Performance and mechanism of sand stabilization via microbial-induced CaCO$_3$ precipitation using phosphogypsum

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Abstract: Phosphogypsum is a solid waste generated during the production of phosphoric acid. Effective utilization of phosphogypsum resources is a complex challenge. In this research, an innovative and eco-friendly sand consolidation technique, i.e., microbial-induced CaCO$_3$ precipitation using phosphogypsum (MICPP), is applied to achieve phosphogypsum mineralization and sand stabilization. Phosphogypsum is employed as a calcium source for sand consolidation. To elucidate the efficiency and the mechanism of sand consolidation through MICPP, a series of experimental tests on the sand columns using varying phosphogypsum dosages and consolidation methods are conducted. The results show a positive correlation between the increase in phosphogypsum dosage and the increase in the compressive strength of the specimens. Concurrently, as the amount of phosphogypsum increased, the permeability coefficient of the sand columns decreased and the production of CaCO$_3$ increased. Notably, the immersion method exhibits a superior curing effect compared to the stirring method. The MICPP-treated specimens significantly mitigated the risk of environmental contamination. The CaCO$_3$ precipitated by the microbial action is predominantly in the form of calcite that effectively fills the voids, bonds surfaces, and bridges gaps in the sand columns, thereby substantially enhancing the performance of sand columns.

Keywords: Sand stabilization, MICPP, Performance evaluation, Mechanistic analysis, Environmental assessment.

1. Introduction

In the context of global warming, a range of measures have been actively implemented by many countries to reduce greenhouse gas emissions, including CO$_2$ $^{[1]}$. Traditional soil reinforcement techniques rely on cementitious materials $^{[2-4]}$. However, the use of conventional reinforcing agents like cement and lime alters the pH of soil, induces alkalinity and creates erosive conditions, which negatively impacts groundwater and surrounding vegetation $^{[5]}$. Additionally, substantial CO$_2$ emissions generated during the production of cement, upon which this technology heavily relies, hinder progress toward the carbon reduction targets $^{[6-8]}$. Recently, Microbially Induced Calcium Carbonate Precipitation (MICP) technology is gaining prominence due to its environmentally friendly, efficient, controllable, and cost-effective attributes, coupled with low energy consumption and sustainability $^{[3, 9, 10]}$. MICP operates on the principle of utilizing microorganisms to decompose urea into carbonate ions. Subsequently, these ions combine with
calcium ions, forming CaCO₃ precipitation and exerting a curing effect [11]. The technology holds significant promises across various civil engineering applications, including foundation reinforcement [3-12,15], crack repair, overlay applications [16-19], soil heavy metal management [20,21], soil permeability enhancement [22,23], slope stabilization, and soil erosion control [24]. The diverse applications underscore the potential of MICP to positively impact the sustainable evolution of the civil engineering sector.

The concept of MICP involves injecting bacteria and cementing solution through alternating grouting cycles to carry out one-dimensional sand column reinforcement [25]. This process notably increases the strength of the column. The mechanism behind microbial-induced CaCO₃ precipitation relies on the urea hydrolysis reaction and involves the enzymatic activity of urease-producing bacteria, which continually decomposes urea. As a result, NH₄⁺ is released, elevating the micro environmental alkalinity. Consequently, continuous CO₂ absorption and conversion into CO₃²⁻ occur, facilitating its combination with Ca²⁺ from the calcium source, leading to the precipitation of CaCO₃ [20,26,27]. Compressive strength is a crucial parameter characterizing the strength properties of MICP curing. Observations of compressive strength and calcite deposition demonstrated a marked improvement in strength correlated with increased calcite deposition [3,28-30]. Moreover, MICP treatment results in varied enhancements of cohesion (c) and the angle of internal friction (φ) in the treated specimens, thereby boosting the shear strength of sandy soil [31,32]. Calcium carbonate precipitation induced by MICP fills granular soil pores, potentially enhancing soil porosity, and permeability, and enabling the sealing of porous materials [33,34]. Ongoing studies utilize calcium chloride, calcium acetate, and calcium nitrate as primary calcium sources, while alternative sources like concentrated seawater, calcium gluconate, calcium lactate, and even eggshells are explored [27,35-38].

