Microbial Precipitation of Calcium Carbonate for Crack Healing and Stabilization of Sandy Soils

Yumi Kim 1,2 and Yul Roh 1,*

1 Department of Earth and Environmental Sciences, Chonnam National University, Gwangju 61186, Republic of Korea; yumikim@jnu.ac.kr
2 Department of Earth and Environmental Sciences, University of Pennsylvania, Philadelphia, PA 19104, USA
* Correspondence: rohy@jnu.ac.kr; Tel.: +82-62-530-3458

Abstract: Microbially induced calcium carbonate (CaCO₃) precipitation (MICP) can improve the shear strength of soil via biocementation while reducing its porosity and hydraulic conductivity. The purpose of this study was to evaluate the effect of the addition of bacterial metabolites and montmorillonite on the crack healing and biocementation of sandy soil during the MICP process. Cracks were generated by drying wet soil samples in Petri dishes, after which they were sprayed with one of four treatments: deionized water, a cementation solution, bacteria mixed with the cementation solution, and bacterial metabolites mixed with the cementation solution. After five cycles of this spray treatment, the surface crack ratio was observed to decrease by about 71% when living cells were used and by about 80% when microbial metabolites were added. However, the crack reduction ratio was relatively low when treated with water (28%) and the cementation solution alone (48%). To investigate the effect of adding a phyllosilicate to improve the strength of sandy soil, MICP was induced in sand mixed with 0–30% montmorillonite (MMT). As a result, the soil strength increased with higher levels of MMT, indicating that MMT contributed to soil stabilization as a colloid for CaCO₃ precipitation and via adhesion between sand grains. Therefore, for the crack healing and stabilization of sandy soil, the addition of bacterial metabolites and montmorillonite may enhance the effectiveness of the MICP process.

Keywords: MICP; soil stabilization; microbial metabolites; Sporosarcina pasteurii; montmorillonite

1. Introduction

Carbonate-forming microbes (CFMs) are capable of decomposing substrates in metabolic processes to produce carbonate, which then reacts with calcium ions to produce calcium carbonate (CaCO₃) [1]. CaCO₃ precipitation via CFM activity is generally considered a form of induced mineralization because the type of mineral produced depends on the environmental conditions [2]. Microbially induced calcite precipitation (MICP) usually occurs in open environments, and the process is often related to the microbial cell’s surface structure and metabolic activity. Metabolisms that can locally increase carbonate saturation and promote MICP are known to rely on enzymes such as urease and carbonic anhydrase (CA). Ureolysis catalyzed by urease enables microorganisms to use urea as a nitrogen and carbon source [3]. CA promotes carbon transport into cells through CO₂–HCO₃⁻ interconversion [4]. Both enzymes generate carbonate anions and increase the pH as a consequence of their activity [5]. In addition, previous studies have reported that microbial extracellular polymeric substances (EPS) can trap and bind calcium to promote CaCO₃ precipitation, thus affecting its morphology and mineralogy [6–8].

MMICP has been employed in a variety of environmental and geotechnical applications [9]. For example, MICP can contribute to the immobilization of contaminants in groundwater, including ionized heavy metals and radionuclides such as strontium [10–12]. In addition, divalent cations have been found to be substituted for Ca or co-precipitated
with the CaCO$_3$ produced by microorganisms. For geotechnical applications, MICP can serve as an environmentally friendly, low-maintenance solution for stabilizing natural biological soils. In previous studies, it has been found that MICP can improve the compressive strength and durability of loose sandy soil [13,14] and soft clay [15], as well as seal cracks in cement and concrete [16]. Additives, such as Portland cement, have been widely used for the improvement of soil, but recently, the demand for the use of natural additives and the development of eco-friendly processes such as MICP has increased.

