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Research Article

Assessing The Potential Of Indigenous *Bacillus cereus* For Soil Crack Healing Via Microbiologically Induced Calcium Carbonate Precipitation

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ABSTRACT

The utilisation of indigenous ureolytic bacteria for the rehabilitation of soil slope cracks through microbial-induced calcite precipitation (MICP) is cost-effective and eco-friendly. This study was designed to assess the soil's crack-healing potential of the indigenous bacteria, *Bacillus cereus*. A solution containing the urease-producing *B. cereus* and *B. megaterium* (control treatment) and a cementation solution was sprayed onto the cracks; this process was repeated five times. *B. cereus* treatment induced soil crack repair, with an average reduction in width of 0.20 ± 0.113 mm from the initial width of 0.45 ± 0.187 mm, but the decrease in cracks was not significantly different compared to that observed for *B. megaterium*. Soil treated with *B. cereus* contained significantly more $CaCO_3$ per 20 g of soil (0.78 ± 0.102 g vs. 0.59 ± 0.220 g) compared to *B. megaterium* and provided optimal growth conditions for the native *B. cereus*. However, X-ray diffraction analysis revealed that only 1.5% of the precipitated $CaCO_3$ was in the form of calcite, while aragonite constituted 28.5% and vaterite accounted for 70% of the different crystal structures. *B. megaterium* was composed of 46% calcite, 24.5% aragonite, and 29.5% vaterite crystals. These findings indicate a positive outlook for the use of indigenous microbial-induced calcite precipitation, especially for fracture repair. Further research is required to determine the mechanism underlying the calcification process of native bacteria and the specific conditions necessary to initiate the formation of calcium carbonate biominerals.

Key words: Aragonite, calcite, crack healing, precipitation, polymorphs, vaterite

INTRODUCTION

Landslides and erosion are two of the soil's geological failures that endanger the populace. A landslip happens when a substantial volume of rock, rubble, or soil slides down a slope due to gravity, resulting in material strength loss (Ayesha *et al*., 2019). Surface water movement can induce soil erosion, either slowly and unobserved or abruptly, resulting in significant topsoil loss. The three basic types of erosion are mass movement erosion, surface erosion, and fluvial erosion. Paramananthan *et al*. (2021) define this form of erosion and deposition as mass wasting. Mass movements are more prone to occur in soils that are loose, weakly compacted, and saturated, especially during heavy rains and earthquakes. To address the possibilities, there is a strategy for preventing natural disasters and stabilising soil. Portland cement, lime, asphalt, sodium silicate, acrylate lignin, and resins are examples of engineered fill or man-made materials that are commonly used to replace low-strength soil deposits. Chemical grouting is becoming more common due to its budgetary advantages (Soon *et al*., 2013). However, the additions commonly change soil pH and can contaminate soils and groundwater. In 1974, acrylamide grout was connected to five incidents of water poisoning in Japan, prompting a ban on practically all chemical grouts, and several countries have recently proposed outlawing all synthetic grouting (DeJong *et al*., 2010).

Implementation of soil stabilisation methods can ensure the progressive development of urbanisation or construction. Current conventional methods for soil stabilisation include mechanical compaction, soil replacement, ground consolidation, and chemical methods, but they fall short of sustainability expectations due to their high costs, laborious processes, and negative environmental effects (Anusha, 2020). In Malaysia, soil stabilisation is important due to the large proportion of tropical residual soils; thus, several fundamental studies have been conducted regarding the relationship between microbial metabolism activity and soil strength in tropical residual soils (Phang *et al*., 2022). Microbial-induced calcium carbonate precipitation (MICP) is a new soil stabilisation method that promotes microbial biomineralisation of calcium carbonate ($CaCO_3$) crystals (Dardau *et al*., 2021). The principle of MICP is to use the urease produced by ureolytic bacteria to catalyse the hydrolysis of urea into carbonate (Ca^{2+}) and ammonium (CO_3^{2-}) via the metabolic process leading to $CaCO_3$ formation. The precipitated $CaCO_3$ in the soil grains binds the soil particles together and increases the shear strength and unconfined compressive strength while reducing the permeability of the soil (Aliyu *et al*, 2023b). It is a natural biological process that has been applied to reduce heavy metal contamination (Rajasekar *et al*., 2021), artificial sandstone formation (Konstantinou *et al*., 2021), soil strengthening (Sharma & Ramkrishnan, 2016), concrete crack remediation (Golovkina *et al*., 2020), and water stability and erosion resistance in slopes

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(Tian *et al*., 2022). It offers a green and sustainable approach in construction and geotechnical engineering to combat issues with soil quality deterioration and crack desiccation (Liu *et al*., 2020), potentially lowering the cost of maintenance and necessitating less concrete material manufacturing.

