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Effect of Temperature, pH, and Reaction Duration on Microbially Induced Calcite Precipitation

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Featured Application: This work can be used to determine the optimal conditions for microbially induced calcite precipitation (MICP).

Abstract: In this study, the amount of calcite precipitate resulting from microbially induced calcite precipitation (MICP) was estimated in order to determine the optimal conditions for precipitation. Two microbial species (*Staphylococcus saprophyticus* and *Sporosarcina pasteurii*) were tested by varying certain parameters such as (1) initial potential of hydrogen (pH) of urea-CaCl₂ medium, (2) temperature during precipitation, and (3) the reaction duration. The pH values used for testing were 6, 7, 8, 9, and 10, the temperatures were 20, 30, 40, and 50 °C, and the reaction durations were 2, 3, and 4 days. Maximum calcite precipitation was observed at a pH of 7 and temperature of 30 °C. Most of the precipitation occurred within a reaction duration of 3 days. Under similar conditions, the amount of calcite precipitated by *S. saprophyticus* was estimated to be five times more than that by *S. pasteurii*. Both the species were sensitive to temperature; however, *S. saprophyticus* was less sensitive to pH and required a shorter reaction duration than *S. pasteurii*.

Keywords: MICP; optimal condition; *Staphylococcus saprophyticus*; *Sporosarcina pasteurii*

1. Introduction

Microbially induced calcite precipitation (MICP) is a bio-grouting method that can improve geotechnical properties by precipitating calcite using microbes. MICP is considered an eco-friendly technology used in the field of soil improvement as it involves biological treatment rather than mechanical or chemical ones. However, this technology is not completely applicable to geotechnical practice because the field condition is not favorable for cultivating microbes. Furthermore, injecting nutrients and medium, and utilizing microbes requires a high level of skilled manpower and involves high costs. Nevertheless, the technology looks promising with the researchers solving some of the problems that they have encountered. Microbial activity and reproduction rate are governed by many factors including availability of nutrients, water, or other environmental factors. The environmental factors consist of pH, redox potential, temperature, presence of predatory microorganisms, which may limit bacterial population, and space limitations [1,2]. These factors have been studied extensively to determine the optimum environmental conditions for soil improvement and to verify the applicability of the technique in the field of geotechnical engineering [3–14].

Temperature, pH of the environment, and the reaction duration are some of the major factors influencing calcite precipitation [15–18]. The influence of temperature and pH on MICP is complicated because they affect various processes including microbial activity/growth, urease activity, and the CaCO₃ solubility. Urease was found to be active at temperatures ranging from 10–60 °C; the activity usually peaked at a temperature around 60 °C [9,19,20]. However, precipitation was not found to be

at its peak at this temperature. It was reported that the microbial activity and growth was slightly higher at 30 °C than at 20 °C [21]. Likewise, an increased calcite production was reported when the temperature was increased from 20 to 50 °C, while there was no precipitation at a temperature of 60 °C or higher [22,23]. Some researchers indicate that urease activity does not have a strong correlation with the engineering properties of MICP treated soil. For instance, the strength of MICP treated coarse sand was found to be higher when soil improvement was performed at a relatively lower temperature of 20 °C [15]. Interestingly, even with the same amount of calcium carbonate precipitated in soil, the strength of MICP treated soil would differ. Cheng et al. (2016) measured the unconfined compressive strength of the soil at temperatures of 4, 25, and 50 °C, and reported that soil improvement was most effective at 25 °C for the same calcium carbonate content in the treated soil [12]. Moreover, more calcite precipitation does not necessarily result in higher strength if the precipitation occurs at a different temperature [24]. This is likely due to the crystal size resulting from a change in CaCO₃ solubility, which varies with temperature and pH [25]. The CaCO₃ solubility decreases with an increase in temperature and pH, thus affecting calcite precipitation.

Another factor that highly influences MICP is the pH, with an alkaline environment known to be favorable to the process [24,26]. Through hydrolysis, urea (CO(NH₂)₂) is hydrolyzed by the urea enzyme into ammonium (NH₄⁺) and carbonate ions (CO₃^{2−}). This CO₃^{2−} is combined with calcium ions (Ca²⁺) from the supplied medium to form calcium carbonate (CaCO₃).