The surging expansion of the phosphate industry aligns with the swift growth of agricultural industry. Phosphogypsum constitutes a considerable solid waste residue, emanating as a byproduct from the production of wet phosphoric acid. Present estimations unveil a significant worldwide accrual of phosphogypsum, averaging 4.5-5.5 tons for every ton of produced phosphoric acid [39,40]. Regrettably, a majority of phosphogypsum is disposed in stockpiles or landfills, with limited application in specialized sectors such as construction materials and fertilizer production. This practice poses significant challenges to both environmental sustainability and resource management [41]. Consisting primarily of calcium sulfate dihydrate (CaSO₄·2H₂O) [42], researchers have dissolved phosphogypsum using alkaline solutions. Subsequent experiments involving carbonization at room temperature and pressure have demonstrated high efficiency in producing CaCO₃ precipitates. Further exploration shows
complete transfer of trace elements from phosphogypsum waste to the resulting calcite precipitate [43]. At present, MICP technology is rapidly advancing and reaching a state of maturity. However, there is a lack of research focusing on the utilization of phosphogypsum as a calcium source in MICP. This study introduces an innovative approach by utilizing phosphogypsum as a calcium source within the framework of MICP technology. Through the action of microbial processes, phosphogypsum undergoes transformation into a viable material suitable for engineering applications, which is termed microbial-induced calcium carbonate precipitation using phosphogypsum (MICPP). As shown in Fig. 1, an initiative is directed towards providing a sustainable pathway for the utilization of phosphogypsum resources, with the potential to alleviate phosphogypsum accumulation and mitigate environmental stress, as well as to utilize phosphogypsum as a source of calcium in MICPP, which also offers economic benefits of this technology. Nevertheless, phosphogypsum exhibits a more acidic nature, featuring a pH range of approximately 2-5 [44], diverging from the alkaline environments favorable for urease-producing bacteria typically employed in MICP [45], which may hinder the viability and metabolic functions of bacteria within phosphogypsum substrates. However, as a nutrient source, urea undergoes hydrolysis to produce $\text{CO}_3^{2-}$ and $\text{NH}_4^+$, consequently increasing the pH and creating an optimal environment for bacterial proliferation. This, in turn, enhances bacterial activity in phosphogypsum solutions.

In this investigation, unconfined compressive strength tests and impermeability assessments are conducted to evaluate the macroscopic properties of the sands stabilized by MICPP. Additionally, leaching analyses are executed to assess the potential environmental impact of the specimens in terms of detected hazardous elements. Scanning Electron Microscope (SEM) and X-ray Diffractometer (XRD) are employed for microscopic examinations of the treated specimens. Furthermore, the generated $\text{CaCO}_3$ is quantified. The use of both macroscopic and microscopic analyses aims to comprehensively understand the macroscopic attributes and reinforcement mechanisms linked with MICPP-treated sands. Ultimately, our findings aim to provide valuable insights and guidance for the advancement and practical implementation of the MICPP technology utilizing phosphogypsum as a calcium source.
2. Test materials and experimental schemes

2.1. Materials and reagents

2.1.1. Phosphogypsum and sands

The phosphogypsum originated from Yichang, China, of which the primary constituent is CaSO$_4$$\cdot$2H$_2$O, displaying a dark gray color. The pH measurement is approximately in the range of 4.0-5.0. The content analysis shows a calcium sulfate dihydrate concentration of 89.23%. Phosphogypsum also contains impurities, including phosphorus, fluorine, and organic matter. The chemical composition of the untreated phosphogypsum is detailed in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Chemical composition of phosphogypsum (%)</th>
<th>SO$_3$</th>
<th>CaO</th>
<th>SiO$_2$</th>
<th>Fe$_2$O$_3$</th>
<th>P$_2$O$_5$</th>
<th>Al$_2$O$_3$</th>
<th>K$_2$O</th>
<th>BaO</th>
<th>SrO</th>
<th>TiO</th>
<th>CuO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51.40</td>
<td>36.11</td>
<td>8.98</td>
<td>0.68</td>
<td>0.59</td>
<td>0.57</td>
<td>0.56</td>
<td>0.47</td>
<td>0.38</td>
<td>0.24</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 2 provides the fundamental physical characteristics of the river sands. Fig. 2 illustrates the particle grading curve of the sands subjected to testing. The sands exhibit a fineness modulus of 1.05, classified as fine sands.

Table 2

<table>
<thead>
<tr>
<th>The physical properties of sands.</th>
<th>Water content (%)</th>
<th>Apparent density (g/cm$^3$)</th>
<th>Bulk density (g/cm$^3$)</th>
<th>Void fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2.66</td>
<td>1.54</td>
<td>46.35</td>
</tr>
</tbody>
</table>
The chosen bacterial strain for this experimental investigation is Bacillus pasteurii, a Gram-positive bacillus ubiquitously present in diverse environmental matrices, including soil, water, and air. Bacillus species exhibit robust metabolic capabilities, environmental benignity, and high urease activity. The strain used in this study is Bacillus pasteurii (GDMCC 1.083) from Guangdong Microbial Culture Collection Center. In the experimental procedure, a liquid medium with a composition detailed in Table 3 was utilized.

| Table 3 |
|-----------------|-----------------|
| Reagents        | Content of liquid medium components |
| Casein Peptone  | 15g              |
| Soy Peptone     | 5g               |
| NaCl            | 5g               |
| Urea            | 20g              |
| Distilled Water | 1L               |

