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From CO_2 Mineralization to Large-Scale Ureolytic Biocementation

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ABSTRACT: Microbially induced calcite precipitation (MICP) is an emerging bio-based technology offering sustainable solutions for soil stabilization, CO_2 sequestration, and protective treatments. This study integrates two complementary research efforts addressing both metabolic pathway selection and large-scale implementation. The first focuses on Bacillus megaterium, a dual-enzyme bacterium capable of activating either the ureolytic or carbonic anhydrase (CA) pathway depending on CO_2 availability. Laboratory experiments revealed that under atmospheric CO_2, ureolysis dominated, producing calcite as the main polymorph, whereas in CO_2-enriched conditions, isotopic analysis confirmed that 94% of the precipitated $CaCO_3$ originated from inorganic CO_2, indicating CA pathway dominance. The CA pathway also produced a wider variety of crystal morphologies, including rhombohedral, spherical, and amorphous forms, with organic carbon content influencing the presence of vaterite. The second component addresses the environmental screening, selection, and scale-up of high-performing ureolytic Sporosarcina pasteurii strains for field-relevant MICP applications. Banked strains, particularly the nhaC variant, achieved the highest urease activity, with conductivity plateaus around 70 mS/cm and rapid hydrolysis kinetics. These strains were successfully upscaled in 900 L non-sterile bioreactors over six consecutive batches without contamination, maintaining consistent performance. Application to a 1.5 m × 0.5 m sand column produced a structurally stable mass with undrained Tresca strengths of 90–140 kPa across most of the height, confirming uniform cementation over approximately 1.35 m. By combining mechanistic insights from the CA pathway with demonstrated scalability of the ureolytic pathway, this work outlines a pathway-dependent design framework for MICP. The ureolytic route enables rapid mechanical improvement but requires ammonium management, while the CA pathway offers CO_2 valorization and a reduced nitrogen footprint. A hybrid treatment approach—rapid ureolytic cementation followed by CA-mediated carbonation—could deliver both engineering performance and environmental benefits, supporting applications from geotechnical infrastructure to heritage conservation in line with carbon-neutral construction goals.

KEYWORDS: Microbially Induced Calcite Precipitation (MICP), ureolysis, carbonic Anhydrase, CO_2 sequestration, biocementation

1 INTRODUCTION

The increasing demand for sustainable geotechnical solutions has intensified interest in microbially induced calcite precipitation (MICP) as a viable alternative to conventional binders such as cement, lime, or synthetic polymers, which are associated with high CO_2 emissions and significant environmental impacts. MICP is a bio-mediated process in which microorganisms catalyze the formation of calcium carbonate ($CaCO_3$), effectively binding soil particles and enhancing the mechanical stability of granular materials. This natural mineralization process is versatile, enabling applications ranging from ground improvement and erosion control to crack sealing, dust suppression, and the protection of built and natural heritage structures (Phillips et al., 2013, Murugan et al. 2021).

Two enzymatic pathways are primarily responsible for MICP. The ureolytic pathway, mediated by the enzyme urease, hydrolyzes urea into carbonate and ammonium ions. In the presence of Ca^{2+}, the rapid generation of carbonate results in the precipitation of $CaCO_3$. This pathway is characterized by high reaction rates and high yields, making it attractive for engineering applications requiring rapid strength gains. However, it generates ammonium (NH_4^+) as a byproduct, which can lead to environmental concerns if not properly managed (Mori and Uday, 2021). Mitigation strategies, such as ammonium recovery and valorization into fertilizers, are therefore essential for sustainable implementation. The second pathway, mediated by carbonic anhydrase (CA), catalyzes the hydration of CO_2 into bicarbonate (HCO_3^-), which subsequently reacts with Ca^{2+} to form $CaCO_3$. While generally slower than ureolysis, the CA pathway offers the advantage of incorporating inorganic CO_2 into stable carbonates, thereby reducing the nitrogen footprint and contributing directly to carbon sequestration.

Recent research has largely focused either on enhancing the performance and scalability of ureolytic MICP for large-scale ground improvement (Harran et al., 2023, Terzis et al., 2025) or on advancing CA-mediated biomineralization for climate-positive applications that capture atmospheric or industrial CO_2 streams (Cappa et al., 2025). This study combines both perspectives, presenting mechanistic insights into pathway activation in Bacillus megaterium, which can selectively engage ureolytic or CA activity depending on CO_2 availability, alongside environmental screening, selection, and successful scale-up of high-performing Sporosarcina pasteurii strains for meter-scale biocementation. By integrating pathway-specific advantages, this work provides a framework for tailoring MICP strategies to achieve both engineering performance and environmental sustainability.