The urea enzyme is optimally active at a specific range of pH; the optimum pH for active enzyme ranges between 7 and 8.0 [9,27–29]. Its activity peaked at a pH close to 8.0 and gradually decreased at a pH of 8 or higher. It was reported that the optimum pH for the action of most of the microbial ureases is around 7 [30], while that for *Sporosarcina pasteurii*, which is the most common microbe for MICP, is 8 [27,31]; and the precipitation amount increased with pH and converged to a certain value at a pH range of 8.7 to 9.5 [27,32–34]. Other microbes such as *Bacillus megaterium* and *Bacillus sphaericus* have an optimum pH value of 7 and 8, respectively [29,35]. Although an optimal pH has been reported for various microbial species, the initial pH of the medium increased during precipitation, thus changing the environment for optimum precipitation. The increase in pH was due to the hydroxide ions (OH[−]) generated during ureolysis, which changed the pH of the soil to alkaline [36,37]. When a source of ammonia was added, a temporal increase in pH was found in slightly acidic soil, but the difference was insignificant in slightly alkaline soil [38].

In this study, *Staphylococcus saprophyticus*, the microbe isolated from calcareous beach sand, and *S. pasteurii*, (ATCC 11859), the most commonly used microbe in MICP, were used to determine the optimal conditions for calcite precipitation. Each microbe was injected into the urea-CaCl₂ medium of different pH and cultured at various temperatures. The effects of the pH of the urea-CaCl₂ medium, the temperature during precipitation, and the reaction duration on the amount of calcite precipitated were investigated.

2. Materials and Methods

2.1. Microorganisms

One of the microbes studied was *S. saprophyticus*, which was isolated from the calcareous sand of Daecheon beach located at the western coast of South Korea. The other microbe was *S. pasteurii* (ATCC 11859), which was purchased from the Korean Collection for Type Cultures in South Korea. *S. pasteurii* has been extensively used with great success in MICP research [16,39,40]. On the other hand, MICP using *S. saprophyticus* has rarely been reported, even though its urease activity was estimated to be higher than that of *S. pasteurii* in Reference [41]. In the aforementioned study, the urease activities of both the microbial species were measured using the phenol-hypochlorite urease assay, and the optical density at a wavelength of 620 nm (OD₆₂₀) of *S. saprophyticus* was much higher than that of *S. pasteurii*.

2.2. Experimental Procedures

Figure 1 shows the experimental process to measure the amount of calcite precipitation. The urea- CaCl_2 medium was prepared using 500 mL of distilled water, 1.5 g nutrient broth (Difco), 5 g NH_4Cl , and 1.06 g NaHCO_3 (equivalent to 25.2 mM). The initial pH of the medium was varied between 6–10 with hydrochloric acid and buffer solution. After autoclaving at 121 °C for 15 min, the medium was cooled and 14 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 1 g urea were added.

Figure 1. Experimental process for measuring the calcite precipitation.

The measurement of calcite precipitation was performed according to the Standard Test Method for Rapid Determination of Carbonate Content of Soils [42]. A calibration curve was plotted using 1 N hydrochloric acid (80 mL HCl and 720 mL distilled water) and 0.2, 0.4, 0.6, 0.8, and 1.0 g of calcite powder (CaCO_3 ; Showa Chemicals Inc. (Tokyo, Japan)). The calcite powder in the reaction cylinder reacted with HCl, and the carbon dioxide (CO_2) pressure generated by this reaction was measured to plot the calibration curve. It was assumed that the pressure of CO_2 was proportional to the amount of calcite precipitated. Using this method, calcite precipitation was measured and the optimal conditions for precipitation were determined by varying different parameters like pH, temperature, and reaction duration.

The testing conditions included different pHs (6, 7, 8, 9, and 10) of the medium, temperatures (20, 30, 40, and 50 °C), and reaction durations (2, 3, and 4 days). First, the two species under investigation were activated by culturing in 35 mL of tryptic soy broth (TSB; Difco Laboratories, Detroit, MI, USA, pH 7.2) for 2–3 days at 28 °C (Figure 2a)). The microbes were then incubated for 3 days at 30 °C before injecting them into the urea- CaCl_2 medium (Figure 2b). For this, 50 mL of urea- CaCl_2 medium with a pH ranging from 6–10 was prepared in Teflon beakers, and inoculated with 2 mL of 108–109 CFU/mL microbes. Then the beaker was covered and placed in the incubators, temperatures of which were set to 20, 30, 40, and 50 °C. For twenty different sets of pH and temperatures, the tests were performed for different reaction durations of 2, 3, and 4 days. Subsequently, the beaker containing the medium was oven-dried at 105 °C for 2 days (Figure 2c). It was placed in the reaction cylinder as in Figure 1, and 20 mL of 1 N HCl was poured into it. The calcite that had precipitated at the bottom of the beaker chemically reacted with HCl, emitting CO_2 gas. According to the ASTM D4373 [42], the pressure of the CO_2 gas was measured to determine the amount of calcite precipitation.