The activated bacterial solution is introduced to the liquid medium at a volumetric ratio of 1:50. In particular, 10 ml of the bacterial solution is carefully pipetted into a conical flask containing 500 ml of sterile liquid medium. Subsequently, the mixture is placed in a constant-temperature shaking incubator set at 30°C and agitated at a speed of 120 rpm for a duration of 48 hours. The spectrophotometer is employed to assess the OD600 value of the bacterial solution. OD600 serves as a standardized method for quantifying microbial growth in liquid media, characterizing bacterial concentration based on the absorbance of solution at a wavelength of 600 nm. Urea undergoes hydrolysis into NH$_4^+$ and CO$_3^{2-}$ during metabolic processes, catalyzed by the urease produced by Bacillus pasteurianus. The resultant metabolic change influences the conductivity within the reaction system, enabling an evaluation of bacterial activity through
variations in electrical conductivity (EC). The formula for calculating urease activity is shown in Eq. (1) \[25\]. Urease activity is quantified at around 8 mmol/(L×min) using a conductivity meter.

\[
A \times E \times t \times n = \Delta
\]

where: \(A\) represents urease activity in \(\text{mmol/(L×min)}\); \(E\) denotes conductivity in \(\text{ms/cm}\); \(t\) is time in minutes; and \(n\) denotes dilution.

To elucidate the influence of the Bacillus pasteurianus metabolic activities on the pH dynamics of the growth medium, pH measurements of the liquid medium are taken throughout bacterial cultivation, aiming to observe the pH trends and interpret the bacterial adaptation mechanisms. Fig. 3 illustrates the temporal evolution of the pH value of the bacterial solution. It is evident that the bacterial solution reaches full maturation with its pH stabilizing between 9 to 9.5.

**Fig. 3.** pH value of the bacterial solution over time.

The MICPP reaction is a multifaceted process governed by many influencing factors. Assessing \(\text{Ca}^{2+}\) conversion rate is pivotal for the effective application of the MICPP technique. Preliminary experiments are conducted then to ascertain the \(\text{Ca}^{2+}\) conversion rate prior to commencing the formal experiments. The \(\text{Ca}^{2+}\) conversion rate is defined as the proportion of the mass of \(\text{Ca}^{2+}\) incorporated into the calcium carbonate precipitate produced through MICPP to the initial mass of \(\text{Ca}^{2+}\) in the calcium source. Fig. 4 illustrates the temporal evolution of \(\text{Ca}^{2+}\) conversion for various calcium source solutions, with the \(\text{Ca}^{2+}\) conversion ultimately determined to be approximately 80%. Despite that phosphogypsum has a lower \(\text{Ca}^{2+}\) conversion rate compared to other calcium sources, the 80% \(\text{Ca}^{2+}\) conversion rate, as shown in Fig. 4, is undoubtedly noteworthy.
As shown also in Fig. 4, the initial phase of the MICPP reaction manifests a diminished Ca\(^{2+}\) conversion rate. This phenomenon can be attributed to the initially acidic pH value of the solution, indicative of subdued bacterial activity. With the advancement of the reaction, the hydrolysis of urea becomes more pronounced, resulting in a substantial augmentation in the generation of CO\(_3^{2-}\) and NH\(_4^+\). The elevation in pH value creates an optimal milieu for bacterial proliferation, consequently amplifying bacterial activity within the phosphogypsum solution.

### 2.2. Preparation of sand columns

Two distinct curing methods are applied in the preparation of the sand columns: (1) Stirring Method: In accordance with the predetermined ratios, the sand sample and phosphogypsum are accurately weighed and subsequently blended with the bacterial solution and a 1 mol/L urea nutrient solution. This mixture is then thoroughly stirred prior to its introduction into the mold to formulate the sand column. (2) Immersion Method: The sand sample and phosphogypsum are weighed based on the pre-established proportions, thoroughly amalgamated, and subsequently introduced into the mold to create the sand column. Following this, the column is submerged in a nutrient solution comprising bacterial solution and 1 mol/L urea for ongoing maintenance. The flow chart for the preparation of the sand columns is shown in Fig. 5. The mold is a PVC pipe with an inner diameter of 4.8 cm and a height of 5 cm that is sterilized to eliminate potential interference with experimental results by external impurities. In the process of preparing the specimens, a layer of petroleum jelly is initially applied to the inner wall of the PVC pipe to facilitate the subsequent demolding of the specimens. A layered filling process is followed, ensuring that each layer undergoes proper vibration and compaction. All the materials utilized in the experiments, including the sand samples, bacterial fluid, and phosphogypsum, are measured for the predefined proportions and thoroughly mixed through adequate agitation. Following the
preparation stage, the specimens are subjected to a curing period of 7 days, followed by a drying phase at 60°C for 48 hours. Following the preparation stage, the specimens undergo a curing period of 7 days, followed by a drying phase at 60°C for 48 hours.