2 MATERIALS AND METHODS

2.1 *Ureolytic pathway – environmental screening and scale-Up*

Over 50 isolates were obtained from:

(i) microorganism banks (S. pasteurii wild type, strain nhaC),

(ii) large-batch reactor enrichments, and
(iii) ammonia-rich soils from Ticino, Switzerland.

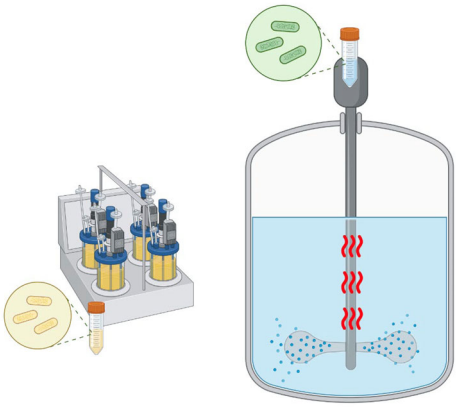

Figure 1. Schematic representation of the 2-step batch growth of *S. pasteurii*, including (a) an initial inoculum of 8 L where the strains were sampled and (b) a 1000 L reactor, with air supplied as the means of agitation and aeration as well as constant temperature maintenance at 30 °C. Source: Terzis et al., 2025 (CC by 4.0)

Safety screening excluded Bacillus cereus group strains harboring toxin genes. Positive urease activity was confirmed on Christensen's urea agar. Conductivity monitoring quantified urea hydrolysis, with kinetic parameters determined as maximum slope (r_m) and plateau (y_m). Strains S. pasteurii wt and nhaC exhibited the highest activities, reaching EC plateaus ~70 mS/cm. Selected nhaC cultures were grown in two stages: a first inoculum of 8 L, composed of yeast extract (20 g/L) and ammonium sulfate (20 g/L) for 48 h under 2000 rpm agitation and (ii) production batches where the inoculum was mixed with 900 L water and 50kg of urea, in non-sterile reactors. Aeration via bottom air pump was provided and temperature was maintained at 30 °C using heaters. Sequential batches reused 50 L inoculum from previous runs to enhance kinetics (figure 1).

A 1.5 m × 0.5 m sand column (0–1 mm) was inoculated from the 900L batch. After 12 h attachment, three 8 h infiltration cycles of equimolar 1 M urea–$CaCl_2$ solution were applied. Strength mapping followed casing removal using a 10 × 10 cm grid.

2.2 *Carbonic anhydrase pathway – B. megaterium CO_2 mineralization*

Bacillus megaterium harbors genetic determinants for both urease and carbonic anhydrase, enabling activation of either pathway depending on CO_2 availability. Pathway selection was investigated under controlled conditions (figure 2). In the open-system ureolysis experiment (U test), cultures at OD_{600} = 0.7 were incubated in growth medium containing 1.5 M urea and $CaCl_2$ under atmospheric CO_2 at 30 °C for four days. In the closed-system CO_2 mineralization experiment (CA test), closed vials were prepared with C^{13}-labelled urea and equimolar $CaCl_2$, then injected with 1.24 × 10^{-3} mol CO_2; pH was maintained above 8 for the four-day incubation period. Precipitates from both tests were washed, dried, and analyzed via Fourier Transform Infrared Spectroscopy (FTIR) to identify $CaCO_3$ polymorphs, Scanning Electron Microscopy (SEM) to examine morphology, and the double-capsule isotopic method to quantify the proportion of carbon originating from urea versus CO_2.

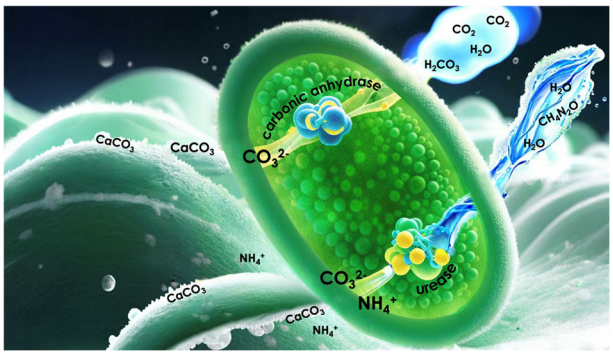

Figure 2. Illustration of urease and CA-induced MICP in *B. megaterium* using urea and CO_2 as reactants, respectively. Source: Cappa et al., 2025 (CC by 4.0)

3 RESULTS

3.1 *Ureolytic pathway*

In the ureolytic pathway experiments, screening showed that the banked strains Sporosarcina pasteurii (wild type) and nhaC exhibited the highest urease activity, outperforming both environmental isolates and those obtained from large-reactor enrichments (figure 3).

While environmental isolates e1 and e6 displayed lower activity, their performance remained within the operational range required for MICP applications. Scale-up trials in 900 L reactors maintained consistent ureolytic activity across six production runs without any signs of contamination, with all reactor cultures confirmed as S. pasteurii. The reuse of 50 L inoculum from preceding batches improved hydrolysis kinetics, shortening the time to reach the plateau in electrical conductivity.