Figure 2. Testing procedure: (a) insert strain into 35 mL TSB; (b) urea- CaCl_2 medium and microbes; and (c) oven-dried sample.

3. Test Results

Sixty different combinations of pH, temperature, and reaction duration were used for each microbial species, and the amount of calcite precipitation was measured for each combination. The test results are tabulated in Table 1. Figure 3 shows the effect of temperature on the amount of calcite precipitates for *S. saprophyticus* and *S. pasteurii*. It is clear that under all the different testing conditions, the *S. saprophyticus* precipitates considerably more than *S. pasteurii*. The amount of precipitation is approximately five times more in the majority of conditions used for testing in *S. saprophyticus*. Although the amount of precipitation significantly differs between the two microbes, the optimal condition for maximum precipitation was identical. Maximum precipitation was observed at a temperature of 30 °C and a pH 7 of the urea- CaCl_2 medium. At higher temperatures, the precipitation significantly dropped for both microbes. Figure 4 shows calcite precipitation at a specific condition relative to that of the optimal condition at reaction duration of 3 days. Both *S. saprophyticus* and *S. pasteurii* were found to be very sensitive to the change in temperature during precipitation, showing a considerable drop of approximately 60%. On the other hand, the effect of pH was found to be different between the microbes. The productivity of *S. saprophyticus* is less influenced by a change in pH, while that of *S. pasteurii* is strongly influenced. The difference in the precipitation was at the most around 25% for *S. saprophyticus* (see Figure 4c) while it was around 60% for *S. pasteurii* (see Figure 4d). It should be noted that the measurement tolerance is 0.0018 g, which is about 1.2% and 4.5% of the maximum calcite precipitation of 3 days for *S. saprophyticus* and *S. pasteurii*, respectively.

Table 1. The estimated calcite precipitation at different pHs, temperatures, and reaction durations.

Reaction Duration (Day)	pH Temp.	Amount of Calcite Precipitation (g)									
		<i>Staphylococcus saprophyticus</i>					<i>Sporosarcina pasteurii</i>				
		6	7	8	9	10	6	7	8	9	10
2	20	0.1102	0.1193	0.1065	0.1010	0.0991	0.0184	0.0220	0.0147	0.0147	0.0147
	30	0.1193	0.1285	0.1230	0.1157	0.1120	0.0220	0.0330	0.0220	0.0165	0.0165
	40	0.0918	0.1010	0.0918	0.0863	0.0826	0.0129	0.0202	0.0147	0.0092	0.0092
	50	0.0551	0.0661	0.0569	0.0496	0.0477	0.0110	0.0165	0.0129	0.0092	0.0055
3	20	0.1138	0.1230	0.1120	0.1065	0.1010	0.0220	0.0275	0.0239	0.0184	0.0184
	30	0.1432	0.1469	0.1285	0.1285	0.1249	0.0330	0.0404	0.0312	0.0220	0.0220
	40	0.1010	0.1138	0.1010	0.0955	0.0918	0.0202	0.0294	0.0184	0.0147	0.0129
	50	0.0643	0.0734	0.0606	0.0551	0.0551	0.0165	0.0239	0.0147	0.0092	0.0092
4	20	0.1120	0.1249	0.1102	0.1065	0.1065	0.0239	0.0294	0.0239	0.0184	0.0184
	30	0.1322	0.1432	0.1285	0.1230	0.1193	0.0312	0.0422	0.0294	0.0239	0.0220
	40	0.1047	0.1102	0.1010	0.0955	0.0973	0.0184	0.0312	0.0202	0.0147	0.0147
	50	0.0643	0.0753	0.0588	0.0569	0.0551	0.0165	0.0220	0.0129	0.0129	0.0092

Figure 3. Effect of temperature on precipitation.

