Exploring the Capabilities of Sporosarcina Pasteurii in Microbially Induced Calcite Precipitation

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1 Abstract

Microbially induced calcite precipitation (MICP) is a method employed to create self-healing bio-concrete, utilizing ureolytic bacteria to fill concrete cracks and bind aggregates like sand and soil through calcium carbonate ($CaCO_3$) production. This technique holds significant implications for sustainability by enhancing concrete durability, stabilizing soil, and facilitating carbon dioxide sequestration from the atmosphere. The present study aimed to assess the quantity and temporal dynamics of the MICP process, along with its effectiveness in soil and sand stabilization. Results indicate that between 0.0431 to 0.0531 g of calcium carbonate could be precipitated over a 10-day period, with precipitation decreasing after approximately 8 days. Additionally, the decrease in optical density of the medium was observed, attributed to bacterial aggregation for enhanced survival, as well as a decrease in pH which could be responsible for the precipitation decrease. Moreover, sandy soil was determined to be more conducive to MICP, and MICP was more prevalent at the surface of the soil because the bacteria was introduced via the pouring method.

2 Introduction

Global warming, driven significantly by elevated levels of carbon dioxide (CO_2) in the atmosphere, exacerbates the greenhouse effect and contributes to climate change. To mitigate these impacts, it is crucial to explore methods for removing CO_2 from the atmosphere. One major contributor to CO_2 emissions is the production of concrete, which accounts for approximately 5-8% of global CO_2 emissions. Microbial Induced Calcite Precipitation (MICP) presents a promising solution to both of these environmental challenges. MICP involves the introduction of ureolytic bacteria into cement, which then catalyze the formation of calcium carbonate ($CaCO_3$) from urea and CO_2 dissolved in water. This process not only endows concrete with self-healing properties but also sequesters CO_2 from the atmosphere (Carter et al., 2023). In addition to its environmental benefits, MICP technology is particularly relevant in Taiwan, where it can help prevent mudslides and soil liquefaction caused by earthquakes by binding loose particles, such as sand and dirt, into more solid structures with $CaCO_3$.

The MICP process contains several key chemical reactions (Bandyopadhyay et al. 2023).

Ureolysis:
The ureolytic bacteria produce the enzyme urease, which catalyzes the breakdown of urea into ammonia and carbonic acid (H_2CO_3) (Carter et al., 2023).

$$CO(NH_2)_2 + 2\,H_2O \longrightarrow NH_2COOH + NH_3 \quad (1)$$

Carbon Sequestration:
H_2CO_3, derived from CO_2 dissolved in water, dissociates into bicarbonate (HCO_3^-) and hydrogen ions. The MICP reaction shifts the equilibrium to the right, promoting the removal of CO_2 from the atmosphere.

$$CO_2 + H_2O \longrightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \quad (3)$$

Production of $CaCO_3$:

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad (4)$$

$$2\,NH_3 + 2\,H_2O \rightleftharpoons {}_2NH_4^+ + OH^- \text{ (pH increase)} \quad (5)$$

$$HCO_3^- + H^+ + 2\,NH_4^+ + 2\,OH^- \rightleftharpoons CO_3^{2-} + 2\,NH_4^+ + 2\,H_2O \quad (6)$$

$$CO_3^{2-} + Ca^{2+} \longrightarrow CaCO_3 \text{ (carbonate precipitation)}$$

(7)

$CO(NH_2)_2 + 2H_2O + Ca^{2+} \longrightarrow 2NH_4^+ + CaCO_3$ (overall reaction) (8)

The ammonia generated raises the pH of the surrounding environment, facilitating the accumulation of $CaCO_3$ and minimizing the release of CO_2 back into the atmosphere. The alkaline environment further encourages the dissolution of atmospheric CO_2 into water and its conversion into carbonates (Okyay et al., 2015). The production of $CaCO_3$ helps to bind loose aggregates, stabilizing soil and sand (Carter et al., 2023).