Natural phyllosilicates, also known as clay minerals, have also been proposed as natural additives for soil improvement to replace Portland cement due to their low costs, high reactivity, and large surface areas. Phyllosilicates are a class of silicates with a characteristic stratified structure formed by tetrahedral (T) and octahedral (O) sheets [17]. For example, montmorillonite (MMT) is a 2:1-type layered silicate consisting of packets of a dioctahedral 2:1 phyllosilicate consisting of two tetrahedral sheets and one octahedral sheet (T:O:T) with adjacent margins. These sheets retain a negative charge, which is neutralized by exchangeable cations such as Na$^+$ or Ca$^{2+}$ located in the interlayer spacing and on the surface [18]. Therefore, MMT has excellent adsorption capacity and high cation exchange and is known to induce biomineralization by interacting with bacteria [19]. In particular, it has been reported that MMT can effectively promote the growth of microorganisms by buffering the pH in biological processes and contribute to microbial nucleation, thus facilitating mineral formation [1,20,21]. Chen et al. (2017) demonstrated that the addition of MMT to an MICP process for the stabilization of uranium tailings controls the crystal form of CaCO$_3$, balances the acidity and ions of the tailings, and reduces toxicity [1]. In addition, when 6% of MMT was added to the uranium tailings, the maximum strength of the cement body reached 2.18 MPa, representing an increase of 47.66% when compared with its absence. Recently, Tang et al. (2021) reported the effects of the addition of Na-MMT to an MICP process on the precipitated minerals and soil mechanical properties [22]. The addition of Na-MMT accelerated the Ca$^{2+}$ ion reaction and led to higher levels of CaCO$_3$. It also significantly reduced the hydraulic conductivity of the consolidated sandy soil.

Though it is a phyllosilicate mineral, montmorillonite can be used as an additive to enhance the MICP effect in loose sandy soils; few experiments have been conducted to verify this. Therefore, the objective of this study was to evaluate the effects of bacterial metabolites and MMT on the crack healing and biocementation of sandy soil in the MICP process.

2. Materials and Methods

2.1. Bacterial Cultivation

The Gram-positive bacterium *Sporosarcina pasteurii*, an endospore-forming facultative anaerobe, can produce urease to decompose urea into carbonate that reacts with calcium to form calcite, which is the key to biomineralization [23]. In this study, *S. pasteurii* (KCTC 3558) was obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Republic of Korea) for the experiment. The fully assembled genome sequence of *S. pasteurii*, KCTC 3558, is deposited in the NCBI database (NCBI Genome UID = 30565) and the JGI database (JGI Genome ID = Gp0384160). The genome size of *S. pasteurii* KCTC 3558 was determined to be 3.3 Mb. The growth medium for *S. pasteurii* cultivation and carbonate precipitation contained 10 g/L of yeast extract, 5 g/L of proteose peptone, 1 g/L of glucose, and 24 g/L of NaCl [10]. The medium was autoclaved at 121 °C and 1.2 kgf/cm$^2$ for 20 min. The pH of the medium was about 6.6. *S. pasteurii* KCTC 3558 was cultivated in the growth medium modified with 20 g/L of urea (sterilized using a 0.2 μm filter) under aerobic conditions at 30 °C for 7 d. During the microbial growth period, *S. pasteurii* KCTC 3558 hydrolyzed urea into ammonium, increasing the pH of the medium. To measure the optical density of cell culture solutions, a disposable cuvette with a path length of 10 mm was used. After 3 d of microbial growth, the OD$_{600}$ value reached 1 (approx. $10^7$ cells/mL) and the pH increased to 9.
2.2. Preparation of Solutions for the MICP Experiment

For the MICP experiment, a cementation solution (CS) was prepared by adding 50 mM of CaCl$_2$ and 330 mM of urea (20 g/L) to the growth medium for the microorganisms. The CS solution was used as a negative control without microbial influence. For comparison of microbial growth and metabolites, a bacterial solution (B) including living cells was prepared by culturing $S$. pasteurii in the microbial growth medium for 7 d, while a solution of bacterial metabolites (BM) was prepared by autoclaving the bacteria-enriched medium to remove the living cells. According to previous studies, a culture supernatant containing microbial metabolites can be used as biological additives to induce calcium carbonate precipitation without active cells [24,25]. Both the B and BM solutions were alkaline (pH 9) and contained bacterial substances such as EPS.

