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Research Article

Investigating the Factors Affecting the Properties of Coral Sand Treated with Microbially Induced Calcite Precipitation

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Microbial-induced carbonate precipitation (MICP) can be used to cement coral sand to improve its engineering properties to protect coastal structures. In this study, a series of laboratory tests were conducted to test the effect of the MICP method by using an ureolytic bacterium (*Sporosarcina pasteurii*). In order to determine the activity of bacteria, the growth properties of the microbial strain were observed under different culture conditions (different pH and temperature). The effect of partial size distribution and nutrient concentration on the soil permeability and unconfined compressive strength was then examined in coral sand. The results showed that the pH had less effect on the bacteria growth compared to temperature. The bacteria can growth well at pH over 8 and temperature higher than 20°C. The well-degraded soil has higher unconfined compressive strength (1.91–2.61 MPa) than poor-degraded soil (1.31 MPa). The similar trend was also found in permeability reduction. The unconfined compressive strength increased as the biocement solution concentration increased to 1 mol/L and then decreased at 1.5 mol/L.

1. Introduction

Coral sand is widely distributed on coral reefs and seashores in South China Sea. In coastal engineering, there are many buildings and breakwaters or other structures constructed on coral sand, or using coral sand as backfill materials for road embankment or airport runways. However, the coral sands consist of shells and corals with high void ratio. The strength is rather low compared to silica sand and can be easily crushed under load. So it is necessary to improve its strength before it is used as foundation or backfill materials. There are many traditional technologies such as pile driving, grouting, and vibrocompaction, which have been successfully applied to other soils. However, these methods are ineffective when applied to calcareous sand due to its high carbonate contents and low strength. For the grouting method, it may also cause pollution to the marine environment. Other methods may cause the breakdown of coral sand [1, 2].

In recent years, a novel ground improving method was proposed to minimize the environmental problems by microbial-induced carbonate precipitation (MICP [3, 4]). The MICP technique involves two biochemical reactions: (1) the hydrolysis of urea by the urease enzyme produced by bacteria and (2) the precipitation of calcite with the presence of Ca^{2+}. With the production of calcite, the soil properties were changed. The chemical reaction equation can be expressed as follows:

$$CO(NH_2)_2 + H^+ + 2H_2O \xrightarrow{\text{bacteria}} 2NH_4^+ + HCO_3^- \quad (1)$$

$$Ca^{2+} + HCO_3^- \longrightarrow CaCO_3 + H^+ \quad (2)$$

MICP has a broad application in various conditions because it can reduce the permeability of soil and improve soil strength, mitigating liquefaction, and stabilizing costal sand dunes [5–14].

It has been well studied by the researchers to optimize the treatment in the laboratory or field experiments on silicon sand [7]. Harkes et al. reported that the unconfined compression strength (UCS) varied from 0.2 to 20 MPa according to the amount of calcite precipitation [15]. Others also found similar results that the UCS ranges from 1 to

12 MPa for silicon sand [16]. Meanwhile, the MICP effect quite relied on the experimental conditions, such as the pH, temperatures, and chemical species concentrations [6, 8, 17]. The change of the conditions can influence both the activity of the bacteria and the chemical reaction rate and then affect the precipitation of calcite. It is difficult to develop an injection approach that can generate homogeneous distributed calcite in the soil. Previous studies have reported non-homogeneous distribution of calcite in soil especially in long distance injection. In order to achieve a homogeneous calcite fill in the soil, they have tried to slow down the injection flow or inject with some fixation solution with bacteria. But these works did not completely solve the problem, more detailed researches are still required to get a better strategy.

Although soil improvement using MICP has shown a great promise in silicon sand, a few studies have been reported to apply the MICP effect on coral sand [13, 18, 19]. Based on the previous studies of the silicon sand, this study aimed at determining the efficiency of MICP treatment to improve the properties of coral sand. First, the growth characteristics of the bacteria were measured under various culture conditions. Then, a series of tests were conducted to explore the effect of particle size distribution and cementation solutions on the efficacy of MICP. Soil permeability and UCS tests were conducted on the biocemented soil columns.