Among the various bacteria used for MICP, *Sporosarcina pasteurii* is the most commonly employed due to its high ureolytic activity. This bacterium performs effectively when supplemented with nickel, calcium chloride, urea, and an oxygenated medium (Ma et al., 2020). It thrives in the high pH environment created by the MICP process and is not adversely affected by ammonia or salts (Carter et al., 2023).

This study aims to investigate the capabilities of *S. pasteurii* in precipitating $CaCO_3$ and to identify optimal conditions for MICP through two experimental approaches. The first experiment examines the rate of $CaCO_3$ accumulation in solutions containing *S. pasteurii*, calcium ions, and various nutrients. The second experiment evaluates the effectiveness of *S. pasteurii* in different concentrations of dirt and sand to determine the optimal conditions for MICP.

3 Materials and Methods

Cultivation of *S. pasteurii*

The pure *S. pasteurii* strain was cultivated in a medium composed of 6.5 g of nutrient broth and 25 g of urea dissolved in 500 mL of distilled water. This medium is referred to as the "culture medium" in this study. The nutrient broth and water were sterilized using an autoclave at 121°C. After the medium was autoclaved, the urea was sterilized through a 0.22 µm filter and added in a laminar flow hood. The bacteria were grown in 50mL of culture medium and were transferred to fresh culture medium every two days. Bacterial growth was monitored by measuring optical density (OD) at 600 nm using an Eppendorf BioPhotometer, with readings taken every two hours.

Preparation of Flasks

The growth curve of *S. pasteurii* indicated that optimal growth occurred between 10 to 12.5 hours. Bacteria harvested at this growth phase were washed with phosphate-buffered saline (PBS) to remove excess urea, and then transferred to 100 mL of the "working medium" at an inoculum concentration of 8 log CFU/mL. The working medium was prepared using the following recipe: 20 g/L urea, 3 g/L nutrient broth, 20 µg/L Ni^{2+} ions, 250 mM $CaCl_2$, 10 g/L NH_4Cl, and approximately 0.016 g NaOH to adjust the pH to 6.6. This formulation was adapted from Šovljanski et al.(2022). The PBS buffer contained 1L of DI water, 8g $NaCl$, 1.44 g Na_2HPO_4, 0.2g KCl, 0.24 g K_2HPO_4, and HCl was added to pH 7.4. This recipe followed the one given by (How to Prepare Phosphate Buffered Saline (PBS) 2024).

Twelve flasks containing *S. pasteurii* in the working medium were prepared and incubated at room temperature. On days 4, 6, 8, and 10, three flasks were selected for analysis of pH, bacterial growth, and $CaCO_3$ precipitation. Optical density was measured at 600 nm using the Eppendorf BioPhotometer. $CaCO_3$ precipitation was quantified by filtering the suspension through a vacuum filter flask, drying the residue at 105°C, and weighing the dried precipitate. pH was measured using a Thermo Scientific pH probe. We selected days 4, 6, 8, and 10 because the $CaCO_3$ that had precipitated by day 2 was negligible.

Preparation of Soil Samples

To determine the optimal sand-to-soil ratio for $CaCO_3$ precipitation and aggregate stabilization, five test tubes were prepared with different sand-to-soil ratios: 90:10, 80:20, 70:30, 50:50, and 30:70. Soil collected from Hsinchu was filtered through a 2 mm sieve, while sand (0.15-0.30 mm) was purchased and filtered through a 0.297 mm sieve. Each soil-sand mixture was added into three 50 mL test tubes, and 10 mL of *S. pasteurii* in working medium, adjusted to a concentration of 8 CFU/mL, was added to two of those tubes, while DI water was added to the other, to function as a control. The tubes were incubated at room temperature for 8 days and then examined qualitatively for cylinder structure and quantitatively for amount of $CaCO_3$ precipitated. The amount of $CaCO_3$ precipitated in each sample was determined using Gravimetric Acid Digestion, which relies on the following reaction.

$CaCO_3 + 2HCl \longrightarrow CaCl_2 + H_2O + CO_2$ (9)

Samples were dried and weighed, then treated with hydrochloric acid (HCl) to dissolve the $CaCO_3$. The samples were filtered to remove the liquid, dried again, and reweighed. The loss in mass was attributed to the amount of precipitated $CaCO_3$.