2.3. Healing of Desiccation Cracks on the Soil Surface Using S. pasteurii and Its Metabolites

MICP has been used to improve soil strength through sealing naturally occurring cracks in sandy soil surfaces. Figure 1A presents an overview of the experimental process. Following the method reported by Liu et al. (2020), 90 mm Petri dishes were first lined with a grid containing holes with a diameter of about 2 mm in order to disperse the solution, and a paper filter was then placed on top of the grid [26]. The soil samples used in the experiment were sandy loam collected from different sites in Muan-gun, Jeollanam-do. These samples were sieved to only retrieve particles with a diameter of 2 mm or lower after removing the organic matter using hydrogen peroxide. To create artificial cracks, 10 g of the soil was placed in the Petri dish and sprayed with distilled water until it reached sufficient wetness. The samples were then dried in an oven at 30 °C for 24 h to generate cracks.

**Figure 1.** Schematic diagram of the experimental process for (A) the surface crack healing and (B) stabilization of sandy soil.

Soil samples in different Petri dishes were sprayed with either deionized water, a cementation solution, bacteria mixed with the cementation solution, or bacterial metabolites mixed with the cementation solution to a sufficient wetness and placed in an incubator at 30 °C for 1 week. This process was carried out once a week for 5 cycles. In order to investigate the crack healing, the soil surface was photographed each week to observe...
the morphological changes in the surface cracks. Image analysis using the open-source software ImageJ was subsequently conducted to quantify the change in the ratio of cracks to the soil surface area. To quantify the crack resistance of the soil, the crack reduction rate ($R_{cr}$) was calculated using Equation (1) [27]:

$$R_{cr} = \frac{R_{sc, u} - R_{sc, ti}}{R_{sc, u}} \times 100\%$$

where $R_{sc, u}$ is the surface crack ratio of the soil sample at the end of preliminary treatment, and $R_{sc, ti}$ is the surface crack ratio of the soil sample after each cycle, with $i$ varying between 1 and 5.

2.4. Effect of MMT on the Stabilization of Sandy Soil Using MICP

To investigate the effect of MMT in terms of improving the strength of sandy soils under MICP, artificial sandy soils were prepared by mixing standard Jumunjin sand (poorly graded sand, SP) and MMT (sand:MMT = 9:1, 8:2, or 7:3) (Figure 1B). According to USDA soil texture classification, the artificially prepared sandy soil samples were classified as loamy sand with 10% of MMT (i.e., sand:MMT = 9:1) and sandy loam with 20–30% of MMT (i.e., sand:MMT = 8:2 to 7:3). Changes in the soil texture can affect mechanical properties, such as soil strength and permeability, but this study focused on investigating whether MMT in sandy soils enhances the MICP effect and soil stabilization. The MMT used in the present study was bentonite from Samchun Chemicals (Seoul, Republic of Korea). X-ray diffraction analysis (XRD) revealed that the MMT had a d-spacing (001) for the main peak located at a $2\theta$ of 5.9° of 1.505 nm (Figure S2). The chemical properties of MMT were analyzed using X-ray fluorescence analysis (XRF), showing that the CaO content (1.5%) was higher than that of MgO (1.36%) and Na$_2$O (0.97%) (Table S1). It was thus confirmed that the MMT used in the experiment was Ca-rich MMT (Ca-MMT). A Brunauer–Emmett–Teller (BET) analysis revealed that the surface area of MMT was 85.5152 m$^2$/g.

For the soil stabilization experiment, 450 g of sand and the three artificially prepared sandy soils (i.e., containing 10%, 20%, or 30% of MMT) were transferred to separate cylindrical containers (332 cm$^3$) with a height of 6 cm. In order to induce MICP, 110 mL of the CS containing bacteria (1% v/v) was added to the soil and mixed by stirring with a stick until it was homogeneous. Previous studies have reported that this mixing method is more efficient than the pouring method when the soil particles are small [28]. Each container was placed in an incubator at 30 °C for one month to induce microbial growth and the precipitation of CaCO$_3$. After four weeks, soil stabilization was confirmed by the formation of a soil cake, after which the soil hardness and the amount and mineralogical properties of CaCO$_3$ were evaluated. The amount of CaCO$_3$ in the soil cake was measured using a leaching method with 0.5 M hydrochloric acid [29]. The CaCO$_3$ content (%) was determined using Equation (2):