However, a sustainable and cost-efficient method is required to promote MICP in both soil and concrete. Numerous studies on MICP application on soil (Sharma & Ramkrishnan, 2016; Zheng *et al*., 2022) and concrete (Oyediran & Ayeni, 2020; Marin *et al*., 2021) have focused on costly commercially available urease-positive bacteria such as *Bacillus* sp. and *Sporosarcina sp*. (Zomorodian *et al*., 2019). Such bacteria may not be suited to the soil conditions (Burbank *et al*., 2011) and may adversely affect nearby soil microbial communities (Badiee *et al*., 2019), whereas indigenous bacteria can adapt to the local environment and are more cost-effective for MICP applications (Wang *et al*., 2014). Indigenous bacteria reduce operational costs by eliminating the need for large-scale cultivation, handling and minimising potential ecological disturbances associated with the introduction of non-native species into soil environments (Gomez *et al*., 2017). Furthermore, indigenous bacteria are naturally adapted to local conditions, and exhibit enhanced survival against threats such as competition, predation, abiotic stresses, nutrient limitation and temperature variation (Evans *et al*., 1993). Consequently, the utilisation of indigenous ureolytic bacteria improves the reliability and sustainability of MICP processes.

This study evaluated whether *Bacillus cereus*, an indigenous ureolytic bacterium isolated from agricultural soil in Selangor, Malaysia, is effective at healing cracks in soil in comparison to an established MICP agent, *Bacillus megaterium.*

MATERIALS AND METHODS

Bacterial culture

The indigenous bacterium *B. cereus* was assessed for its capability for crack healing compared to the control *B. megaterium. B. cereus* was isolated from substandard soil on a farm in Universiti Putra Malaysia in Selangor, Malaysia (https://www.ncbi.nlm.nih.gov/nuccore/ NR_115714.1), and *Bacillus megaterium* ATCC 33679 was from a laboratory collection.

Biocementation treatment

The biocementation solution was prepared by mixing the ureolytic bacteria cultures of *B. cereus* and *B. megaterium* (1×10^7 cells/mL) with a cementation solution consisting of 30.03 g/L of urea, 55.49 g/L of calcium chloride, $CaCl_2$ and 3 g/L of nutrient broth according to Liu *et al*. (2020).

The soil crack healing capability was assessed as described by Liu *et al*. (2020) with several modifications. First, a 1 mm diameter soil sample was autoclaved and compacted evenly (5 mm thickness) in a perforated plate with 3 mm diameter holes to obtain a dry density of 1.00 g/cm^3. Before the treatment, 40 mL of sterile distilled water was sprayed on the soil samples and air dried at 37 ± 0.5°C for 2 hr to induce crack formation. Three treatments were sprayed onto the soil surface: 40 mL of distilled water as a control treatment, 40 mL of *B. cereus* biocemention solution, and 40 mL of *B. megaterium* biocemention solution before being left at 37 ± 0.5°C. The spray process was repeated every 24 hr for 5 days, and a photo was taken after each treatment for crack pattern analysis using J-image software.

Quantification of crack width

The soil images were processed using digital image processing software to generate a binary image to enhance the crack definition. ImageJ software was utilised to quantify the mean width of nine selected crack spots by converting the pixelated image to its corresponding actual measurement in the image.

Quantification of calcium carbonate in treated soil

The calcium carbonate content in the treated soil samples was determined by gravimetric analysis of acidified samples (Soon *et al*., 2014). Briefly, 20 g soil samples were oven-dried at 105 ± 0.5°C for 48 hr before being mixed with excess 2 M HCl to induce effervescence. The soil residues were filtered through a 150-mm filter paper funnel and then oven-dried at 105 ± 0.5°C for 48 hr and weighed. The weight difference was considered equivalent to the total calcium carbonate content in the soil samples.