4 Results and Discussion

Growth Rate Over Time in Culture Medium
We investigated the growth rate of S. pasteurii by measuring the optical density every two hours. The growth curve, depicted in Figure 1, revealed that the bacteria exhibited the most rapid growth between 10 and 12.5 hours after being introduced into the fresh medium. After this period, the growth rate plateaued around 16 hours. These findings indicate that for optimal bacterial activity, experiments with S. pasteurii should be initiated between 10 and 12.5 hours after inoculation into the medium.

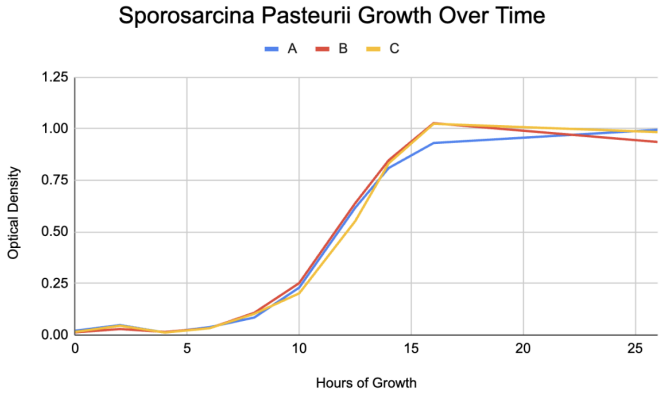

Figure 1: S. pasteurii growth over 26 hours

Calcium Carbonate Precipitation Over Time
We wanted to measure the mass of calcium carbonate over time to understand both the quantity of $CaCO_3$ *S. pasteurii* was capable of producing and an appropriate time scale for this precipitation to occur. Upon introducing the active bacteria into the working medium, calcium carbonate production commenced via the MICP (Microbially Induced Calcite Precipitation) process. Measurements of calcium carbonate precipitation over time revealed a steady increase up to day 8, after which the levels began to decline slightly (see Figure 3). These findings suggest that the optimal duration for maximum MICP activity is approximately 8 days.

The observed decline in calcium carbonate precipitation after day 8 was unexpected. A potential explanation for this could be the slight decrease in pH levels, as a more acidic environment may lead to the dissolution of calcium carbonate, thereby reducing the overall CaCO3 content.

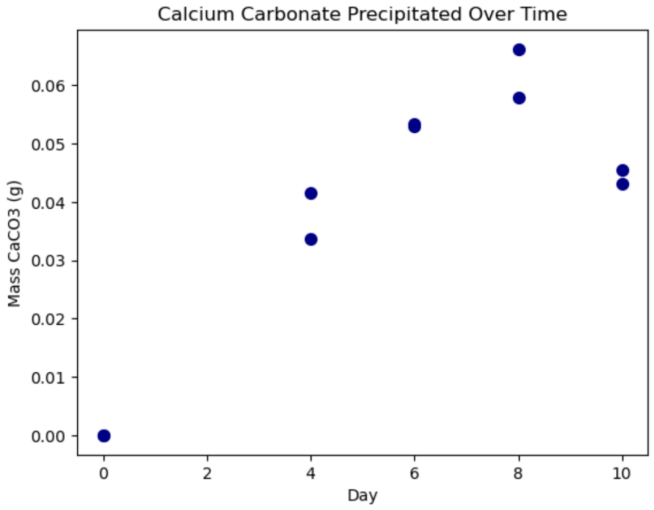

Figure 2: Calcium Carbonate Precipitation Over Time

Bacterial Concentration Over Time in Working Medium
Over the course of 10 days, the optical density of the working medium showed a decreasing trend, as depicted in Figure 3. This decline is likely attributed to bacterial aggregation, or flocculation, where bacteria clump together in response to environmental stresses such as nutrient depletion (Trunk et al., 2018). Given the experimental setup, it is reasonable to assume that nutrient levels progressively decreased, as they were not replenished over time. This depletion likely led to a reduction in freely suspended bacteria, with some bacteria adhering to the walls of the medium. However, the bacteria were likely still alive, and thus still active in the MICP process.

