Experimental Study on the Permeability of Microbial-Solidified Calcareous Sand Based on MICP

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Abstract: In the construction of artificial islands in distant seas, calcareous sand has been widely used as a foundation filler due to its excellent mechanical properties and extensive availability in the marine environment. How to store more fresh water on the artificial islands by reducing its permeability is currently a great challenge. Microbial-induced carbonate precipitation (MICP) has always been considered as a great potential method to improve the cemented properties of calcareous sand, but the effect of grain gradation on the permeability of MICP-improved calcareous sand remains unclear. In this research, a self-made device was developed to conduct MICP grouting and permeability tests, where the permeability coefficient (k) under different grain gradations (curvature coefficient (C_c) and uniformity coefficient (C_u)) was measured. A CT scan was conducted to investigate the variation in the porosity (n) of sand samples before and after MICP treatment. The weighting method was adopted to measure the content of induced calcium carbonate (M). A scanning electron microscopy (SEM) technique was used to further study the micromechanism of the MICP treatment. Finally, the correlations between the k of MICP-treated sand and C_u, as well as C_c, were semiquantitatively analyzed. The results show that the magnitude of M, k and n changes are closely related to C_c and C_u. The reduction amount of k and n increased with the rise in C_c and C_u, and the increased amount of M increased with the rise in C_c and C_u. The SEM results show that the particle surface became rough due to the coating effect of CaCO_3 crystals, and the pore spaces were reduced because of the partially filling effect of the crystals, which was responsible for the decrease in permeability and porosity. Furthermore, k fitted well with C_u and C_c, respectively, and the fitting curve reveals that larger C_u (C_u ≥ 6.0) and smaller C_c (2.0 > C_c > 0.5) were more suitable for MICP treatments and lead to a large reduction in permeability. The above results indicate that the grain gradation of calcareous sand had a significant influence on its permeability improved by MICP.

Keywords: MICP; calcareous sand; grain gradation; permeability coefficient; porosity

1. Introduction

The construction of artificial islands is in full swing all over the world due to space limitation in coastal areas [1,2] and transfer transportation requirements in distant seas (e.g., Fiery Cross Island in the South China Sea). On the artificial islands, the storage and access of fresh water are important fundamental conditions for human living [3,4]. In China, it has been reported that the cost of transporting high-quality tap water from the mainland to one island reaches USD 370 thousand per year [5]. How to store more fresh water on the artificial islands is of great significance to cost savings. Previous efforts have found that the permeability of island-building materials dominates the water storage on the island. General consensus has been achieved that the application of less permeable island-building materials can increase freshwater storage [6,7].

Regarding island-building materials, calcareous sand, formed by the accumulation of coral debris and other biological debris, has been widely used as a foundation filler [8–10] due to its excellent mechanical properties and extensive availability in marine environments [11,12]. However, the reclamation in distant seas using sand is often carried out via
dredgers, and the foundations are formed using the rainbow method on the water surface or the bottom dumping method [13,14], which are generally unfeasible to meet the required permeability [15]. This problem is especially prominent when using calcareous sand as a foundation material after a hydraulic fill. To solve this problem, island builders propose the rolling method, dynamic consolidation and vibroflotation to treat calcareous sand foundations. However, the rolling method is only suitable for low depth foundations, and dynamic consolidation and vibroflotation require large machinery, which is inconvenient and costly to transport. Large-scale mechanical operations may also cause great damage to the ecological environment of the island reef. Therefore, there is an urgent need to develop an efficient, economic and environmentally friendly technology to reduce the permeability of calcareous sand foundations.

Microbial-induced calcite precipitation (MICP), an emerging soil improvement technology, uses urease-producing microorganisms to hydrolyze urea to form carbonates, and then calcite formed with calcium ions is added. It can cement loose sand bodies [16–19], increasing sand strength and decreasing its permeability at the same time. This technique presents the advantages of low grouting pressure, viscosity and concentration and, thus, less disturbance and pollution to the formation compared with conventional grouting reinforcement technology [20–23]. Currently, MICP has been widely used to improve the mechanical properties of sands [24–31], and some field-scale applications have been successful [31–34], but these studies mainly focused on common silica sand. Calcareous sand, as a kind of marine sediment, has the characteristics of fragility, high compressibility and a low bearing capacity [35], which is quite different from silica sand. Pioneering works applied MICP to such special sand and found that the mechanic strength of calcareous sand can also be improved after cementation. For instance, Liu [36] found that the dynamic shear stress of cemented calcareous sand was 50% higher than that of uncemented calcareous sand. Khan [37] found that artificial beach rock, made from coral sand solidified by MICP, achieved the expected unconfined compressive strength of 20 MPa.

