcium carbonate formed a solid shell on the sand surface.

## 3. Models for Predicting the Curing Process of MICP in the Field

The technology of microbial solidification of sand is relatively mature in the laboratory. For example, Phillips et al. [56] repaired sandstone fractures using two grouting methods and the experiment showed that multiple grouting methods promote the even distribution of $CaCO_3$ deposit in sandstone along the inflow direction. However, excessive repeated treatment leads to deposition blockage near the injection point [57]. Microbial solidification of sand has also been used in the field. Cuthbert et al. [58] applied MICP technology to fractured rocks and showed that when bacterial fluid and urea are simultaneously injected into fractured rocks, the addition of $CaCl_2$ solution promotes the formation of $CaCO_3$ precipitation to repair cracks, thus significantly reducing the permeability of rocks.

However, field tests need to overcome difficulties caused by many environmental factors and are very expensive [59]. Therefore, field tests are rare. Harkes et al. [60] used the two-stage method of adding bacterial solution and fixation solution in order to make the calcium carbonate generated by MICP evenly distributed in the sand, but the operation is complicated and the economic cost is high. Due to the complexity of the natural environment, some phenomena are difficult to explain. Ohan et al. [61] found that after applying MICP, the pH value of groundwater decreased, which contradicted the normal pH value increase.

The researchers found that models could be used to better analyze the dynamic changes and reaction mechanisms of the MICP process. A good model can predict the mechanical properties of MICP solidified sand samples and is helpful for the engineering design [62]. **Table 1** lists some models that predict the curing process of MICP. Fauriel et al. [63] proposed a prediction model of the microbial grouting response based on the changes in porosity, permeability and density of soil after microbial grouting. Connolly et al. [64] introduced urease genes into *P. aeruginosa* AH298 and *E. coli* AF504gfp to construct two urease strains of *Pseudomonas aeruginosa* MJK1 and *Escherichia coli* MJK2 that had a characteristic of expressing green fluorescent protein (GFP), and used the Gompertz function to model the bacterial population density. It was found that the urealytic rate of the two strains was not high, *Escherichia coli* MJK2 grew faster, and *Pseudomonas aeruginosa* MJK1 had a higher urealytic rate. Gai et al. [65] established a model to evaluate the mechanical properties of MICP-solidified sandy soil, which clearly reflects changes in the mechanical properties of solidified sand soil, and the analysis of model parameters and the law of mechanical properties change are helpful to understand the process of MICP solidification. The results showed that mechanical properties are related to $CaCO_3$ content. Wang et al. [66] established a biochemical-hydraulic model, which proposes the concept of porosity to reflect the change of permeability. Their results showed that the pore structure has an important influence on the curing rate, the maximum urease rate has an indispensable influence on the hydraulic response of MICP, and the MICP reaction rate is influenced by the concentration of the bacterial and cementation solutions. Martinez et al. [67] proposed a biological reaction migration model, which coupled UCODE-2005 with TOUGHREACT sequence, and its practicability was confirmed in the MICP prediction experiment. TOUGHREACT numerical simulation program was used to reflect the reaction rate of urea hydrolysis and $CaCO_3$ generation in the MICP process. UCODE-2005 model was used to correct and verify the MICP experimental data. The results showed that the actual experimental results are close to the predicted data in the half-meter sand column experiment and dynamic changes in the MICP process can be seen.

**Table 1.** Models for predicting the curing process of MICP.

| Model Names | The Role of Models | References, Year |
| --- | --- | --- |
| **Aquifer conceptual model** | **Finding that the sedimentation rate of calcite is closely related to the hydrolysis rate of urea** | **[68], 2005** |
| **A three-dimensional (3D) discrete element method (DEM)-based numerical model** | **Simulating the macroscopic mechanical properties of $CaCO_3$ sediment-solidified sandy soil induced by micro-organisms under the condition of no triaxial compression of the drainage system** | **[69], 2019** |
| **A loose sandstone numerical model based on a one-dimensional advective dispersion model** | **Predicting the movement of micro-organisms in soil and rock** | **[70], 2014** |
| **A pore-scale network model** | **Simulating the $CaCO_3$ precipitation process and the influence of different operations on $CaCO_3$ precipitation** | **[71], 2016** |
| **Thermal conductivity predictive models** | **Predicting the thermal conductivities of MICP-treated sands** | **[72], 2020** |
| **A small repeated five-point treatment model** | **Predicting solidification treatment in large-scale field experiments** | **[73], 2014** |
| **The biogrouting foam model** | **Simulating key solidification processes such as on-site bacterial solution perfusion and adhesion and urea hydrolysis** | **[74], 2019** |

The solidification effect and mechanical properties of MICP can be simulated by the model. Before the large-scale outdoor experiment, model analysis of the existing laboratory-scale experimental data can be carried out to predict the results of the large-scale outdoor experiment. The combination of model analysis and laboratory data is conducive to the smooth implementation of large-scale field experiments [27][75]. At present, researchers have established many models, and each model has its own role. In the future, multiple models can be combined to improve the accuracy of the model prediction.

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