pH Changes Due to MICP
After the MICP process initiates, the production of ammonia in the soil typically causes an increase in pH, sometimes reaching levels as high as 9 (Carter et al., 2023). Figure 4 illustrates the results of this experiment: the pH initially rose by approximately 1.2 points, from an initial value of 6.6 on day 0 to an average of 7.82 on day 4, before gradually decreasing to an average of 7.55 by day 10. While the initial pH increase is consistent with my hypothesis, the subsequent gradual decline was unexpected. The observed

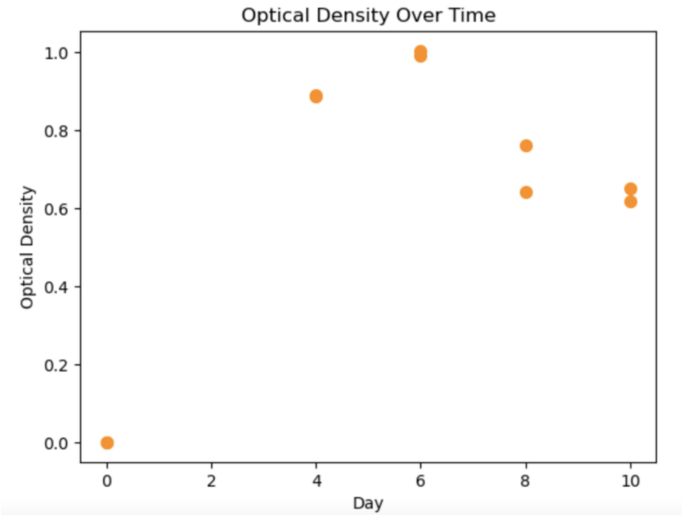

Figure 3: Optical Density Over Time

timeline suggests that the majority of MICP activity occurred between days 0 and 4, yet this does not correlate with the observed calcium carbonate precipitation patterns over time.

One possible explanation for the pH decrease could be the activity of ammonia-oxidizing bacteria, which are known to lower pH through ammonia oxidation and may also reduce calcium carbonate concentration as discussed earlier (Gat et al., 2015). Although this hypothesis aligns with the observed decline in calcium carbonate precipitation, it contradicts the fact that our experimental setup used pure cultures, where such bacterial contamination should not occur.

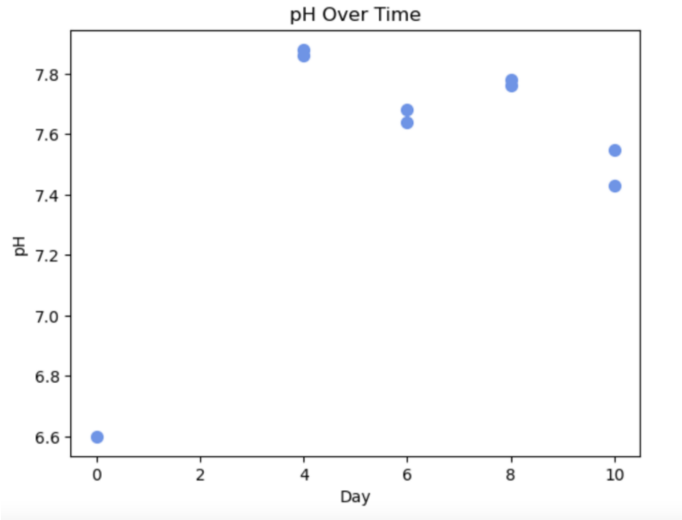

Figure 4: pH Over Time

Optimizing Soil and Sand Concentrations

We combined ureolytic bacteria, working medium, and varying concentrations of sand and soil and left them for eight days to investigate the conditions that could lead to MICP strengthening soil and ideally producing soil columns. This revealed that amounts of calcium carbonate precipitated appeared to increase when the soil was sandier, because the difference between the samples and the controls widened as seen in figure 5. Though the actual mass decreased, the masses of the controls decreased as well, indicating perhaps that soil with more dirt was more inclined to falsely overreport calcium carbonate precipitation. Other research also indicates that medium-sized sand precipitates more calcium carbonate when the medium was poured onto the substrate from above (Chae, et al. 2020). The qualitative data also supported this conclusion: Figure 6 demonstrates that the sandier columns appear to have a more stable structure than the ones with higher soil concentrations. This can be observed best by looking at the amount of dirt which has detached from the main column structure.