2. Materials and Methods

2.1. Bacteria Cultivation. The test bacteria *Sporosarcina pasteurii* (ATCC 11859) was used in this study. The bacterial strain was cultured in the liquid medium under various conditions (pH and temperature) and found the optimal growth conditions. The cell density was quantified by measuring the absorbance of the suspension using a spectrophotometer at 600 nm wavelength (OD_{600}). The bacteria were first grown on the plate media and incubated at 30°C. The cultivation solution ingredients are listed in Table 1. After the plate growth, the bacteria were harvested and inoculated in the liquid media to grow for 24 h at 180 rpm with an aeration of 1 : 2.5 (200 mL of the media in a 500 mL flask) to an optical density of 600 nm (OD_{600}) of 1–1.3. This OD_{600} value can ensure the bacteria had high urease activity during the experiment.

The following two factors were analyzed to investigate the growth condition of the bacterial strain: (1) the pH of the liquid culture media ranged from 8 to 11 and (2) the culturing temperature varied from 5 to 35°C.

2.2. Soil Column Preparation. The sands used in this study were collected from Nansha Island. The sands were crushed down and sieved through 5 mm sieve to be used in the experiments. Three different particle size distributions (PSDs) were prepared by mixing different size sands. In Figure 1, soil #1 and #2 are classified as well graded, and #3 is poorly graded. The mean particle size of soil #1 is fine, and #3 is coarse. The summary of the properties (porosity e, dry density ρ_d, coefficient of uniformity C_u, and coefficient of curvature C_c) is presented in Table 2. The relative density of soil was around 2.7–2.85 g/cm^3.

Table 1: Summary of the microbial-induced carbonate precipitation recipe.

Solution	Constituents
Growth media	0.5% peptone 0.3% yeast extract 2% urea 10 mg/L $MnSO_4{\cdot}H_2O$ with agar for plate growth
Cementation media	$CaCl_2$ Urea

The soil columns were prepared in a PVC column with 50 mm in diameter and 120 mm in height. A mesh and filter paper were placed at the bottom of the column to minimize the loss of soil particles during the test.

2.3. MICP Treatment. A peristaltic pump was used to inject the bacteria and cementation solutions to the soil column from the bottom to the top and then let it drain from the top to the bottom. The flow rate was set to 1 mL/min. Each sample was flushed by 1.5 pore volumes of bacteria-0.5 M $CaCl_2$ solution first. The bacteria-$CaCl_2$ solution was kept within the sample for 6 h to allow the bacteria to attach to the soil surface. Then, 1.5 pore volume cementation solutions (urea and $CaCl_2$) were pumped to the soil sample and kept for 12 h. The bacteria and cementation solution were injected four times as described before.

To determine the effect of various conditions on the UCS of the specimen, the following conditions were considered: the concentrations of urea and $CaCl_2$ used were 0.5 mol/L, 1.0 mol/L, and 1.5 mol/L. Each sample was prepared in triplicates.

3. Results and Discussion

3.1. Bacteria Growth Conditions Tests. The growth curve of the microbial strain in different culture conditions (pH and temperature) was obtained to investigate the effects of various conditions on bacterial growth.

3.1.1. The Effect of pH. The cell densities were reflected by the value of OD_{600}. OD_{600} obtained at different pH values from 8 to 11 is shown in Figure 2. The results showed that the OD_{600} can reach to above 1 after about 8 h. The data presented showed that there is only a slight difference between pH over 9 especially at the stable stage of the bacteria growth curve. It means that these bacteria prefer alkaline environment and can be used in the environmental pH at 9–11. For the biochemical reaction, the calcium carbonate precipitated when pH is above 8.3 and increased up to 9, and the pH tends to lower back to neutral afterwards [10, 20]. As the coral sand mainly exists in seawater, the pH is always larger than 7. So it is possible to apply these bacteria to coral sand from the aspect of the pH value.