However, for calcareous sand foundations on artificial islands, in addition to the mechanical properties, its permeability that determines freshwater storage on the islands needs attention. In particular, when physical reinforcement measures are adopted to treat calcareous sand foundations, the large-size calcareous sand particles are prone to breaking under external forces [38–41], leading to changes in the gradation and pore structure [42]. This process will inevitably cause changes in the permeability of the calcareous sand foundations. Some scholars have conducted related studies on the permeability properties of calcareous sands [12,43]; in addition, some MICP-based permeability characteristics were investigated [44], but there are few studies on the effects of particle gradation on the permeability properties of MICP-improved calcareous sands.

Altogether, there exists the need for an understanding of the permeability variation in MICP-improved calcareous sand with various gradations. Possibly, different particle gradations could change the pore space structure between the sand particles, thus affecting the reinforcement effect of the MICP treatment. The novelty of our work lies in testing this hypothesis through a series of penetration tests, CT scan tests and microstructure tests. Based on a self-made grouting and seepage device, cemented sand columns were formed. The effects of grain gradation, with indicators including the curvature coefficient ($C_u$) and the uniformity coefficient ($C_c$), on permeability were studied. A CT scan was conducted to investigate the porosity of the cemented sand. The content of the induced calcium carbonate was measured using a weighting method. A scanning electron microscopy (SEM) technique was adopted to further study the micromechanism of the MICP treatment in sands of different gradations. Finally, the correlation of the permeability coefficient with $C_u$ and $C_c$ was semiquantitatively analyzed.
2. Materials and Methods
2.1. Materials
2.1.1. Test Sand

The calcareous sand used in the experiment was taken from a reef in the South China Sea. It was mainly composed of the accumulation of pieces of dead madrepore and halobios. The content of calcium carbonate exceeded 97%, and the main mineral components were aragonite and high-magnesian calcite. It was uncemented in loose shape and was cleaned, dried and sieved to remove the sand particles > 5 mm. The oven temperature was less than 70 °C to prevent calcareous sand from disintegrating and producing fine particles. Then, the obtained calcareous sand was sieved into five particle size groups of 0.075~0.25 mm, 0.25~0.5 mm, 0.5~1 mm, 1~2 mm and 2~5 mm. In this study, calcareous sand with 6 kinds of grain gradations were prepared, namely, group A, group B, group C, group D, group E and group Y, respectively. Group Y is the original gradation level with particles less than 5 mm. The other five kinds of grain gradation (A–E) were achieved through recombination of each particle size group, and their effective particle size \(d_{10} = 0.25 \text{ mm}\) was the same. The cumulated curve and particle size distribution histogram of calcareous sand is shown in Figures 1 and 2, respectively.

![Figure 1. Particle-accumulated curve of calcareous sand with different gradations.](image1)

![Figure 2. Particle size distribution histogram of calcareous sand with different gradations.](image2)
By measuring the grain density ($\rho_s$, obtained using AccupyC II 1340 fully automatic true density meter (Micromeritics instrument corporation, Norcross, GA, USA)), maximum dry density ($\rho_{\text{max}}$, obtained using JDM-2 electric relative density meter, Xinkeyiq Co. Ltd., Hebei, China) and minimum dry density ($\rho_{\text{min}}$, obtained by adopting cylinder inversion method) of calcareous sand, the minimum void ratio ($e_{\text{min}}$) and maximum void ratio ($e_{\text{max}}$) were calculated using the following equations:

$$e_{\text{min}} = \frac{\rho_s - \rho_{\text{max}}}{\rho_{\text{max}}}$$

$$e_{\text{max}} = \frac{\rho_s - \rho_{\text{min}}}{\rho_{\text{min}}}$$

where $e_{\text{min}}$ is the minimum void ratio of calcareous sand; $\rho_s$ is the grain density of calcareous sand, g/cm$^3$; $\rho_{\text{max}}$ is the maximum dry density of calcareous sand, g/cm$^3$; $e_{\text{max}}$ is the maximum void ratio of calcareous sand; and $\rho_{\text{min}}$ is the minimum dry density of calcareous sand, g/cm$^3$.

With the internal volume of the mold known, the mass of sand was controlled and compacted to three relative densities ($D_r = 30, 60$ and $85\%$). They are denoted as suffixes 1, 2 and 3; for example, A1 represents the sand sample of A gradation level with relative density of 30%. $D_r$ can be controlled using the following equations:

$$e = \frac{V_v}{V_s}$$

$$D_r = \frac{e_{\text{max}} - e}{e_{\text{max}} - e_{\text{min}}}$$

where $e$ is the controlled void ratio of sand sample, and $\rho$ is the natural density of calcareous sand, g/cm$^3$.

The basic parameters of sand samples are shown in Table 1.