$$\%\text{CaCO}_3 = \frac{m_{\text{dry soil before}} - m_{\text{dry soil after}}}{m_{\text{dry soil before}}} \times 100\%$$

where $m_{\text{dry soil before}}$ is the initial soil weight after oven drying at 105 °C for 24 h and $m_{\text{dry soil after}}$ is the soil weight after acid washing and drying until no more gas bubbles occur. To investigate the effect of the CS on the cementation of sandy soils via MICP, the same experiment was conducted without the bacterial cells and the results were compared with those from the MICP experiment.

2.5. Analytical Methods

ImageJ was used to quantitatively compare the effects of the various treatments on the cracks that developed during the drying of the soil. The original color images of the crack patterns were converted to gray-level images, and the noise was removed using filtering. The ratio of the cracked area and the total surface area of the soil were then
measured to calculate the surface crack ratio (Rsc). X-ray fluorescence (XRF) analysis for the chemical characterization of MMT was performed using an XRF-1800 spectrometer (Shimadzu, Kyoto, Japan), and Brunauer–Emmett–Teller (BET) analysis for MMT’s surface area was conducted using a 3Flex specific surface area analyzer (Micromeritics, Norcross, GA, USA). Precipitation of CaCO\(_3\) in the soil was confirmed using XRD and field-emission scanning electron microscopy (FE-SEM) with energy-dispersive X-ray spectroscopy (EDS). The precipitate for the XRD and SEM analyses was collected by centrifuging the suspension at 3000 rpm for 5 min. After the removal of the supernatant from the suspension, the remaining residue was carefully rinsed with distilled water twice and then dried in an oven at 60 °C to obtain solid CaCO\(_3\). XRD analysis was performed using an Empyrean 3D high-resolution X-ray diffractometer (Malvern Panalytical, Malvern, UK) with Cu K\(\alpha\) radiation (40 kV, 30 mA) at a scan speed of 5 \(^\circ\)/min. FE-SEM analysis was conducted using a Hitachi S-4700 FE-SEM (Hitachi, Tokyo, Japan) at an accelerating voltage of 15–20 kV with EDS (Phillips, Eindhoven, the Netherlands) to determine the morphology and elemental composition of CaCO\(_3\). Cross sections of the sand cake were observed using a stereomicroscope S8APO (Leica, Wetzlar, Germany) at 10× and 40× magnifications. The soil hardness was measured using a 351-EN pocket cone penetrometer (Fujiwara, Wakayama, Japan).

3. Results

3.1. Healing of Desiccation Cracks on the Soil Surface via MICP

Figure 2 presents the changes in the desiccation crack patterns at the end of each cycle for the four treatments (water [W], CS, CS + B, and CS + BM). After the preliminary water treatment and drying, the soil was divided by cracks into separate clods (preliminary row, Figure 2). The soil samples exhibited different degrees of initial cracking (2.2–7.8%) due to differences in the shapes of the cracks (Figure 3A). For this reason, it was difficult to calculate and statistically compare the crack healing between the treatments, but the changes with each cycle could be tracked. With the water treatment (column W, Figure 3), few morphological changes were observed in the cracks, though the crack connectivity increased, with the crack ratio on the surface decreasing by up to 28.8%.

The samples treated with the CS (CS, Figure 3) healed more cracks than those treated with water, resulting in a crack reduction of up to 48%. In the MICP treatment (CS + B, Figure 3), the crack ratio decreased by up to 70.9% after all of the treatment cycles, thus confirming its efficacy in reducing the desiccation cracks of soil. This was similar to the results of a previous study that employed MICP to heal shrinkage cracks in clayey soil [26]. On the other hand, the treatment with microbial metabolites but no live cells (CS + BM, Figure 3) healed up to 79.7% of the soil cracks through the precipitation of CaCO\(_3\). Therefore, the chemical change in the medium due to bacterial growth and the incorporation of bacterial-secreted substances such as EPS led to rapid CaCO\(_3\) precipitation that assisted in crack healing without the presence of live bacterial cells.
3.2. Stabilization of Sandy Soils Using MMT and MICP