X-ray diffraction (XRD) analysis

The soil samples (2 g) were carefully scooped from the area of MICP activity, which was determined from the presence of white precipitate for XRD analysis to verify calcium carbonate deposition (Dhami *et al*., 2016). A Shimadzu 6000 diffractometer was used to determine the composition of crystalline phases and distinguish between the amorphous and crystalline forms of $CaCO_3$ (calcite, vaterite, or aragonite), utilising Cu K radiation and a Panalytical Empyrean reflectometer operating at an ambient temperature of 28 ± 0.5°C. The exploration range (2θ) was adjusted at a rate of 2.00° per min, ranging from 20° to 80°. The Cu anode was set at 0.0530°, with step sizes of 30 kV and 30 mA for the voltages and currents, respectively. The data were further analysed in Xpert Highscore.

Statistical analysis

This study used the Statistical Package for the Social Sciences (SPSS). The Analysis of Variance (ANOVA) test was conducted to compare the results and identify significant differences between groups. If differences were found, a Tukey's Honestly Significant Difference (HSD) test was used to determine which groups varied. Results were presented as a mean ± standard error (SE) to ensure accuracy.

RESULTS AND DISCUSSION

The drying effect or capillary attraction, which causes the soil to lose moisture, leads to the formation of surface cracks, which can lead to the formation of larger cracks by causing water loss in deeper parts of the soil. The formation of these small cracks causes the loss of soil integrity and the soil structure to become loose; therefore, the formation of small cracks shall be reduced or prevented. Figure 1 presents the soil crack formation patterns at the end of the five-day treatment cycle, showing that the cracks widen, forming finer cracks that intersect the larger crack lines, forming a discernible, intricate crack network.

After five treatment cycles, the cracks treated with 40 mL of distilled water remained unremediated. The crack network became more visible, with the main crack widening and more intricate fine cracks forming throughout the soil samples. In contrast, crack lines formed and developed continuously until the second cycle in the soil samples treated with *B. megaterium* biocement (BMC), then white precipitates began to form on the crack edges, eventually covering the entire crack line by the fifth cycle. Similar results were observed for the samples treated with *Bacillus cereus* biocement (BCC), but the white precipitation did not form until the third cycle. However, after 120 hr of treatment, the BCC treatment had a larger area of white precipitate than the BMC treatment (Figure 1). Both soil samples treated with *B. cereus* and *B. megaterium* experienced crack remediation, with an average width reduction of 0.20 ± 0.113 mm and 0.013 ± 0.035 mm, respectively, from the initial measured crack width of 0.45 ± 0.187 mm and 0.45 ± 0.257 mm. Even though *B. megaterium* showed better performance in sealing cracks through the faster production of white precipitation following the second cycle compared to BCC, , there was no significant difference compared to the indigenous ureolytic bacteria *B. cereus*.