**Table 1.** The experimental scheme and the physical parameters for various gradations of sand samples.

| Groups | $C_c$ | $C_w$ | $D_r\%$ | $D_{10}/\text{mm}$ | $D_{30}/\text{mm}$ | $D_{60}/\text{mm}$ | $\rho_s/\text{g cm}^{-3}$ |
|--------|------|------|---------|-----------------|-----------------|----------------|------------------|
| A1     | 1.5  | 2    | 30      | 0.25            | 0.36            | 0.5           | 2.81             |
| A2     | 1.5  | 2    | 60      |                 |                 |               |                  |
| A3     | 1.5  | 2    | 85      |                 |                 |               |                  |
| B1     | 1.5  | 8    | 30      | 0.25            | 0.71            | 2             | 2.78             |
| B2     | 1.5  | 8    | 60      |                 |                 |               |                  |
| B3     | 1.5  | 8    | 85      |                 |                 |               |                  |
| C1     | 2    | 8    | 30      | 0.25            | 1.00            | 2             | 2.80             |
| C2     | 2    | 8    | 60      |                 |                 |               |                  |
| C3     | 2    | 8    | 85      |                 |                 |               |                  |
| D1     | 0.5  | 8    | 30      | 0.25            | 0.5             | 2             | 2.80             |
| D2     | 0.5  | 8    | 60      |                 |                 |               |                  |
| D3     | 0.5  | 8    | 85      |                 |                 |               |                  |
| E1     | 1.5  | 6    | 30      | 0.25            | 0.75            | 1.5           | 2.79             |
| E2     | 1.5  | 6    | 60      |                 |                 |               |                  |
| E3     | 1.5  | 6    | 85      |                 |                 |               |                  |
| Y1     | 0.8  | 3.3  | 30      | 0.17            | 0.28            | 0.56          | 2.78             |
| Y2     | 0.8  | 3.3  | 60      |                 |                 |               |                  |
| Y3     | 0.8  | 3.3  | 85      |                 |                 |               |                  |
2.1.2. Microbial Suspensions

Solutions of *Bacillus pasteurii* (ATCC 11859) were used for MICP treatment. Bacteria were first grown on a sterilized Petri dish containing solid medium, after which bacteria were inoculated in a liquid medium to expanded culture with shaking at 30 °C for a 24 h period steriley (see Table 2 for media composition). Both mediums were prepared with deionized water and autoclaved at 121 °C for 30 min. Then, urea solutions were sterilely filtered into the mediums. Note that all the inoculation operations were performed on the clean bench.

| Constituent      | Liquid Medium | Solid Medium |
|------------------|---------------|--------------|
| Agar (g)         | /             | 20           |
| Sodium chloride (g) | 5             | 5            |
| Casein peptone (g)  | 15            | 15           |
| Soya peptone (g)  | 5             | 5            |
| Urea (g)         | 20            | 20           |
| Deionized water (mL) | 1000         | 1000         |

Table 2. Media components.

Enough urease bacteria can ensure the full hydrolyzing of urea and provide sufficient carbonate ions for the precipitation of calcium carbonate (CaCO₃). The biomass was detected by measuring the absorbance of the bacteria solution using an L5S UV-Vis spectrophotometer (Inesa instrument Co Ltd., Shanghai, China) at 600 nm wavelength (OD₆₀₀), and the measurement result is shown in Figure 3. It can be seen that the absorption values gradually rose and kept stable for 24–32 h. Hence, the bacteria solutions at the stable phase were used for ureolysis-induced calcification.

Figure 3. The growth curve of *Bacillus pasteurii*.

The growth and reproduction of microbes are generally affected by the pH of the environment. Excessive acidity or alkalinity will denature the surface proteins of the microbes and eventually reduce the number of microorganisms by inhibiting their survival. In the study, 1 M NaOH/HCl was used to prepare the liquid medium with different pH to investigate the optimum pH for the growth and reproduction of Bacillus. The biomass of various pH was measured, and the result is shown in Figure 4. From the figure, it is clear that the highest OD₆₀₀ was found at pH = 9, which is optimal for the growth and reproduction of microbes. Therefore, the microbes were cultured at pH = 9 in the study.
2.1.3. Fixation and Cementation Solutions

Harkes [45] found that fixation fluid including 50 mM calcium chloride can achieve the immobilization of bacteria using the flocculation of calcium ions. Our previous work shows that the cementation solution with 1 M equimolar concentration of urea–calcium chloride is more conducive to inducing CaCO$_3$ [46]. Therefore, 50 mM calcium chloride and 1 M equimolar concentration of urea–calcium chloride were used for fixation and cementation solutions, respectively.