**Fig. 5.** Flow chart for the preparation of sand columns.

### 2.3. Experiment design

After the curing process, the sand columns are tested to evaluate the unconfined compressive strength, impermeability, and leaching of the specimens. Following that, evaluations are carried out on the CaCO₃ generation and microstructural properties of the specimens, under different curing conditions, with the aim of uncovering micro-mechanisms. The combination of macroscopic observations and micro-mechanical insights shows the inherent reinforcement mechanism. The details of the experiments for microbial-induced CaCO₃ precipitation using phosphogypsum and the microstructure analysis adhered to the standardized experimental design, are shown in Table 4.

**Table 4**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bacterial solution (OD₆₀₀)</th>
<th>Phosphogypsum (g)</th>
<th>Sand (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSP-20</td>
<td>26</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>BSP-40</td>
<td>52</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>BSP-60</td>
<td>1.5</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>BSP-80</td>
<td>104</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>BSP-100</td>
<td>130</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SP-20</td>
<td>26</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>SP-40</td>
<td>52</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>SP-60</td>
<td>0</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>SP-80</td>
<td>104</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>SP-100</td>
<td>130</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BIP-20</td>
<td>26</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>BIP-40</td>
<td>52</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>BIP-60</td>
<td>1.5</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>BIP-80</td>
<td>104</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>BIP-100</td>
<td>130</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IP-20</td>
<td>26</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>IP-40</td>
<td>52</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>IP-60</td>
<td>0</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>IP-80</td>
<td>104</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>IP-100</td>
<td>130</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Note: B represents use of bacterial solutions; P denotes the addition of phosphogypsum; S and I indicate the stirring method and immersion method, respectively. The numbers in groups represent the percentage of phosphogypsum admixture.

2.4. Experimental methods

2.4.1. Unconfined compressive strength

Consistent with the well-established methodologies in sand reinforcement assessment, unconfined compressive strength is one of the primary properties in evaluating sand strength after microbial-induced CaCO₃ precipitation using phosphogypsum. The strength of the material is evaluated by gradually applying axial pressure until the specimen ruptured, and the maximum of the applied pressure is then deemed as the unconfined compressive strength. The unconfined compressive strength is evaluated for different amounts of phosphogypsum and curing methods. To mitigate potential testing errors, the upper and lower surfaces of the specimens are carefully sanded prior to the testing. Three independent specimens of each set of ratios are made and tested to ensure consistency. The compressive load is applied uniformly in the direction of compression. The specimen is placed on the lifting table of the WAW-Y1000C Universal Testing Machine and loaded at a rate of 1 mm/min.

2.4.2. Permeability

Owing to the filling of the pore spaces by the products of microbial mineralization reactions and the cementation of sand particles, a substantial reduction in the permeability coefficient is observed in comparison to that of the pre-reinforcement state. The variable head test method is used to evaluate this reduction. Eq. (2) is the equation used in the variable head permeability test to calculate the permeability coefficient of sand columns.

\[ k = 2.3 \frac{aL}{At} \log \frac{H_1}{H_2} \]  

where, \( k \) represents permeability coefficient (cm/s); \( a \) signifies cross-sectional area of the variable head pipe (cm²); \( L \) denotes path of penetration, i.e., the height of the specimen (cm); \( H_1 \) represents the head at the start (cm); \( H_2 \) signifies the head at termination (cm); \( t \) represents time (s); and \( A \) signifies cross-sectional area (cm²).

2.4.3. Determination of CaCO₃ content
To accurately quantify the CaCO$_3$ content generated within the sand column during microbial-induced CaCO$_3$ precipitation using phosphogypsum, a custom-designed testing system (Fig. 6) is employed. This system facilitates the determination of CaCO$_3$ content by subjecting it to dilute hydrochloric acid within the sand column, triggering the release of carbon dioxide gas in accordance with the principles of the Carbonate Content Test Method. The CaCO$_3$ content is dependent of the air pressure and the formula calculates the percentage of CaCO$_3$ content ($W$), as shown in Eq. (3) [47].

$$W = \frac{PVM}{RTm} \times 100\%$$

where $P$ represents the measured air pressure in pascals (Pa); $V$ signifies the volume of generated CO$_2$ gas; $M$ denotes the molar mass of CaCO$_3$ in grams per mole (g/mol); $R$ is the gas constant, having a standard value of 8.314 J/(K•mol); $T$ is the gas temperature within the device, measured in Kelvin (K), where 0°C is equivalent to 273.15K; and $M$ signifies the mass of the specimen powder in grams (g).

![Micro-precision digital carbonation measuring instrument.](image)

Fig. 6. Micro-precision digital carbonation measuring instrument.