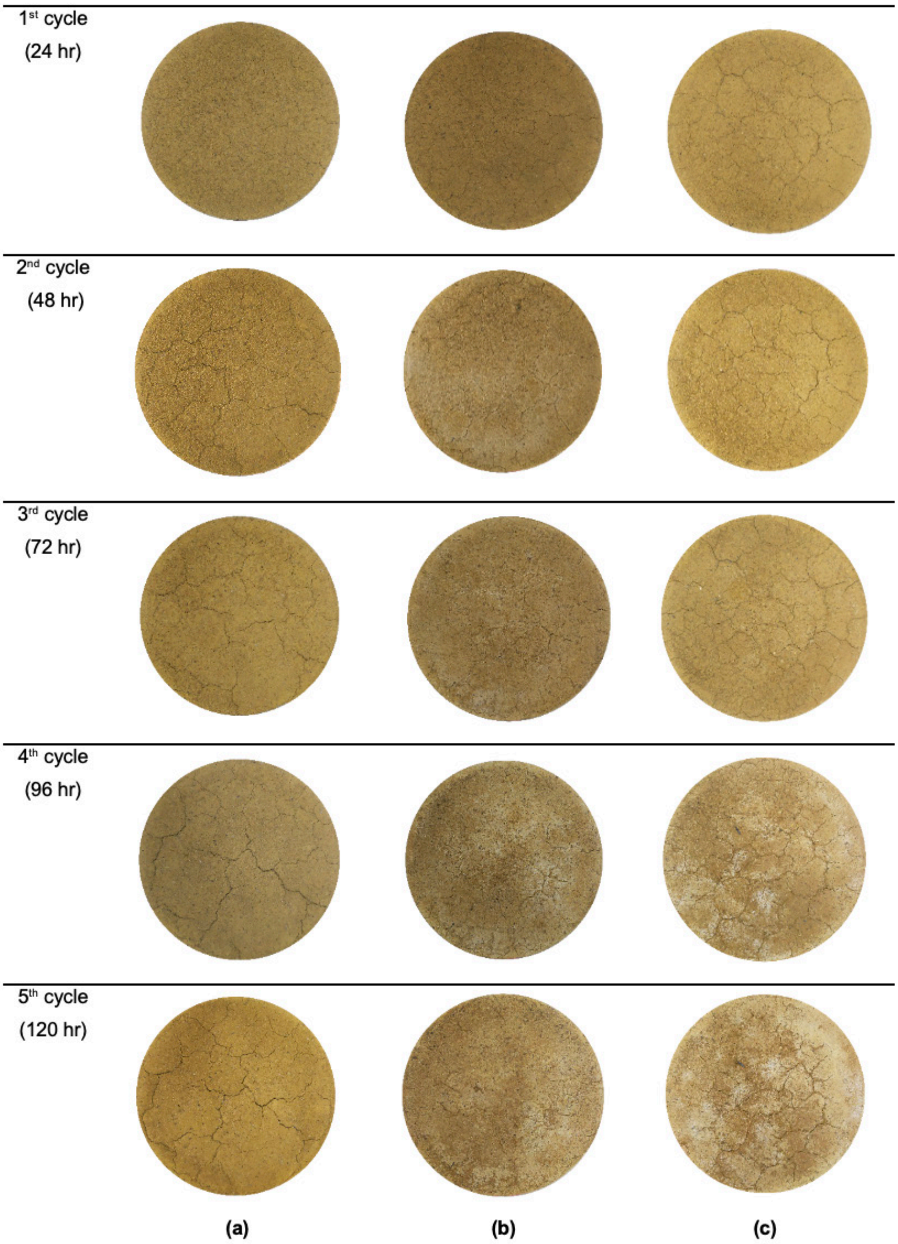


Fig. 1. Crack pattern changes of three soil sample groups underwent five cycles of treatment: (a) cycles of distilled water (Control); (b) *Bacillus megaterium* biocemention (BMC); (c) *Bacillus cereus* biocemention (BCC).

The ureolytic bacteria cumulatively formed white precipitates, which covered the crack lines and reduced the crack connectivity (Figure 2). Calcium carbonate precipitates were observed on cracks close to the edge of the soil sample, with cracks fully healed as they were covered with calcium carbonate crystals. Liu *et al*. (2020) observed comparable findings, where the existence of calcium ions, along with the formation of calcium carbonate crystals, caused the soil particles to adhere together, resulting in a biofilm of white calcium carbonate precipitate on the soil surface. Quantification of $CaCO_3$ showed that the soil treated with *B. cereus* contained 0.78g ± 0.102 g of $CaCO_3$ per 20 g of soil (3.86%), whereas *B. megaterium*-treated soil contained only 0.59 ± 0.220g of $CaCO_3$ (2.94%). This study's soil type favoured the indigenous *B. cereus* due to its better adaptation to the soil environment compared to *B. megaterium*. XRD analysis was performed on a sample since it is expected that these white layers are a part of the calcium carbonate patch.