2.2. Apparatus

A self-made device was developed to conduct induced mineralization and permeability tests, consisting of a column-shaped mold, mold support frame, scale plate, HD camera, effluent collection container, etc. (Figure 5). The column-shaped perplex mold has a prefabricated recess for easy separation, and its top and bottom are sealed with a perforated rubber stopper. The contact interface between the sand and rubber stopper is padded with geotextile to prevent deformation of the sand surface and reduce the loss of fine particles.

Figure 4. OD600 value of Bacillus pasteurii at different pH.

Figure 5. Experimental set-up for induced mineralization and permeability tests.
Two mold support frames of the same size (length × width × height = 42 cm × 8 cm × 53 cm) were used to place eight sand molds for tests. The HD camera was placed in a fixed position to record the whole process of the infiltration test and provides detail involving time and scale changes according to the image data; thus, the permeability coefficient of the sand sample can be calculated according to Equation (5).

A scale plate (2 m in height) with scale lines and perplex tubes was used to transport liquid, where the head difference between the scale plate and the mold can provide the driving force to the MICP grouting and permeability test. All the solutions first flow from the top to the bottom of the scale plate, then flow through the sand bodies from the bottom of the mold and finally, flow out from the top of the mold to the effluent collection container.

2.3. Experimental Strategy

2.3.1. MICP Treatment

MICP treatment was carried out according to the grouting sequence of bacterial suspension, fixation fluid and cementation solution. The grouting volume of all the solutions was set to 1.2 times that of the pore volume of the calcareous sands. Before the first grouting, deionized water was injected to fully saturate the calcareous sand to minimize the bubbles between sand particles. Combined with previous results [47], this research proposed the cycle of MICP treatment to be 7 times.

2.3.2. Permeability Test

As shown in Figure 5, the grouting device was also adopted to carry out the variable head permeability test, where the perplex pipe on the scale plate is used as a variable head pipe. By utilizing the head difference, seepage flows can be formed in the mold from the bottom to top.

The initial permeability coefficient and after each cycle of MICP treatment, all sand samples were measured according to the standard for variable head penetration tests [48]. The procedure of permeability test was as follows:

1. The sand sample in the mold was sealed to be watertight and fully saturated.
2. The deionized water was injected into the variable head pipe until the fluid was overflowing without bubbles, then the pinchcock was closed.
3. The variable head pipe was injected with deionized water to the required height (1~1.5 m). Then, the pinchcock was opened, and the changing of the water head and time was recorded. The measurement was repeated 2~3 times at the same water head.
4. The measurement was repeated 5~6 times at different water heads for each sand sample. When the permeability coefficient measured was within the allowable error (±2.0 × 10⁻ⁿ cm/s), the test was ended. The permeability coefficient (k) can be calculated using the following equation:

$$k = 2.3 \frac{a L}{A (t_2 - t_1)} \log_{10} \frac{h_1}{h_2}$$

where k is the permeability coefficient of the sand sample, cm/s; a is the cross-sectional area of standpipe, cm²; L is the center distance between two pressure-measuring tubes, cm; A is the cross-sectional area of sand column cm²; t₂ is the ending time of permeability test, s; t₁ is the beginning time of permeability test, s; h₁ is the initial water head, cm; and h₂ is the ending water head, cm.

2.3.3. The Content of Induced Calcium Carbonate in Sand Columns

Conventional methods for measuring the content of induced calcium carbonate (CaCO₃), such as titration, pickling, ASTM standards, etc., are not suitable for calcareous sand with calcium carbonate as the main component [49]. Therefore, we adopted the weighting method to determine the content of CaCO₃ crystals, coving the initial mass and after each MICP treatment. In this study, the percentage increase in mass of MICP-treated
sand was used to represent the content of CaCO$_3$ crystals, and it can be calculated using the following equation:

$$ M = \frac{M_3 - M_2}{M_1} $$

where $M$ is the content of CaCO$_3$, and $M_3$ is the mass of sand sample and mold after each MICP treatment, g. Note that the sand particles flowing out of the mold in the process of MICP grouting and permeability test were counted into $M_2$; $M_2$ is the mass of the sand sample and mold before first MICP treatment, g; and $M_1$ is the mass of sand sample in the mold before the first MICP treatment, g.

2.3.4. X-ray Computed Tomography Scan (CT)

X-ray computed tomography (Sanying precision Instruments Co Ltd., Tianjin, China) imaging, with a minimum resolution of 3 µm, was adopted to obtain the porosity changes in sand samples before and after MICP treatment. As shown in Figure 6, the meso-structure of sand was first inspected with the spatial resolution of 13.43 µm, and the 2D images of the samples at different sections were obtained. Then, the layer-by-layer porosity was obtained from the computer based on the image information of the sand samples, and thus the overall porosity of the sample was calculated.

![X-ray Computed Tomography Scan](image_url)

Figure 6. X-ray computed tomography (CT) scan: 2D images of the sample at different sections.