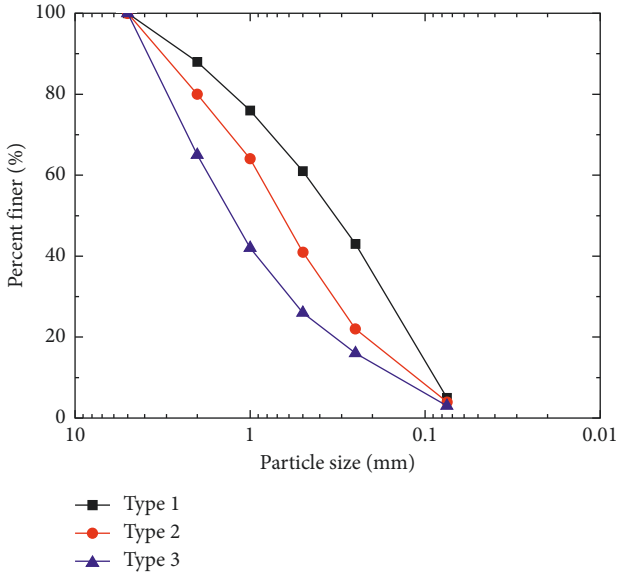

Figure 1: The soil particle distribution curve for 3 types of soil.

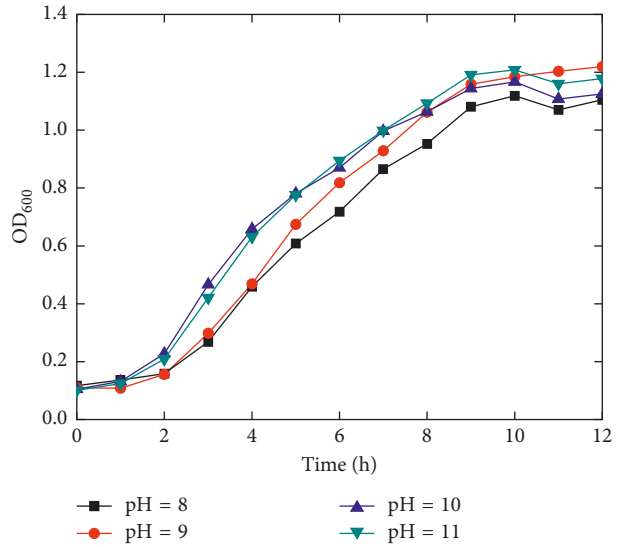

Figure 2: The OD_{600} value of the bacteria solution at different pH values.

Table 2: Initial porosity and parameters for soils.

Parameter	Soil #1	Soil #2	Soil #3
e	0.91	0.85	1.11
ρ_d	1.46	1.51	1.35
C_u	4.82	6.93	9.56
C_c	1.03	1.30	1.38

3.1.2. The Effect of Temperature. In the tests, the bacteria were cultured at 5, 10, 20, 30, and 35°C to measure the growth curve. The bacteria densities increased with the increase in temperature. As shown in Figure 3, at low temperatures (5–10°C), the final OD_{600} was less than 1.0, which was rather low to apply in soil. The best temperature for the bacteria growth is 30–35°C. It has also been reported by Whiffin [21] and van Paassen [16] that the urease activity increased with temperature up to 60°C.

From the aspect of temperature, the bacteria are not applicable to deep seabed but can be used in the offshore area. Or indigenous bacteria which can produce urease enzyme may be used to precipitate calcite in deep seabed.

The temperature had a more significant influence on the bacteria growth ability compared to the pH value (comparing Figures 2 and 3). So it is more important to control the temperature when applying the MICP technique in engineering applications. Thus, the bacteria used in the following soil column experiments were grown at 30°C with pH at 9.

3.2. Permeability. The soil permeability was measured during the MICP process after each injection of cementation solution.

3.2.1. The Effect of Sand Particle Distribution. Three different SPDs of coral sand were used in the experiments. The concentration of the cementation solution in these tests was