2.4.4. Leaching test for harmful elements

At elevated concentrations, fluoride, a prevalent groundwater contaminant, presents potential health hazards. Water acidity or alkalinity, which may profoundly influence the aquatic ecosystem, is dependent on pH. Leaching tests are conducted by dissolving phosphogypsum in water, where the soluble contaminants are dissolved and diffused into the leaching solution, and the amount of soluble contaminants in the leaching solution is measured. The objective of this process is to ascertain concentration of pollutants in the leaching solution, appraise the harmful characteristics of the waste, and assess its environmental impact. In this study, the standards for water pH determination (HJ 1147-2020) [48] and ion-selective electrode method (GB/T 7484-1987) [49] are strictly followed. The chosen leaching agent is deionized water, and its volume is determined to achieve a liquid-to-solid ratio of 10:1.

2.4.5 Microscopic analysis
The CaCO$_3$, formed primarily via microbial-induced CaCO$_3$ precipitation using phosphogypsum, exhibits properties described by its crystalline structure and morphology. In conducting the microstructural examinations, a JSM-7500F scanning electron microscope manufactured by Nihon Electronics is used to provide magnification in the order of tens of thousands of times. Furthermore, qualitative analysis of the specimens is conducted using a SmartLab X-ray diffractometer manufactured by Nippon Rigaku. This facilitates a comparison of the compositions between the MICPP treated specimens and their untreated counterparts.

3. Results and discussion

3.1. Unconfined compressive strength of stabilized sands

3.1.1. Effect of phosphogypsum dosage

The unconfined compressive strength of the specimens, prepared using two curing methods (stirring and immersion), for different phosphogypsum dosages, is illustrated in Fig. 7. The compressive strength of the sand columns increases gradually with the increase of the amount of phosphogypsum. In the absence of microbial induction, the unconfined compressive strength of sand columns is about 0.1 MPa at 20% dosage. However, with the use of MICPP, the strength of the specimens prepared using the stirring method increases to 0.4 MPa, while the strength of the specimens prepared using the immersion method increases to 0.63 MPa. The compressive strength of the sand columns manufactured through immersion surpasses that of the columns manufactured through mixing by 57.5%. With an 80% phosphogypsum dosage, the stirring method yields a compressive strength of 0.98 MPa, while the immersion method results in 1.19 MPa. These findings are consistent with previous studies on sand treated with MICP, as summarized in Table 5 [50]. The results illustrate a significant improvement induced by MICPP technology in phosphogypsum mineralization. Nevertheless, with a 100% phosphogypsum dosage, the unconfined compressive strength does not increase significantly from that of the specimens with an 80% dosage.

![Fig. 7. Unconfined compressive strength of specimens at various phosphogypsum dosages.](image-url)
Table 5
Previous studies on the unconfined compressive strength of MICP-treated sand.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Urease activity</th>
<th>Sand Type</th>
<th>Density (g/cm³)</th>
<th>Compressive strength (MPa)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporosarcina pasteurii</td>
<td>0.23 mS/min</td>
<td>Iterbeck sand</td>
<td>1.56</td>
<td>0.19-0.574</td>
<td>[30]</td>
</tr>
<tr>
<td>Sporosarcina pasteurii</td>
<td>0.8-1.2 (OD600)</td>
<td>British sand</td>
<td>0.2-1.0</td>
<td>0.37-2.95</td>
<td>[51]</td>
</tr>
<tr>
<td>Bacillus sphaericus</td>
<td>10 U/ml</td>
<td>Ottawa sand</td>
<td>1.62-1.63</td>
<td>0.180-2.27</td>
<td>[52]</td>
</tr>
<tr>
<td>S. pasteurii and urease</td>
<td>0.3-1.5 (OD600)</td>
<td>Ottawa sand</td>
<td>1.58-1.64</td>
<td>0.098-2.145</td>
<td>[53]</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>10 U/ml</td>
<td>Silica sand</td>
<td>1.63-1.85</td>
<td>0.037-2.112</td>
<td>[54]</td>
</tr>
<tr>
<td>S. pasteurii</td>
<td>0.6-1.2 (OD600)</td>
<td>Xiamen sand</td>
<td>-</td>
<td>0.048-1.872</td>
<td>[55]</td>
</tr>
</tbody>
</table>

3.1.2. Failure mode

Fig. 8 illustrates the damage morphology of a representative specimen from the MICPP group. In the SP and IP specimens, the regions near the ends are more prone to loosening due to the relatively weak bonds, making them susceptible to initial damage under pressure. The lack of microbial-induced mineralization in these regions leads to structural weakness and the subsequent fracture under external loading, reducing the compressive strength of the samples and leading to severe fracture. The lack of effective calcium carbonate cementation, low cohesion between particles, and the insufficient internal friction angle to provide adequate resistance make these samples susceptible to structural loosening and cracking when subjected to compression.