2.3.5. Scanning Electron Microscopy (SEM) of MICP-Treated Sand

After completion of the above tests, all sand samples were left under room condition (about 30 °C) to dry by air for 30 days. Then, scanning electron microscopy (SEM) tests were performed on the sand cores from dried samples to investigate the micromechanism of MICP treatment in different grain gradations of sand.

3. Results and Discussion

3.1. The Effect of Gradation on the Permeability Coefficient

Figure 7 shows the permeability coefficient difference ($\Delta k$) vs. the cycles of MICP treatment (MT) curves for calcareous sands with different gradations and relative densities ($D_r$). $\Delta k$ refers to the difference in the permeability coefficients before and after each MICP treatment cycle. Note that the initial permeability coefficients of all of the sand samples are between $10^{-2}$ and $10^{-4}$ cm/s, which is close to the data of relevant studies [43], proving the credibility of the data provided by the permeability test device. Overall, with the increase in MT, the $\Delta k$ first sharply increases, then rapidly decreases and finally, tends to a relatively stable equilibrium state (MT = 5–7). Such a trend is the process of calcium carbonate (CaCO$_3$) gradually filling the intergrain pore places. That is, in the early grouting
stage (MT = 1–2), the pore places between sand particles are continuously filled, so the permeability sharply decreases (i.e., the $\Delta k$ rapidly increases). With continuous grouting (MT = 3–4), the partial pore places are filled, so the permeability slowly decreases (i.e., the $\Delta k$ rapidly decreases). At the final grouting stage (MT = 5–7), a large number of pore places are basically filled, and the permeability changes little (i.e., the $\Delta k$ finally tends to a relatively stable equilibrium state). In fact, the cementation product filling or coating on the particle surfaces changes the specific surface area of the pores. The pore surface is the interface for the fluid flow in sand particles, and its dimensions affect the flow of fluid. As the number of MICP treatments increased, the pore-specific surface area increased, although the porosity of the samples decreased (Figure 8). As an example, the specific surface area was 24 mm$^{-1}$ without the MICP treatment and 29 mm$^{-1}$ after seven MICP treatments for sample Y2. Usually, the permeability of the soil decreases with the increase in the pore-specific surface area, so the permeability coefficient decreased with the increase in MT. This relationship between permeability and MT was also confirmed by Aamir et al. [50]. Moreover, the small change in permeability (MT = 5–7) can be attributed to the ineffective filling of some cementation products [51]. That is, some CaCO$_3$ crystals fill the particle surfaces rather than the interparticle pore spaces and cannot lead to a reduction in permeability.

**Figure 7.** Permeability coefficient difference ($\Delta k$) vs. cycles of MICP treatment (MT) of sand at different gradations and compactness ($D_r$): (a) $D_r = 30\%$ for samples A1, B1 and E1; (b) $D_r = 60\%$ for samples A2, B2 and E2; (c) $D_r = 85\%$ for samples A3, B3 and E3; (d) $D_r = 30\%$ for samples B1, C1 and D1; (e) $D_r = 60\%$ for samples B2, C2 and D2; (f) $D_r = 85\%$ for samples B3, C3 and D3; (g) samples B and Y.
Figure 7. Permeability coefficient difference ($\Delta k$) vs. cycles of MICP treatment (MT) of sand at different gradations and compactness ($D_r$): (a) $D_r = 30\%$ for samples A1, B1 and E1; (b) $D_r = 60\%$ for samples A2, B2 and E2; (c) $D_r = 85\%$ for samples A3, B3 and E3; (d) $D_r = 30\%$ for samples B1, C1 and D1; (e) $D_r = 60\%$ for samples B2, C2 and D2; (f) $D_r = 85\%$ for samples B3, C3 and D3; (g) samples B and Y.

From Figure 7a–c, it can be seen that for the sand samples with the same $D_r$ and $C_c$ (e.g., groups A1, E1 and B1 or A2, E2 and B2 or A3, E3 and B3), the $\Delta k$ increased with the rise in $C_u$. Similarly, in the case of the same $D_r$ and $C_u$ in Figure 7d–f (e.g., groups B1, C1 and D1 or B2, C2 and D2 or B3, C3 and D3), the $\Delta k$ increased with the rise in $C_c$. These results show that the CaCO$_3$ crystals produced by the MICP treatment filled the pores and cemented the grain particles, resulting in a decrease in $k$ in the sand samples, but the decreasing amplitude of $k$ in the samples with various $C_u$ and $C_c$ is different. In this study, the total particle size interval (0.01~5 mm) and the effective particle size ($d_{10} = 0.25$ mm) were limited, and when the uniformity coefficient is larger (e.g., $C_u = 6, 8$), it can be seen from Equation (7) that $d_{60}$ corresponds to a larger particle size (see Figure 1), i.e., more
coarse particles and less fine particles (see Figure 2). During the sample preparation process, larger pores can be formed between the coarse particles, while the fine particles cannot completely fill these pores. Therefore, the initial pore space of the sand sample is larger when \( C_u \) increases, which is conducive to the penetration of the cementation solution and increases the filling effect of the cementation products, resulting in a larger decrease in the permeability coefficient:

\[
C_u = \frac{d_{60}}{d_{10}}
\]  