In general, the more silt and clay in a soil, the stronger it is. For this reason, it was expected that the addition of small MMT particles would improve the strength of the sandy soil samples in the present study during the MICP process by filling the pores of the sand and acting as a support. It was observed that the surface pores of the sandy soil samples containing MMT (10–30%) were filled with the precipitate formed via MICP, with the MMT levels affecting the efficiency of sand cementation. In particular, the resulting soil cake...
had heights of 4.5 cm, 5.0 cm, and 6.0 cm for MMT mixing ratios of 10%, 20%, and 30%, respectively (Figure 4).

Without MMT, the surface of the sand appeared to be firmly bonded after the MICP treatment, but the shape of the cake collapsed when removed from the cylindrical container, indicating that the sand grains were not cemented evenly.

The hardness of the cemented sandy soil samples was measured using a pocket cone penetrometer, with the compressive strength of the soil cake found to be about 0.2 MPa with 10% of MMT and about 3.3 (±0.4) MPa at 20% and 30% of MMT. The strength of the sand was also improved with the addition of only MMT and CS (i.e., no microbes), but not to the same extent as with MICP. With 10% of MMT, the strength of the sand cake was 0.05 MPa, which was 20.4% of that observed with MICP. Similarly, with 20% and 30% of MMT, the strength rose to 0.15 MPa and 0.68 MPa, but these values only represent 4.6% and 19.3% increases, respectively, of the MICP-induced strength (Figure 5).

The amount of CaCO$_3$ precipitated in the presence of MMT increased by approximately 1.8-fold compared to sand alone (Table 1). In particular, 0.014 g of CaCO$_3$ was found in 1 g
of the 100% sand sample, compared with 0.018 g, 0.022 g, and 0.027 g in the 10%, 20%, and 30% MMT soil samples, respectively.

Table 1. Mineralogical and physical properties of soil cakes formed using MICP treatment.

<table>
<thead>
<tr>
<th>No.</th>
<th>Samples</th>
<th>Calcium Carbonate in Soil (g)</th>
<th>MMT (001)</th>
<th>Compressive Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td>%</td>
<td>2 Theta</td>
</tr>
<tr>
<td>1</td>
<td>Sand</td>
<td>0.014</td>
<td>1.428</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>MMT 10%</td>
<td>0.018</td>
<td>1.774</td>
<td>6.81</td>
</tr>
<tr>
<td>3</td>
<td>MMT 20%</td>
<td>0.022</td>
<td>2.232</td>
<td>6.90</td>
</tr>
<tr>
<td>4</td>
<td>MMT 30%</td>
<td>0.027</td>
<td>2.691</td>
<td>6.95</td>
</tr>
</tbody>
</table>

The increase in CaCO₃ precipitation with a higher MMT content is important for the enhancement of the MICP, in which two mechanisms may be involved. In the first, the microbial activity and urea degradation rate may have been enhanced by MMT. Previous studies have compared the effects of various soil properties on MICP, finding that the urea degradation of microorganisms is promoted by the presence of MMT [30]. In addition, in the second mechanism, the Ca ions present between the MMT layers may have leached out during the reaction process, contributing to the precipitation of CaCO₃. Ca leaching from 2 g of MMT in 40 mL of pure water, the microbial growth medium, or the growth medium containing bacteria was thus investigated. It was found that 36.6 mg/L of Ca was leached from distilled water after 7 d, in addition to large amounts of Na. In the growth medium, the amount of leached Ca was 214 mg/L, compared to only 4.78 mg/L in the growth medium with urea and microorganisms.

![Figure 5](image-url)  
**Figure 5.** Soil compressive strength of soil cakes formed through MICP treatment on sand (MMT 0%) and sand with various levels of MMT (10–30%). This indicated that the dissolved Ca was precipitated by MICP (Figure S1). In a growth medium with a high ionic strength, the Ca between MMT layers can leach out and be replaced with Na, while the growth of microorganisms can produce small organic molecules, such as EPS, which can be inserted between the MMT layers [31]. Therefore, the Ca-MMT present in the soil during the MICP treatment could act as a potential Ca source and contribute to the precipitation of CaCO₃.