In the MICPP-treated specimens (BSP and BIP), cracks gradually expand from the bottom to the top, displaying a typical shear failure mode with a distinct shear slip surface. This failure mode is primarily due to the non-uniform distribution of CaCO₃ precipitation in the spatial location, resulting in overall strength non-uniformity. Specifically, CaCO₃ precipitates increase gradually from the bottom to the top in the vertical direction, leading to a relatively low strength at the bottom of the sample and a higher strength at the top. Although the formation of CaCO₃ enhances cohesion and internal friction angle, improving overall stability and compressive resistance, the uneven distribution of CaCO₃ precipitates results in uneven strength, affecting the uniformity of the force in the material and the stress transfer under external loads. Particularly in the bottom regions with less precipitation, the lower cohesion and internal friction angle make these regions potential locationss of damage initiation, further leading to the emergence of the distinct shear damage modes.
3.2. Permeability coefficient of stabilized sands

The permeability coefficient ($K$) acts as a crucial indicator for assessing sample permeability. The variable head permeability test allows for a more accurate assessment of the influence of MICPP products on the permeability of the specimens, providing crucial insights into the curing process. As depicted in Fig. 9, various phosphogypsum dosages and sand curing methods have a significant impact on the permeability. Initially, the original specimen has a permeability coefficient of $9.928 \times 10^{-4}$ cm/s, commonly associated with the pore structure of the sand columns. Reduced and increased permeability coefficients are often associated with finer particles and higher porosity, respectively. For example, the SP-20 and IP-20 sand columns have a decreased permeability coefficient of around $8.0 \times 10^{-4}$ cm/s. The minimum particle size of the river sands used in the experiment is 0.1 mm, whereas the size of the phosphogypsum particles is smaller. Consequently, the phosphogypsum particles effectively fill the gaps between the sand particles, thereby reducing the permeability coefficient of the sand column. With microbial curing, the permeability coefficient decreases to $8.954 \times 10^{-5}$ cm/s, representing a substantial magnitude of improvement. Particularly, the specimens treated through immersion exhibited an 18% improvement compared to those treated through agitation. At an 80% phosphogypsum dosage, the specimens cured through stirring have a decreased permeability coefficient of $5.901 \times 10^{-5}$ cm/s. However, it is observed that adding any additional phosphogypsum improves permeability only marginally. The immersion-treated group with 80% phosphogypsum shows a permeability coefficient of $4.089 \times 10^{-5}$ cm/s, signifying an almost 50% improvement in impermeability compared to the stirring method. These observations align with the outcomes of existing research on MICP-treated sand, as shown in Table 6 [50]. Overall, MICPP can significantly enhance impermeability, particularly, when the immersion method is used.
Permeability coefficients of specimens with varied phosphogypsum dosages.

![Permeability coefficients graph](image)

**Table 6**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Urease activity</th>
<th>Soil Type</th>
<th>Density (g/cm³)</th>
<th>Permeability (m/s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporosarcina pasteurii</td>
<td>0.8 to 1.2 (OD600)</td>
<td>British sand (R.D)</td>
<td>0.2 to 1.0</td>
<td>1.18×10⁻⁶</td>
<td>[51]</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>10 U/ml</td>
<td>Silica sand (Australia)</td>
<td>1.60 to 1.61</td>
<td>4.22×10⁻⁵</td>
<td>[52]</td>
</tr>
<tr>
<td>Sporosarcina pasteurii</td>
<td>3.7 mM/min</td>
<td>Ottawa sand</td>
<td>1.65</td>
<td>1.16×10⁻⁵</td>
<td>[56]</td>
</tr>
<tr>
<td>Sporosarcina pasteurii</td>
<td>0.8 to 1.2 (OD600)</td>
<td>Sand</td>
<td>-</td>
<td>2.54×10⁻⁶</td>
<td>[57]</td>
</tr>
</tbody>
</table>

**3.3. Assessment of CaCO₃ production**

The quality of CaCO₃ deposition within a specimen is a crucial indicator when assessing the effectiveness of the MICPP technology. It not only reflects the quality of mineralization but also serves as the foundation for optimizing MICPP process parameters, thereby enhancing curing efficiency and effectiveness. As illustrated in Fig. 11, a noticeable correlation exists between CaCO₃ production and phosphogypsum dosage. As phosphogypsum dosages increases, the CaCO₃ content in the specimens cured through the stirring method increases from around 4% to 9%, and those cured through immersion increases from 5% to about 10%. In comparison, with the same phosphogypsum dosage, the CaCO₃ content increases by approximately 25% when using the immersion method compared to that when the stirring method is used. These findings are consistent with existing studies, illustrating a direct linear correlation between CaCO₃ content and the unconfined compressive strength of microbiologically cured specimens [31]. With the increase in CaCO₃ content, there is an increase in the unconfined compressive strength. This highlights the significance of CaCO₃ deposition, which directly influences the strength of the microbiologically treated specimens, confirming its role as a key determinant in the effectiveness of MICP technology [30].
3.4. Assessment of leached harmful elements