(7)

However, for samples with different \( C_c \) (i.e., samples B, C, and D), as the total particle size interval (0.01~5 mm), the effective particle size (\( d_{10} = 0.25 \) mm) and uniformity coefficient (\( C_u = 8 \)) are fixed, it can be calculated from Equation (7) that the \( d_{60} \) is also fixed (\( d_{60} = 2 \) mm, see Figure 1). Therefore, different \( C_c \) leads to different particle content in the three particle size ranges of 0.25~0.5 mm, 0.5~1 mm and 1~2 mm (see Figure 2). As can be seen from Figure 1, when \( C_c \) is smaller (e.g., \( C_c = 0.5 \) for sample D), the gradation curve appears as an upper convex segment, indicating that there are more particles with particle size \( \leq 1 \) mm and less particles with particle sizes of 1~2 mm. Thus the relatively finer particles can properly fill the pores between the coarse particles exhibiting the smaller initial pore space. As \( C_c \) increases (i.e., \( C_c = 1.5 \) for sample B and \( C_c = 2.0 \) for sample C), the gradation curve exhibits a downward concave segment, which indicates an increase in the content of the relatively coarser particles and a decrease in the content of the relatively finer particles, resulting in a decrease in the filling effect by the finer particles, i.e., a larger initial pore space. Therefore, the larger initial pore space facilitates the penetration of the cementation solution and the filling effect of the cementation products, causing the obvious reduction in the permeability coefficient.

In conclusion, the above experimental results confirm the differences in the application of the MICP technique for calcareous sands with different gradations and that soils with larger \( C_c \) and \( C_u \) are better improved in terms of permeability by applying MICP.

Moreover, with the same gradation, the \( \Delta k \) decreased with the rise in \( D_r \). This is because the increase in compactness further compresses the size of the penetrating pore channels inside the sample and reduces the permeability of the sample. Meanwhile, the smaller pore space reduces the filling effect of the cementation product, which also leads to a smaller \( \Delta k \). Otherwise, microbes could not infiltrate deeper into the sand sample due to the reduction in the pore throat [50]. This may lead to a heterogeneous distribution of \( \text{CaCO}_3 \), which was high in the region close to the percolation points, causing the hindered penetration of solutions and poor permeability improvement.

### 3.2. Porosity and Mass Variation

Figure 8 shows the porosity difference (\( \Delta n \)) vs. the content of induced \( \text{CaCO}_3 \) (\( M \)) for MICP-treated sands at different relative densities (\( D_r \)) and grain gradations. \( \Delta n \) refers to the difference in porosity before and after each MICP treatment cycle. Overall, all sand samples showed a certain increase in \( M \) and decrease in \( n \) after the MICP treatments. Specifically, with the increase in MT, the \( \Delta n \) first increases, then decreases and finally, tends to a dynamic equilibrium (MT = 5~7); the variation pattern of \( \Delta n \) corresponds to the changes in permeability (Figure 7). This can be attributed to the fact that pore structure characteristics are the key factors affecting permeability [4]; that is, the induced \( \text{CaCO}_3 \) filled the pores leading to the reduction in porosity, which inevitably reduces permeability. Therefore, the porosity and the permeability exhibit the same trend with the change in MT.
(the explanation of this trend can be found in Section 3.1). Meanwhile, during the MICP treatment, the increase in $M$ was smaller in the late stage ($MT = 5–7$). Such a change trend is shown in Figure 8, with dense data points at the end of the curve and discrete data points in the front section of the curve. The decrease in porosity and the increase in mass suggested that the induced CaCO$_3$ filled the pores between the sand particles, but with the increase in $MT$ ($MT = 5–7$), cementation products filled most of the pores, resulting in small changes in $\Delta n$ and $M$.

It is of interest to note that, from Figure 8a–c, it can be seen that for sand samples with the same $D_r$ and $C_c$ (e.g., groups A1, B1 and E1 or A2, B2 and E2 or A3, B3 and E3), the $\Delta n$ and $M$ increased with the rise in $C_u$. Similarly, from Figure 8d–f, for the sand samples with the same $D_r$ and $C_u$ (e.g., groups B1, C1 and D1 or B2, C2 and D2 or B3, C3 and D3), the $\Delta n$ and $M$ increased with the rise in $C_c$. In addition, taking sample B and the original gradation sample Y as examples (see Figure 8g), it can be found that for samples with the same $D_r$, $\Delta n$ and $M$ are larger when both $C_c$ and $C_u$ increase.