In the column biocementation test, the treated 1.5 m sand column retained its structural integrity after casing removal. Measured undrained strengths ranged from 90 to 140 kPa over most of the column surface, with a reduction only in the top 15 cm, furthest from the injection point, confirming uniform cementation along approximately 1.35 m of its height (figure 4).

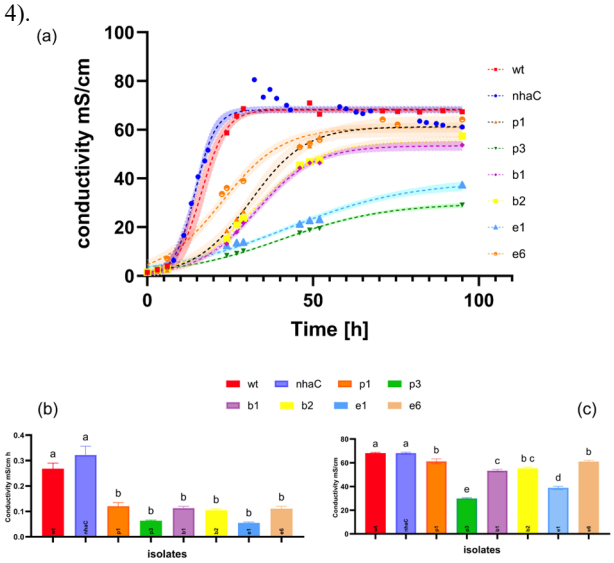

Figure 3. Bench-scale analysis of the ureolytic potential of eight strains. (a) EC monitoring and kinetic parameter assessment, including (b) the maximum slope of the curve (rm) and (c) the maximum value at plateau (ym). The same letters in the conductivity kinetics columns indicate significant differences at P < 0.05 according to one-way

ANOVA and Tukey's post hoc analyses. Source: Terzis et al., 2025 (CC by 4.0)

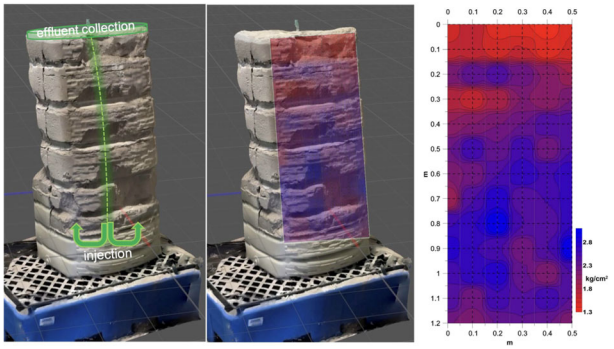

Figure 4. A sand column composed of 0–1 mm aggregates infiltrated via a 25 mm tube was used to apply a bottom-to-top flow path at 5 L/min. The resulting spectrum of strength (kg/cm2) from the surface of the column was assessed using a pocket penetrometer. Source: Terzis et al., 2025 (CC by 4.0)

3.2 *Carbonic anhydrase pathway*

In the carbonic anhydrase pathway experiments, the metabolic route activated by Bacillus megaterium was found to depend strongly on CO_2 availability. Under atmospheric CO_2 conditions in the open-system ureolysis (U test), the urease pathway was dominant, leading to rapid urea hydrolysis and the precipitation of calcite, as confirmed by FTIR spectra showing characteristic absorption peaks at 712, 872, and 1394 cm^{-1}. In contrast, in the closed-system CO_2 mineralization (CA test) with elevated CO_2 levels (1.24×10^{-3} mol injected), isotopic analysis using C^{13}-labelled urea revealed that only about 6% of the precipitated $CaCO_3$ originated from urea, while the remaining 94% derived from inorganic CO_2, indicating a clear metabolic shift towards carbonic anhydrase activity.

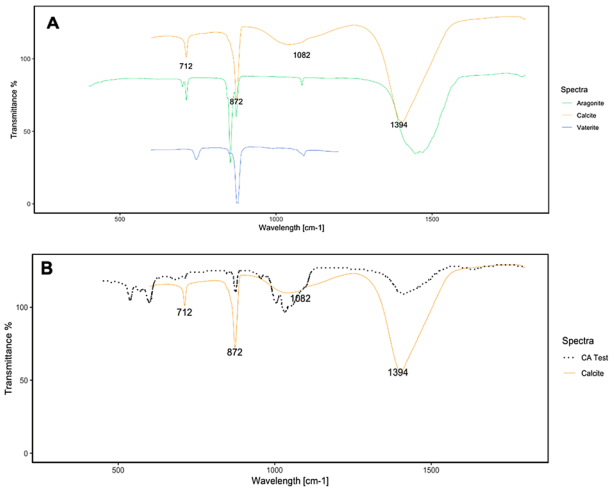

Figure 5. (A) FTIR spectra of calcite, aragonite and vaterite; (B) CA test results compared with those of calcite.