Leaching of harmful elements from the phosphogypsum-cured specimens is studied in this section, with the goal of assessing the environmental safety of MICPP and its benefits for eco-friendly and sustainable development. Fig. 12 (a) illustrates the correlation between fluoride content in the leachate of the sand columns and time. Additionally, Fig. 12 (b) presents the pH values of the leachate. An important observation is the significant decrease in fluoride concentration in the microbially cured sand columns, accounting for approximately 10% of the pre-curing levels. This observation may be attributable to the microbial cell membranes that harbor functional groups such as carboxyl and amino groups. The groups engage with ions via diverse chemical bonds, encompassing hydrogen bonds, van der Waals forces, and ligand bonds. These interactions play a pivotal role in the adsorption process. Metal ions or other multivalent ions may serve as “bridging” ions linking microbial cell surfaces or biofilms to negatively charged fluoride ions, thereby facilitating the indirect adsorption of fluoride. This phenomenon occurs via the formation of ligand or alternative chemical bonds with both surfaces \(^{[58]}\). Furthermore, there exists a potential for fluoride to be encapsulated within the calcium carbonate precipitate generated during the MICPP reaction, leading to a substantial reduction in fluoride concentration in the water.

The leachate from the microbially cured sand columns show higher pH values than the leachate from uncured sand columns. After the microbial mineralization, the leachate approaches to a nearly neutral pH, in contrast to the initially faster pH reduction observed in the leachate from the uncured sand column, which shows a more acidic nature. According to the standard for groundwater quality (GB/T 14848-2017) \(^{[59]}\), the fluoride concentration in the 60-day leachate...
from the MICPP-treated sand column is 1.52 mg/L, surpasses the 1 mg/L standard for Class I groundwater quality but still below the 2 mg/L standard for Class IV groundwater quality. Thus, the leachate from the MICPP treated specimens is suitable for agriculture and certain industrial water applications, implying potential suitability for domestic drinking water after further treatment. The pH value of the 60-day leachate from the cured specimen falls within the Class I groundwater quality range of 6.5-8.5, complying with the established standard. This affirms the effective sequestration of harmful elements within phosphogypsum by microbial curing, thereby reducing water contamination.

![Fluoride concentration change curve and pH change curve of leaching solution.](image)

**Fig. 12.** Fluoride concentration change curve and pH change curve of leaching solution.

### 3.5. Mechanistic analysis of MICPP

SEM is employed to examine the micro-morphology of the untreated phosphogypsum and MICPP-treated sand columns. A magnification of 500 is utilized to achieve a clearer crystal morphology of the untreated phosphogypsum, while a magnification of 1000 is employed to observe the micro-morphology of CaCO₃ in the MICPP-treated sand columns. The micro-morphology of the MICPP-treated sand columns is assessed under different curing conditions, as illustrated in Fig. 13. The crystal morphology of CaSO₄·2H₂O appears as tetragonal thin plates. The MICPP treatment results in the deposition of CaCO₃ precipitates, effectively filling the pores within the specimen. The locally generated CaCO₃ precipitates agglomerate in colloids of larger particle size. With an increase in phosphogypsum, there is a proportional increase in the CaCO₃ precipitates, leading to enhanced inter-crystal cementation. Consequently, pore space is reduced, significantly improving the structural stability and cementation of the sand column. These observations align with the results of the unconfined compressive strength tests, where the BIP group outperforme the BSP group. The stirring method used in the BSP group may lead to an uneven spatial distribution of reactants due to physical stirring limitations, thereby affecting reaction homogeneity and adequacy. In contrast, the immersion method employed in the BIP
group allows for prolonged contact between the sand column and reactants, ensuring sufficient reaction time. The filling of pore and the bonding of sand grains are facilitated by calcium carbonate precipitation. This mechanism reduces microscopic defects, increases compactness, and enhances homogeneity, ultimately improving the overall structural integrity of the sand columns.
Fig. 13. SEM micromorphology of untreated phosphogypsum and sand columns treated with MICPP.

To provide further clarity on the mineralization mechanism induced by MICPP in the phosphogypsum, XRD tests are conducted to compare the composition of the samples undergoing microbial-induced mineralization with those not exposed to the microbes. The XRD results of the sand columns with different phosphogypsum dosages before and after MICPP treatment are illustrated in Fig. 14, wherein the XRD analyses of the sterile fluid samples show two primary phases, i.e., SiO₂ and CaSO₄·2H₂O. This aligns with the expectations, as silica is the primary constituent of sand and calcium sulfate dihydrate is the principal component of phosphogypsum.