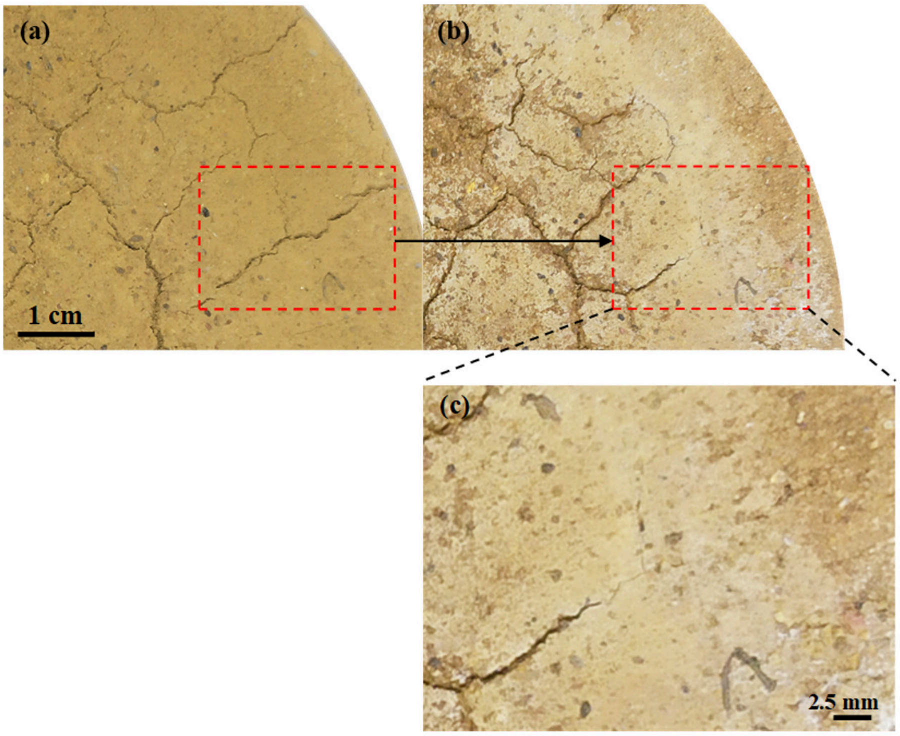
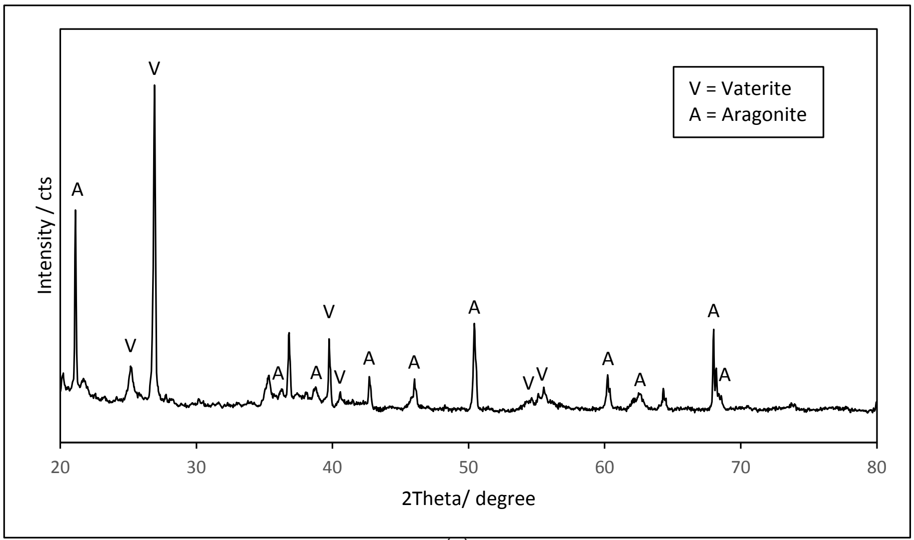


Fig. 2. Microphotographs of an area of soil sample treated with *Bacillus cereus* biocement (a) Day 0; (b) Day 5; (c) enlarged area focusing on crack covered with $CaCO_3$ crystals.