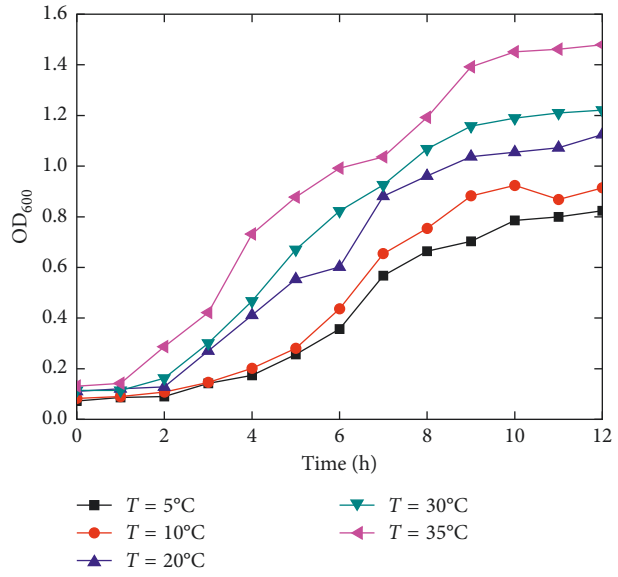

Figure 3: The OD_{600} value of the bacteria solution at different temperatures.

1 mol/L. Figure 4 shows the cemented samples after the MICP process of three soil types. The loose sand was cemented together by the MICP. However, the cementation effect was not that good as there are still some pores at the surface, and the bonding between particles was weak. There were more calcite precipitated at the top of the column and less at the middle and bottom.

The permeability of three soils during the MICP process is shown in Figure 5. There was a significant reduction in soil #2 and a slight reduction in soil #1. For soil #3, the permeability almost remained the same before the MICP process. That was because soil #3 had a high initial porosity and larger particle size. The bacteria were difficult to attach

(a) (b) (c)

Figure 4: The soil column after the MICP process with different soil particle distributions: (a) soil #1; (b) soil #2; (c) soil #3.

to the soil particles and can be washed off by the injection flow even at very low flow rate. Meanwhile, even there were calcite precipitated between the soil particles, the pore space was not completely occupied to block the flow of water. For low porosity and finer sand particles, the soil pore space can easily be blocked by the calcite and then affect the biocement effect. To reach a better cementation effect, the initial pore space cannot be too large or too small. Previous studies had reported that the optimal grain size for the biocementation process is between 50 and 400 μm for sand [22]. The MICP cannot take place in very fine sand, and larger amount of nutrients were required in coarser sand. However, there was less report about the effect of PSD.

3.2.2. The Effect of Solution Concentrations. Figure 6 shows that the cemented samples after the MICP process at three different solution concentrations of 0.5, 1, and 1.5 mol/L. The soil used in these experiments was soil #2. The biocement effect of 1 mol/L and 1.5 mol/L was better than 0.5 mol/L as illustrated in Figure 6.

Figure 7 shows the permeability of soil #2 cemented at three different solution concentrations. The permeability was reduced after the biocement process. When the solution concentrations were low at 0.5 mol/L, the calcite amount in soil was low. The permeability only changed slightly. For 1.0 and 1.5 mol/L, the permeability change almost had the same trend. The higher the solution concentration injected, the better the MICP effect.

3.3. Unconfined Compressive Strength

3.3.1. The Effect of Sand Particle Distribution. The soil was taken out from the column and dried for 7 days after the permeability test for the unconfined compressive strength test. Table 3 shows the UCS strength of three soils during the test. Unconfined compressive strengths were not obtained for every sample due to the poor cementation effect of some samples. Soil 2# was cemented the best compared to 1# and

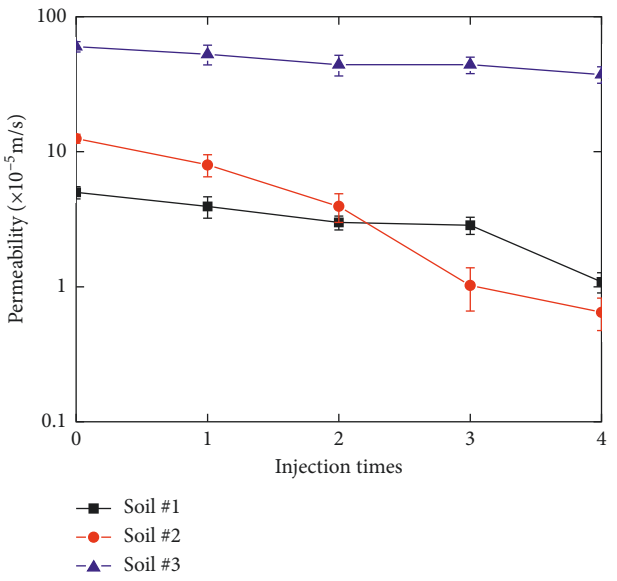

Figure 5: Soil permeability after each injection of the bacteria and cementation solution with different soil particle distributions.