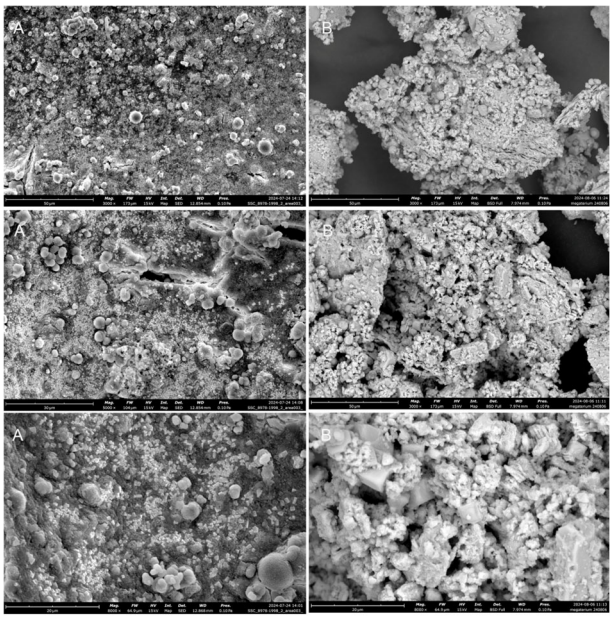

Figure 6. Carbonic Anhydrase test; Scanning Electron microscopic observations at magnifications of 3000x, 5000x, 8000x at 15 kV and 10 Pa; (A) Pellet collected from fresh samples; (B) pellet postdrying and postwashing.

FTIR analysis (figure 5) in the CA test confirmed calcite as the predominant polymorph, though SEM imaging (figure 6) revealed a broader diversity of crystal morphologies, including rhombohedral, spherical, and amorphous structures. This morphological variation suggests the coexistence of different calcium carbonate polymorphs—calcite, aragonite, and vaterite—within the precipitate. In particular, high dissolved organic carbon (DOC) concentrations were associated with a greater tendency for vaterite formation, whereas low DOC favored the stability of calcite. The SEM observations further showed that while microbial cells were not clearly distinguishable in the final precipitate due to washing and drying steps, the residual mineral matrix displayed hierarchical and interconnected crystal networks. These results demonstrate that B. megaterium can switch between ureolytic and CA-driven MICP depending on CO_2 availability, with the CA pathway not only enabling direct CO_2 sequestration but also influencing mineral polymorphism and microstructural characteristics of the precipitate.

4 CONCLUSIONS

The results highlight that the ureolytic pathway, driven by Sporosarcina pasteurii, remains the most efficient option for rapid and high-yield calcite precipitation, making it well-suited for applications where immediate structural improvement is essential, such as foundation stabilization, roadbed reinforcement, and erosion control. The high reaction rates, uniform cementation profiles, and mechanical gains observed at the meter scale confirm its readiness for practical deployment. However, the process inevitably produces significant amounts of ammonium (NH_4^+), which, if left untreated, can pose environmental concerns. The present work addresses this challenge by exploring the valorization of NH_4^+ into ammonium sulfate fertilizer, thereby closing a material loop and creating an additional product stream. In contrast, the carbonic anhydrase (CA) pathway, as demonstrated with Bacillus megaterium, offers a more environmentally benign alternative by directly integrating CO_2 sequestration into the MICP process and eliminating nitrogenous byproducts. While

the slower kinetics of CA-mediated precipitation currently limit its use as a standalone structural treatment, its ability to incorporate inorganic CO_2 into stable carbonates presents a clear advantage in carbon-neutral construction strategies and could be highly valuable in applications where environmental footprint minimization outweighs the need for rapid strength gain. From a scalability perspective, the successful operation of 900 L non-sterile production batches with consistent strain purity and performance marks a decisive step toward the industrial application of MICP technologies. The infrastructure and operational know-how developed for ureolytic cultures could be readily adapted to CA-pathway strains, provided that growth conditions, CO_2 delivery, and pH control are optimized to sustain high mineralization rates. This opens the possibility for hybrid treatment strategies in which a ureolytic phase is used initially to achieve rapid mechanical improvement, followed by a CA-mediated carbonation phase for long-term CO_2 sequestration and enhanced durability. Such a sequential approach could be particularly beneficial in heritage conservation projects, where the balance between mechanical stability and chemical compatibility with existing materials is critical, as well as in low-disturbance geotechnical contexts where environmental impact reduction is a priority. Together, these findings suggest a pathway-dependent design framework for MICP, allowing engineers to tailor treatment strategies to specific technical, environmental, and regulatory requirements.

5 ACKNOWLEDGEMENTS

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