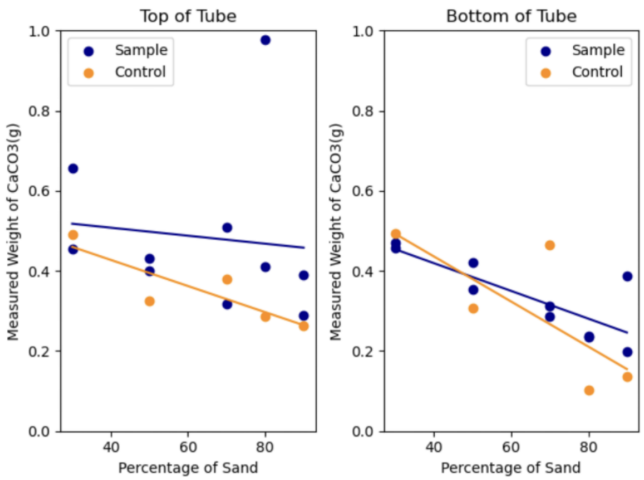

Figure 5: $CaCO_3$ Mass for Different Sand-Soil Concentrations and Soil Depth

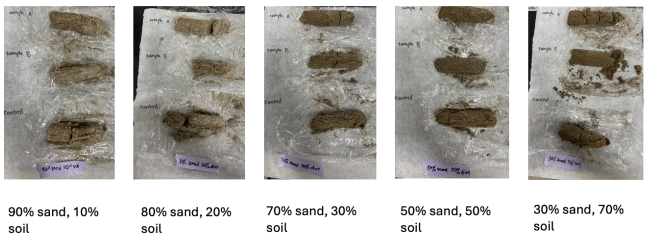

Figure 6: Columns with Different Sand:Soil Ratios

Difference in Sand-Soil Position
In order to determine whether placement within the dirt impacted MICP, I sampled from the top and bottom of each column. In the samples, the CaCO3 precipitated in the top samples were generally consistently higher than those precipitated in the bottom. This can be observed by comparing the sample dots in the left and right charts in Figure 5. This indicates that MICP will generally affect the top layer of sand or soil more than the bottom. This phenomenon was not observed in the controls. This is likely because the bacteria medium was poured from above rather than being mixed in. This leads to a large difference in calcium carbonate precipitation between the top and the bottom of the sample (Chae, et al. 2020). This result is unsurprising because the working medium and bacteria within likely do not penetrate all the way through the bottom layer.

5 Conclusions

In this study, we aimed to investigate the efficiency of Sporosarcina pasteurii in precipitating calcium carbonate and to determine optimal conditions for MICP in various soil and sand concentrations. We also investigated the relation of pH and bacterial concentration to MICP capabilities.

We found that S. pasteurii will precipitate MICP on a time scale of around 8 days, and that continuing beyond 8 days will be ineffective because the calcium carbonate begins to drop, as does the pH and optical density. Because of the drop, further research could continue this experiment beyond 10 days to see if $CaCO_3$ continues to drop. We also found that MICP works best in sandy soil closer to the surface of the soil, particularly if the bacteria has been poured in from above. This indicates that MICP only works on the top layer of substrate, or as far as the implementers mix it into the substrate, which could prove difficult on a larger scale. This indicates that it may be beneficial to further develop technologies and methods for adding S. pasteurii to substrate.

Understanding how to effectively use MICP is important to removing carbon dioxide from the atmosphere, contributing to the longevity of concrete, and preventing soil liquefaction and landslides. This research contributed to showing both the strengths of MICP in holding sandy soil together and precipitating CaCO3 as well as its limitations in quantity and maintenance over time.

6 References

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