In sum, the test results once again confirm the differences in the application of the MICP technique for calcareous sands of different gradations; specifically, soils with larger $C_c$ and $C_u$ have a greater decrease in porosity and more CaCO$_3$ generated within the soil after MICP treatment, thus the decrease in the permeability coefficient is also more significant.

3.3. Scanning Electron Microscopy (SEM) Analyses

Due to space constraints, SEM tests were not conducted on all of the samples in this study, and only representative samples were selected for testing. Therefore, the cored samples, obtained from samples A1 ($C_c = 1.5$, $C_u = 2$) and B1 ($C_c = 1.5$, $C_u = 8$) after drying, were subjected to SEM tests to study the mechanism of the MICP treatment in different grain gradations of sand. Figure 9 show the images of the MICP-treated sands (A1 and B1) at magnifications of $200 \times$ and $4000 \times$, respectively.

From the microscopic scanning results, the surface of the sand grains of the two samples became rough because the sand particle surface was coated with generated CaCO$_3$ crystals, and the particle pore spaces were partially filled with crystals. The CaCO$_3$ crystals were mainly in the form of rhombic crystals, which is consistent with other research results [52]. Meanwhile, the additional observation was the formation of CaCO$_3$ crystal clusters (Figure 9c) on the particle surface or in the interparticles [52]. The clusters or CaCO$_3$ crystals provide a bonding effect in sand grains that can fill pore places to reduce permeability or porosity. However, the cementation product coating on the particle surface is less helpful in reducing permeability and belongs to the ineffective CaCO$_3$ crystals [51]. The cementation products bonding with adjacent particles and filling pore spaces are effective CaCO$_3$ crystals, which can reduce the seepage space. A schematic diagram was drawn to better understand the ineffective and effective CaCO$_3$ crystals, as shown in Figure 10. This phenomenon can partially explain the smaller reduction in the permeability coefficient at $MT = 5–7$ (Figure 7).

It is worth noting that despite the different particle gradations of the samples, the microstructure shows similar filling and coating effects on the surface of sand particles or interparticles after the MICP treatments. This phenomenon indicates that even if the initial pore space is larger (B1), after MICP treatment, the crystal filling allows more improvement in permeability and porosity, thus exhibiting a microstructure similar to that of the sample with smaller initial pore space (A1). Thus, with sufficient MICP treatment cycles, larger $C_u$ provides more pore space for the filling of CaCO$_3$ crystals, thus leading to the significant reduction in pore space and lower permeability. However, larger $C_u$ may also result in
a smaller size of induced CaCO$_3$ crystals, which tends to result in poor solidification effectiveness [53].

Figure 9. SEM images of MICP-treated sand (A1 and B1).

Figure 10. Schematic diagram of ineffective and effective CaCO$_3$ crystals in cemented samples.
3.4. Correlational Analyses

According to the results of the permeability tests, the correlation of the permeability coefficient ($k$) with the uniformity coefficient ($C_u$) and curvature coefficient ($C_c$) was analyzed. To reduce the influence of other factors, the effective particle size ($d_{10}$) of each sand sample was controlled to be a constant value. Therefore, $d_{10}$ was introduced into the fitting equation, based on the experience of the expression of the permeability coefficient established by some scholars [53]. The analysis results are shown in Figure 11, where MT = 1 means the MICP treatment is performed once, and so on.

Finally, to attempt to observe a theoretically more general fitting curve, the values of $C_u$ and $C_c$ are extended in Figure 11b,e, respectively. The results are shown in Figure 12a,b. It can be seen that for $C_u \geq 6.0$, the decrease in $k$ becomes increasingly obvious with the increase in $C_u$ and MT; for $2.0 > C_c > 0.5$, the decrease in $k$ keeps increasing with the increase in $C_c$ and MT; however, when $C_c > 2.0$, the decrease in $k$ remains stable. However, the validity of the reacquired curves need to be verified by repeating permeability tests with a wider range of grain gradations in the future.

For Figure 11a–c, the approximate expression for different $C_u$ is given as the following equation:

$$k = ae^{-C_u/\lg d_{10}} + b$$  \hspace{1cm} (8)
where \( k \) is the permeability coefficient of the sand sample, \( \text{cm/s} \); \( C_u \) is the uniformity coefficient; \( d_{10} \) is the effective particle size, \( d_{10} = 0.25 \text{ mm} \); and \( a \) and \( b \) are the relative coefficients.