In the specimens exposed to microbial influence, in addition to the expected SiO₂ phase, new mineral phases are observed, characterized by the distinctive absorption peaks of calcite (CaCO₃). The experimental results demonstrate that the precipitated CaCO₃ primarily takes on a crystalline form known as calcite that is characterized by a stable crystal structure and high resistance to solubility, signifies durability and long-lasting maintenance of morphology, as it is resistant to dissolution or decomposition under various conditions. Additionally, calcite enhances the strength of the sand columns. The analysis of the XRD results of sand columns with different phosphogypsum dosages shows that the CaSO₄·2H₂O phase is almost absent at 80% phosphogypsum dosage, whereas at other phosphogypsum dosages, the CaSO₄·2H₂O phase is still present. This finding suggests that the conversion from CaSO₄·2H₂O to CaCO₃ in the MICPP treatment is more efficient at 80% phosphogypsum dosage. This discovery aligns with the results above concerning both the unconfined compressive strength and the permeability coefficient.
Bacillus pasteurus is employed in the experiments of the MICPP process. These microorganisms act as catalysts, triggering the decomposition of urea—a fundamental process in the mineralization reactions of MICPP. The MICPP mineralization reaction comprises two primary steps: urea hydrolysis and CaCO$_3$ precipitation. As shown in Fig. 15. Stage I: The initial step involves the action of urease, catalyzing the breakdown of urea (NH$_2$CONH$_2$) into ammonium ions (NH$_4^+$) and carbonate ions (CO$_3^{2-}$). The continued accumulation of NH$_4^+$ and CO$_3^{2-}$ increases the cellular membrane potential and raises the overall pH of the solution. This alkaline environment encourages the continuous accumulation of NH$_4^+$ and CO$_3^{2-}$. Simultaneously, urease is produced through the metabolism of Bacillus pasteurianus, and extracellular polymers are produced [60]. Stage II: The polymers, combined with the double-layer structure of microorganisms, tend to adhere to the surfaces of the sand particles. Functional groups such as hydroxyl, amine, amide, carboxyl, etc. present on the surface of microbial cell membranes carry negative charges. Ca$^{2+}$ released from the phosphogypsum is attracted to this negatively charged surface, leading to substantial Ca$^{2+}$ accumulation on the cell surface. Concurrently, CO$_3^{2-}$ produced through urea hydrolysis is transported extracellularly. The convergence of Ca$^{2+}$ and CO$_3^{2-}$ at the cell serves as a nucleation site, initiating the gradual formation of CaCO$_3$ crystals [61]. As these crystals develop, the microbial bacteria become completely encased by them, restricting nutrient transport and ultimately leading to the demise of the bacteria. Typically, higher specimen strength is associated with the increased CaCO$_3$ content in the specimen. However, strength also depends on the distribution and form of CaCO$_3$ cementation. Cementation is classified into three
categories based on the relationship between CaCO₃ and particle size: (1) Coating Role: CaCO₃ covers particle surfaces, enhancing particle volume and surface area without interparticle contact. (2) Bonding Role: CaCO₃ primarily resides near particle contact points, causing aggregation and bonding between particles. (3) Bridging Role: CaCO₃ accumulates between adjacent particles without direct contact, fostering growth until the particles contact each other. These forms of CaCO₃ cementation enhance specimen strength and reduce permeability. However, structural improvement primarily depends on the bonding and bridging roles of CaCO₃.

Fig. 15. Schematic diagram of the principle of microbial-induced CaCO₃ precipitation using phosphogypsum.

4. Conclusions

In this paper, the MICPP technology is introduced and implemented for the treatment of sand using phosphogypsum as the calcium source. Subsequently, the performance and mechanism of the treated sand columns are analyzed and evaluated, and the conclusions drawn can be summarized as follows:

(1) In the process of MICPP, bacterial and nutrient solutions permeate the pores between the particles. This facilitates hydrolysis of urease, interacting with the free calcium ions extracted from phosphogypsum, thereby initiating the formation of CaCO₃ crystals. The resultant crystals contribute to enhancing the density and uniformity of the sand by bonding the particles together and filling the pores, consequently fortifying the overall structural integrity of the sand.

(2) On a macroscopic scale, MICPP strengthens specimens. A higher phosphogypsum dosage is associated with an elevated unconfined compressive strength and a decreased
The permeability coefficient of the MICPP-treated specimens is ten times lower than that of the untreated specimens. Remarkably, the immersion method exhibits superior curing efficiency when compared with the stirring method.

(3) The leachate from the control group, where phosphogypsum is cured without microbial treatment, surpasses applicable emission standards. In contrast, harmful leaching tests conducted on the leachate of the sand columns show that the MICPP process reduces environmental contaminability.

(4) On a microscopic scale, the increased strength is associated with the expanded pores filled with CaCO$_3$. Microbially induced calcium precipitation predominantly encompasses calcite, providing robust stability and serving as a bonding and bridging agent.

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