MMT can also fill the spaces between sand grains and become cemented during MICP treatment. Because the water-soluble organic matter on the microbial cell membrane and that on the surface of the MMT particles are negatively charged, the formation of CaCO₃ may have been promoted via the chelation of Ca²⁺ [1,32]. In a cross section of the sand cake observed under a stereoscopic microscope, it was found that the spaces between the
sand grains were filled with MMT particles, with the extent of this filling increasing with a higher MMT content (Figure 6). The MMT was supported by the sand grains, and the CaCO$_3$ that formed during the MICP process contributed to the stabilization of the sand by acting as a bridge between the particles. However, in the soil cake with 30% of MMT, the sand and MMT particles were compressed more densely than in the soil cake with 20% of MMT, leading to agglomeration and cracks between the clumps. Therefore, when the MMT content increases, the durability of the soil cake is weakened because shrinkage cracks occur when soils with high clay contents are dried. The width of the shrinkage cracks observed in the micrographs varied between 50 and 250 µm (Figure 6F).

**Figure 6.** Microscopic observation at low (10×) and high (40×) magnification of soil cake cross-sections formed through MICP treatment on sand (MMT 0%) and sand with various levels of MMT (10–30%): (A, B) MMT 10%, (C, D) MMT 20%, and (E, F) MMT 30%. Figure 7 shows the morphological characteristics of the particles in the soil cake observed using SEM. In the sample with 10% of MMT, the large particles (about 600 µm in diameter) were mainly quartz (Si and O), with MMT covering their surfaces (Figure 7A). Under a high magnification, the MMT on the quartz surface consisted of flaky particles primarily containing Si, Al, and O with trace amounts of Fe and Ca. With 10% of MMT, it was observed that the quartz grains and the surface-covering MMT particles were separated (Figure 7B). However, in the sample with 30% of MMT, particle agglomeration with MMT was widely observed, and the quartz particles were covered entirely with MMT. Figure 7C presents the agglomerated MMT particles, while Figure 7D shows calcite crystals partially coated by MMT. An EDS analysis confirmed the presence of calcite based on the high Ca content.
The precipitation of CaCO₃ in the soil samples was investigated using XRD patterns (Figure 8). The sandy soil was mainly composed of quartz, feldspar, and mica, and as the MMT addition increased from 10% to 30%, the peak intensity of MMT also increased. The position of the MMT diffraction peak shifted from a 2θ of 5.9° to 6.9°, and the d-spacing (001) decreased from 1.505 to 1.270 nm as the amount of MMT increased (Figure 8 and Table 1). The MMT d-spacing (001) remained at 1.505 nm in distilled water over 24 h but increased to 1.780 nm in the microbial growth medium containing organic matter such as glucose. However, in the culture containing EPS and microbial metabolites, the XRD analysis revealed that, upon aggregation, the MMT underwent an initial interlayer contraction (Figure S2).

Short-duration experiments of up to 24 h resulted in a decrease in the d (001) value from 1.505 to 1.228 nm due to the biofilm produced by the microorganisms interacting with MMT to form an organo-clay complex. Previous studies have shown that biofilm molecules replace the two interlaminar water layers of smectite, resulting in net contraction along the 001 lattice plane of the smectite. However, it has been reported that, when the reaction time is extended, the interlayer expansion of the clay occurs as a result of the replacement or addition of larger polymer groups [31]. In this experiment, the re-expansion step did not occur because the reaction solution was injected once and then dried.