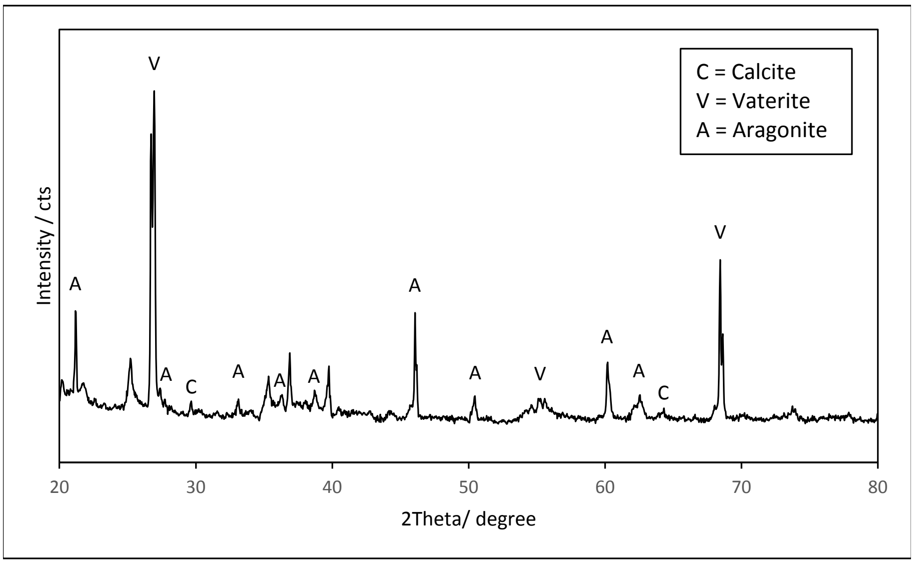
Certain factors affect the effectiveness of MICP to precipitate $CaCO_3$ crystals in the soil. The Bacillus group, known for its ability to survive in harsh environments (Aliyu *et al*., 2023a), exist in a dormant state of bacterial spores to survive until the conditions are suitable for growth (Lindsay *et al*., 2005). Higher MICP efficiency was expected, as evidenced by the amount of $CaCO_3$ produced by *B. cereus* due to its ability to quickly adapt to the natural environment or an environment that closely resembled its natural state, thereby enhancing the strength of sand and soil columns more effectively than introduced species (Marin *et al*., 2021; Phang *et al*., 2022). However, the effectiveness of creating an effective MICP depends not only on the bacterial species used but also on the cementation solution's suitability. The presence of urease-producing bacteria and the necessary nutrients in the cementation solution promote microbial-induced calcite precipitation (Dardau *et al*., 2021). The surplus of urea and calcium ions in the cementation solution increases the calcite content in the soil treated with *B. cereus* and *B. megaterium*. The presence of urea in the cementation solution drives *B. cereus* and *B.megaterium* to undergo urea hydrolysis to ammonium and carbonate ions (Oualha *et al*., 2020; Eberemu *et al*., 2021). The cementation solution fills up the cracks and is later used by the bacteria to precipitate the $CaCO_3$ crystals (Vail *et al*., 2019; Liu *et al*., 2020).

Despite the continuous precipitation of $CaCO_3$ by both *B. cereus* and *B. megaterium* to repair soil cracks, the soil continued to experience simultaneous desiccation cracking due to the constant evaporation of the cementation solution, which decreased the moisture content (Vail *et al*., 2019). This effect was exacerbated by the thin soil layer (5 cm) used in this study, which led to rapid drying and new crack formation even though the cementation solution was applied throughout the experimental period. As moisture evaporates and holes form, the cementation solution in the soil is drawn out, creating a suction force that generates new cracks, explaining why cracks persisted despite undergoing five rounds of BMC and BCC treatment cycles.

Figure 3 shows that the XRD peak profile of soil treated with *B. cereus* primarily consists of aragonite and vaterite (Figure 3a), with a few minor peaks corresponding to calcite. Conversely, the soil sample treated with *B. megaterium* exhibited significant calcite peaks and vaterite (Figure 3b). Subsequent investigation of the XRD polymorph breakdown (Figure 4) showed that 1.5% of the soil treated with *B. cereus* was calcite, with aragonite making up 28.5% and vaterite accounting for 70% of the polymorphs, whereas soils treated with *B. megaterium* showed a composition of 46% calcite crystals, 24.5% aragonite, and 29.5% vaterite.



(a)



(b)

Fig. 3. XRD peak graph of soil sample treated with (a) *Bacillus cereus* in cementation solution and (b) *Bacillus megaterium* in cementation solution after five cycles of treatment.