3#. There were visible voids or deflections in soil 3# as the pores were larger. The UCS value was larger in soil 2# to about 2.61 MPa and 1.31 MPa for soil 3#. During the experiments, the same amounts of nutrients were injected to the column, but soil #1 and #3 had larger pore volumes than soil #2.

3.3.2. The Effect of Cementation Solution Concentration. To investigate the effect of cementation solution concentration on MICP-treated coral sand, the sand column was prepared under three different cementation solution concentrations using soil #2. The soil was selected because of its best MICP efficiency as described before. Table 4 shows the UCS strength of soil biocemented with three solution concentrations. The biocement effect of 1 mol/L and

(a) (b) (c)

Figure 6: The soil column after the MICP process with different solution concentrations: (a) 0.5 mol/L; (b) 1 mol/L; (c) 1.5 mol/L.

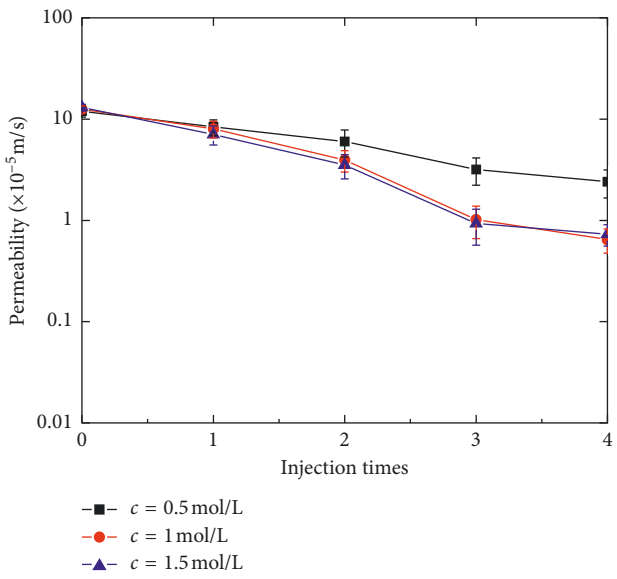

Figure 7: Soil permeability after each injection of the bacteria and cementation solution with different cementation solution concentrations.

Table 3: Soil UCS after the biocement process for 3 soil types.

Soil type	UCS (MPa)
Soil #1	1.93 ± 0.32
Soil #2	2.61 ± 0.51
Soil #3	1.31 ± 0.45

1.5 mol/L was better than 0.5 mol/L. Although the amount of calcite increased as the concentrations increased, the distribution pattern at the pore scale was also affected by the concentration. At higher concentration (1.5 mol/L), there were more calcite deposited at the inlet, while at lower concentration (mol/L), calcite distribution was more homogeneous.

Table 4: Soil UCS after the biocement process for 3 soil types.

Solution concentration (mol/L)	UCS (MPa)
0.5	1.53 ± 0.34
1	2.61 ± 0.55
1.5	2.31 ± 0.47

4. Conclusions

MICP is a complex biochemical process which has been used to improve coral sand properties. Identification of different factors enables the control of MICP in geotechnical engineering. Understanding how different treatments and sand properties could affect MICP is very important. This study describes the influence of pH and temperature on bacteria growth, soil particle size distribution, and solution concentrations on soil permeability and strength. Based on the experimental data, the following conclusions were drawn:

(1) The pH values within 8–11 had little effect on the growth of bacteria.

(2) The temperature had greater effect on the bacteria activity: at low temperature, the bacteria did not have high enough density for the MICP application. The best temperature is over 30–35°C.

(3) The permeability of biocemented coral sand was reduced after the MICP process. However, the well-graded sand and medium porosity sand has a larger reduction in the permeability.

(4) The results of UCS showed that the SPD and solution concentrations have an obvious effect on the MICP-treated coral sand. For well-cemented coral sand, the UCS can reach up to 2.6 MPa.

The MICP process was very complex. The results reported in this paper will be employed to further investigate the use of MICP on improving the soil strength.

Data Availability

All the data supporting the conclusions of this study are presented in the tables of the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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