Figure 7. SEM-EDS images of soil cake fragments formed through MICP treatment in sand with 10% and 30% MMT: (A) Quartz and (B) MMT particles observed at MMT 10% and (C) MMT aggregates and (D) calcite observed at MMT 30%. 
4. Discussion

4.1. Healing of Desiccation Cracks on the Soil Surface Using MICP

This study investigated the healing efficiency of MICP for cracks on the surface of sandy soils produced during the drying process. It was found that these cracks were healed by the microbiologically induced precipitation of CaCO$_3$. In the treatments using microorganisms (CS + B) and microbial metabolites (CS + BM), the crack-healing effects were about 71% and 80%, respectively. These results indicate that biological additives can improve CaCO$_3$ precipitation even without microbial activity. According to previous studies, soluble microbial products (SMPs) act as templates and create a local environment that may favor the attraction of Ca$^{2+}$, leading to carbonate saturation [33]. In addition, CaCO$_3$ has been shown to precipitate even when only the biological additives from cultures of actinobacteria were employed (e.g., mycelium pellets, culture supernatant, and spent cultures) [24]. This is the result of interactions between organic and/or inorganic compounds with an organic matrix that do not require extracellular or intracellular biological activity [34]. This mechanism could be described in terms such as biologically mediated mineralization or organomineralization processes [34,35]. However, the mechanisms underlying the effect that acidic organic molecules (such as polysaccharides, proteins, and amino acids) have on the composition, microstructure, shape, and size of biominerals require more investigation [36]. Overall, microbial metabolites have the potential to promote CaCO$_3$ precipitation for the purpose of crack healing or the stabilization of sandy soil in polluted or extreme environments where microbial activity is very low or absent.

4.2. Combination of MMT and MICP for the Stabilization of Sandy Soil

The cementation of sandy soil by MICP has been proven by many previous researchers, and Table 2 shows examples of recently reported biocementation studies mediated by microorganisms. Dubey et al. (2021) reported that high urea and calcium concentrations can improve the biocementation of sandy soil by increasing calcium carbonate precipitation [37]. On the other hand, this study showed that adding MMT to sandy soil was effective in stabilizing sandy soil by improving the MICP efficiency even at a low calcium concentration and simplified treatment. The effect of the combination of MICP and MMT is summarized in Figure 9. Because the bacterial cell walls, which are composed of peptidoglycan rich in
carboxylate groups, are negatively charged, Ca\(^{2+}\) is attracted and serves as a nucleation site for CaCO\(_3\) [38,39]. MMT, which has excellent adsorption capacity, adsorbs these bacterial cells and promotes CaCO\(_3\) nucleation and mineral crystal growth. This mechanism has been demonstrated in a previous experiment conducted by Tang et al. (2021) [22]. In addition, the biofilm produced by the microorganisms interacted with MMT to form an organo-clay, leading to net contraction along the 001 lattice plane of MMT. It is known that the basal contraction of Ca-MMT can occur even when Na is substituted for Ca in a chemically Na-rich environment [18,40]. However, in the present study, the 001 lattice plane of MMT tended to contract from 1.505 to 1.270 nm as the MMT content increased, even though the concentration of NaCl in the CS was the same. Therefore, it appears that the increase in the MMT content promoted the growth and activity of microorganisms and increased the production of biofilm and its substitution into MMT [1,20,21]. As an example of combining MICP and MMT for the treatment of contaminated soil, Chen et al. (2017) cemented uranium tailings by adding different amounts of MMT to an MICP process, finding that MMT contributed to reducing the toxicity of uranium ions for microorganisms [1]. When the MMT content was 6%, the maximum strength of the cement body reached 2.18 MPa, representing a 47.66% increase compared to the case without MMT. These results indicate that adding MMT to the MICP process for soil stabilization can contribute to CaCO\(_3\) precipitation and contaminant fixation by promoting microbial activity and mineral nucleation. Therefore, this combined process is likely to be effective not only for soils that require lower permeability and greater strength, but also for soils contaminated with pollutants.

![Diagram](image.png)

**Figure 9.** Schematic diagram of the effect of adding MMT to sandy soil on MICP and soil stabilization: acting as a support by filling the pores between sand grains and inducing calcium carbonate precipitation due to the attraction and adsorption of microbial cells and calcium ions.

Nevertheless, the addition of MMT may inhibit the permeability of the solution for the biomineralization of calcium carbonate in soil. Therefore, the effects shown in these results can only be expected to be positive when applied to sandy soil, where it is necessary to reduce the size of pores and lower permeability for stabilization. Additionally, efficiency can be increased if the solution for the biomineralization of calcium carbonate can be mixed with soil particles instead of pouring it during the process. However, if the MMT addition ratio is too high, internal cracks may occur due to the agglomeration of sand grains, so the addition amount needs to be adjusted according to the physical characteristics of the soil in the field.
Table 2. Biocementation studies of sandy soil mediated by microorganisms.