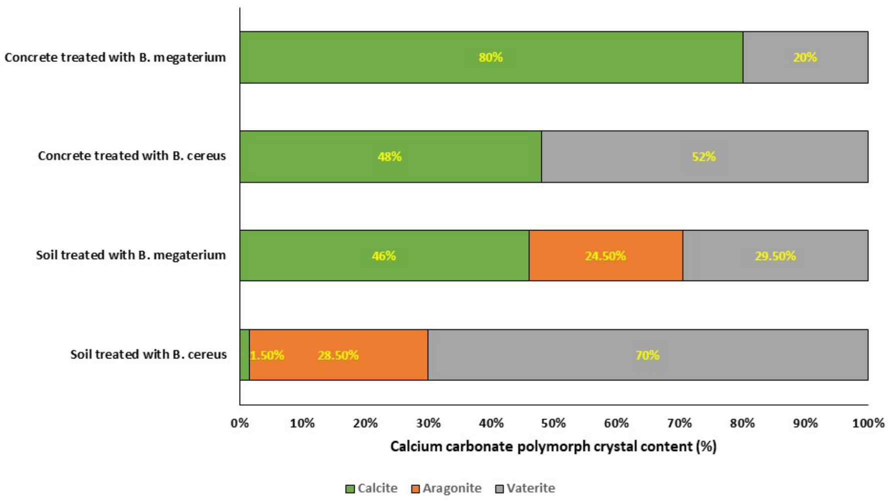


Fig. 4. Percentage of calcium carbonate polymorph in soil sample treated with *Bacillus cereus* in cementation solution and *Bacillus megaterium* in cementation solution after five cycles of treatment.

Although *B. cereus* produced a much smaller amount of calcite than *B. megaterium*, it precipitated all three $CaCO_3$ polymorphs in this study, suggesting that *B. megaterium* excels in transforming the most stable form of $CaCO_3$, calcite. $CaCO_3$ precipitation is the initial step in the development of metastable spherical vaterite crystals, which eventually transform and disintegrate to form calcite crystals (Al-Thawadi & Cord-Ruwisch, 2012). Calcite and vaterite were the most common types of polymorphs formed (Al-Thawadi & Cord-Ruwisch, 2012; Algaifi *et al*., 2020), with 70% of those polymorphs being vaterite. Given that the study period was only five days, the existence of vaterite was expected, as vaterite is the simplest form of $CaCO_3$, which ureolytic bacteria produce in a short time (Akyol *et al*., 2017). Although the ultimate goal is to produce more calcite, the fact that *B. cereus* produces 70% of vaterite is an advantage. Numerous studies have documented the occurrence of vaterite crystals and reported the synthesis of vaterite in the first precipitates formed by ureolytic bacteria in the presence of urea (Reeksting *et al*., 2020). *B. cereus* with a significant urease activity has made it simpler for kinetically favourable vaterite crystals to form. The amount of energy needed to change amorphous calcium carbonate (ACC) to vaterite is lower than the energy needed to change ACC to calcite; vaterite formation is more likely to occur compared to calcite (Yi *et al*., 2021). There is a correlation between urease activity and the rapid precipitation of vaterite crystals conversion into calcite crystals as vaterite becomes less stable over time (Kakelar *et al*. 2016). It is important to note that the pace of change from vaterite to calcite is substantially impacted by the degree of supersaturation and the pH of the surrounding environment (Spanos & Koutsoukos, 1998; Oral & Ercan, 2018). However, this is not to deny the benefits that calcite offers in terms of its crystallinity and stability, as most studies concluded that calcite is the most common type of biomineralisation because it is more stable at low solution supersaturations (Achal *et al*., 2013). Calcite is the material of choice for MICP applications because of its exceptional thermal stability and its capacity to provide superior cementation while simultaneously enhancing mechanical qualities (Aliyu *et al*., 2023b; Xu *et al*., 2024). Nonetheless, this study demonstrates that indigenous *B. cereus* is capable of forming calcium carbonate on and within the soil grains, demonstrating its potential for use as a ureolytic bacterium to stabilise soil through MICP.

Current findings showed the *B. cereus* capability as a bio-cementation agent. Overall, *B. tropicus* has emerged as a promising candidate with its superior urease activity and sustained calcium carbonate production. The ability of *B. tropicus* to promote the precipitation of calcite, a thermodynamically stable form of $CaCO_3$, is positioned. These crystals can effectively seal microcracks and reduce water permeability in soil. The fact that crystal formation can occur without prolonged bacterial presence suggests the potential to develop more efficient, single-use bacterial inoculation procedures. This could help mitigate operational costs and logistical challenges in field applications. The findings of this research contribute valuable insights into the use of indigenous microbial strains for an eco-friendly and self-sustaining MICP agent.

CONCLUSION

Bacillus cereus, an indigenous bacterium that produces urease, demonstrated crack-healing capability via $CaCO_3$ precipitation similar to the established MICP agent *B. megaterium*. The ability of *B. cereus* to reduce the size of soil cracks and the amount of $CaCO_3$ deposition after five days of treatment was not significantly different from that of *B. megaterium,* but it took longer to produce the more stable $CaCO_3$ polymer, calcite.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

Not applicable.

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