For Figure 11d–f, the approximate expression for different \( C_c \) is given as the following equation:

\[
k = -a'e^{C_c/l0d_{10}} + b'
\]

where \( k \) is the permeability coefficient of the sand sample, \( \text{cm/s} \); \( C_c \) is the curvature coefficient; \( d_{10} \) is the effective particle size, \( d_{10} = 0.25 \); and \( a' \) and \( b' \) are the relative coefficients.

Equations (8) and (9) show that the permeability coefficient is an exponential function of \( C_u \) and \( C_c \), respectively. Overall, \( k \) is roughly proportional to \( C_u \) and \( C_c \) [53], and most curves show a high fitting degree (\( R^2 > 90\% \)). Notably, with the case of \( MT < 5 \), the curves involving \( C_u \) showed a concave trend, while the curves involving \( C_c \) showed a convex trend. This result indicates that when increasing the MICP treatment cycles, the higher the \( C_c \) (\( C_u \geq 6.0 \)) and the lower the \( C_c \) (\( 2.0 > C_c > 0.5 \)), more of a decrease in \( k \) will be observed. Indeed, larger \( C_u \) and smaller \( C_c \) often imply well grain gradation. Therefore, it can be inferred that only fewer MICP treatment cycles are needed to achieve the target permeability coefficient when the calcareous sand shows a well grain gradation.

In addition, Figure 11 also presents an interesting result in that the curves with poor fitting results (\( R^2 < 90\% \)) are mainly concentrated on \( MT = 5~7 \). It is deduced that after a number of cycles of MICP treatment (\( MT \geq 5 \)), the interparticle pores of the calcareous sand are basically filled by the induced CaCO_3, and the difference in the pore structure caused by the different particle gradations (\( C_u \) and \( C_c \)) has been weakened. Therefore, the permeability coefficient does not correlate well with \( C_u \) or \( C_c \) after a number of cycles of MICP treatment (\( MT \geq 5 \)).

Finally, to attempt to observe a theoretically more general fitting curve, the values of \( C_u \) and \( C_c \) are extended in Figure 11b,c, respectively. The results are shown in Figure 12a,b. It can be seen that for \( C_u \geq 6.0 \), the decrease in \( k \) becomes increasingly obvious with the increase in \( C_u \) and \( MT \); for \( 2.0 > C_c > 0.5 \), the decrease in \( k \) keeps increasing with the increase in \( C_c \) and \( MT \); however, when \( C_c > 2.0 \), the decrease in \( k \) remains stable. However, the validity of the reacquired curves need to be verified by repeating permeability tests with a wider range of grain gradations in the future.

**Figure 12.** The reacquired curves for a wider range of \( Cu \) and \( Cc \): (a) \( 2 < Cu < 10 \); (b) \( 0.5 < Cc < 8 \).

### 4. Conclusions

This study investigates the effects of grain gradation on the permeability coefficient (\( K \)) for calcareous sand improved by microbial-induced calcite precipitation (MICP) technology. Permeability tests, CT scans and SEM analyses were conducted to reveal the law and mechanism of permeability variations in MICP-improved sand samples with different grain gradations. Finally, the correlation of the permeability coefficient with the uniformity coefficient (\( C_u \)) and the curvature coefficient (\( C_c \)) was analyzed. The main conclusions from this study are as follows:
1. The grain gradation of cemented sand influences its permeability. In the case of the same \( D_r \), the decreased amount of the permeability coefficient of each MICP treatment cycle increased with the rise in \( C_u \) and \( C_c \).

2. All sand samples showed a certain increase in induced calcium carbonate and a decrease in porosity after the MICP treatments. In the case of the same \( D_r \), the decrease amount of the porosity and increase amount of the cementation content of each MICP treatment cycle increased with the rise in \( C_u \) and \( C_c \).

3. From the SEM analyses, after MICP treatment, the microstructures of the sand samples with different gradations were similar. The calcium carbonate crystals not only coat on the particle surfaces but also fill the particle pores, thus reducing permeability and decreasing porosity.

4. The permeability coefficient \((k)\) is proportional to \( C_u \) and \( C_c \). When the MICP treatment cycle is less than five, the larger \( C_u \) (\( C_u \geq 6.0 \)) and smaller \( C_c \) (\( 2.0 > C_c > 0.5 \)) will lead to a more significant reduction in permeability, and permeability was fitted well with \( C_u \) and \( C_c \). However, the permeability coefficient does not correlate well with \( C_u \) or \( C_c \) after a number of cycles of MICP treatment (\( MT \geq 5 \)) because the interparticle pores are basically filled by calcium carbonate crystals.

5. This work provides a reference for the freshwater storage of calcareous sand foundations and the antiseepage of calcareous sand structures on island reefs. The targeted application of MICP according to soil gradation makes permeability a controlled variable, which not only saves the cost of construction but also avoids damaging the local ecosystem due to a permeability which is too low.

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