<table>
<thead>
<tr>
<th>Used microbes</th>
<th>This Study</th>
<th>Dubey et al. (2021) [37]</th>
<th>Tang et al. (2021) [22]</th>
<th>Chen et al. (2017) [1]</th>
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<td>Urea</td>
<td>330 mM</td>
<td>500 mM</td>
<td>30.03 g</td>
<td>0.05–0.15 mol/L</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>50 mM</td>
<td>500 mM</td>
<td>55.5 g</td>
<td>(Ca²⁺/urea)</td>
</tr>
<tr>
<td>Temperature</td>
<td>30 °C</td>
<td>32 ± 3 °C</td>
<td>25 °C</td>
<td>30 °C</td>
</tr>
<tr>
<td>Soil</td>
<td>sand</td>
<td>Sand, loamy sand</td>
<td>Sand</td>
<td>Uranium tailings</td>
</tr>
<tr>
<td>Additives</td>
<td>Montmorillonite</td>
<td>-</td>
<td>Na-montmorillonite</td>
<td>Montmorillonite</td>
</tr>
<tr>
<td>Metabolite</td>
<td>Bacterial suspension, soluble microbial products</td>
<td>-</td>
<td>Bacterial suspension</td>
<td>Injection of mineralizing liquid</td>
</tr>
<tr>
<td>Treatment method</td>
<td>Mixing</td>
<td>Spraying</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Treatment cycles</td>
<td>1</td>
<td>1–3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Minerals formed</td>
<td>Calcite</td>
<td>Calcite</td>
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<td>Calcite, vaterite</td>
</tr>
<tr>
<td>Soil compressive strength</td>
<td>0.24–3.7 Mpa</td>
<td>1.67–5.3 Mpa</td>
<td>-</td>
<td>1.2–2.18 Mpa</td>
</tr>
</tbody>
</table>

5. Conclusions

In this study, the effect of bacterial metabolites and montmorillonite on the surface crack healing and biocementation of sandy soils was evaluated to improve the efficiency of the MICP process. Crack healing efficiency rates of about 71% and 80% were observed via CaCO₃ precipitation promoted by living microorganisms (CS + B) and microbial metabolites (CS + BM), respectively. The precipitation of CaCO₃ induced by microbes and their metabolites indicated that the healing of shrinkage cracks in sandy soils can occur in both microbial-growth-conducive and extreme environments. The addition of MMT to sandy soils for the MICP process also resulted in changes to the soil texture and increased its compressive strength. However, the effects of 20% and 30% of MMT on the strength of the sandy soil were similar, but at levels higher than 30% of MMT, desiccation cracking of clay occurred, indicating that the stability of the cementation effect may be weaker in the long term. The large surface area and adsorption properties of MMT were found to contribute to the improvement of MICP efficiency via the adsorption of microorganisms and Ca ions and the promotion of microbial activity and mineralization. Ca-rich MMT acted as a Ca source through the leaching of Ca ions from the mineral interlayers. In this study, the mixing ratio of MMT with sand was simplified to clarify its influence and characteristics, but for field applications, it will be necessary to adjust the mixing ratio of MMT more precisely based on the purpose of the MICP process and the site environment. In addition, further investigations on the roles of the constituents and organic molecules in the metabolites of *S. pasteurii* in precipitating calcium carbonate will be required to clarify the mechanism. Overall, the addition of biological additives and/or montmorillonite to the MICP process can be effective for sandy soils that require the healing of desiccation cracks, reduced permeability, and greater strength, as well as for environments where the spread of contaminants is a concern.
Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app14041568/s1, Figure S1: Ca leaching amount (mg/L) from montmorillonite treated in various solutions; Figure S2: X-ray diffraction analysis of MMT samples in various solutions (water, microbial growth media, and microbially enriched cultures); Table S1: Result of X-ray fluorescence analysis of montmorillonite.

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