The calcite precipitations for both the species appear to reach a maximum value at a reaction duration of 3 days under most of the tested conditions. An average increase of about 10% was observed in *S. saprophyticus* when the reaction duration was changed from 2 to 3 days. An increase of 14% was seen when optimal pH (7) and temperature (30 $^{\circ}\text{C}$) were used. However, precipitation significantly increased for *S. pasteurii*, with an average increase of 37% and 22% for the optimal condition, indicating that reaction occurred more slowly for *S. pasteurii* than for *S. saprophyticus*. Although *S. pasteurii* reaches exponential phase of growth within 12 hours, the increase in the calcite precipitation was measured at a reaction duration of 2 days because cell viability is not solely related with urease activity and calcite precipitation. In fact, urease and its activity might not be associated with an active microbial population (cell viability) since there was no significant correlation between urease activity and respiration in soil bacteria [43]. This is because urease is a cytoplasmic protein [30,44]. For example, cell fractionation

studies generally demonstrate that urease partitions with the cytoplasmic fraction [44]. Thus, the urease enzyme activity could increase after cell lysis, which might result in an increase in calcite precipitation even after 2 days.

Figure 4. Relative calcite precipitation of the two microbial species at a reaction duration of 3 days: (a,b) the precipitation relative to that at a temperature of 30 °C; (c,d) the precipitation relative to that at a pH of 7.

Previous research along with this study reveal that the optimum pH and temperature for urease activity, calcite precipitation, and bacterial growth are the values as shown in Figure 5 [19–22,27,29,31,35,45–50]. The optimum pH of *S. pasteurii* for urease activity ranges widely from 7 to 9, with a negligible difference reported by Lauchnor et al. [45]. On the other hand, the pH required for optimum bacterial growth was nearly 9. Calcite precipitation is a complex process resulting from various factors, with the optimum pH not coinciding with either urease activity or bacterial growth. For the two microbes used in this study, the optimum pH for calcite precipitation was 7 (neutral), which is same for *B. megaterium* and lower than *B. sphaericus*. Urease activity and bacterial growth tend to favor a highly alkaline environment. Since CaCO_3 solubility decreases with increasing pH, calcite precipitation is likely to increase with increasing pH. However, the pH was measured to be optimum at 7 because increasing the pH makes the medium alkaline during ureolysis. The optimum temperature for urease activity varies from 30 to 70 °C for *S. pasteurii*. Previous research has shown that the optimum temperature for calcite precipitation is 50 °C [22], which is higher than 30 °C obtained from this study. However, it should be noted that the previous research adopted only two temperatures (22 and 50 °C), so the optimum temperature was not thoroughly investigated.

Figure 5. Optimal pH and temperature for urease activity, calcite precipitation, and bacterial growth.

4. Conclusions

In this study, the effects of temperature, pH of medium, and the reaction duration on calcite precipitation were evaluated for two microbial species (*S. saprophyticus* and *S. pasteurii*). The amount of precipitated calcite was quantified indirectly by measuring the pressure of CO₂ gas, which is generated as a result of the reaction between calcite and HCl. The following conclusions were drawn from the test results:

1. The optimal conditions for calcite precipitation for both the species were identical: the optimum pH of the urea-CaCl₂ medium was 7 and the optimum temperature during precipitation was 30 °C. The precipitation of *S. saprophyticus* was five times more than that of *S. pasteurii* under identical conditions.
2. Both microbial species were strongly influenced by the temperature of the environment during precipitation, showing a significant decrease with varying temperatures. The least precipitation was measured at a temperature of 50 °C, which was as little as 30–40% of that seen at the optimal temperature (30 °C).
3. *S. saprophyticus* was found to be less sensitive to a change in pH of the urea-CaCl₂ medium than *S. pasteurii*; the decrease in precipitation by varying the pH was less than 20% for *S. saprophyticus*. On the other hand, pH significantly affected the precipitation of *S. pasteurii*, reducing it by 60%.
4. For *S. saprophyticus*, a meaningful increase in precipitation was observed between the reaction durations of 2 and 3 days, but little difference was measured for longer durations, indicating that maximum precipitation occurred within 3 days. Likewise, most of the precipitation occurred within 3 days for *S. pasteurii*.

Author Contributions: G.K. performed the laboratory experiments; J.K. analyzed testing results; H.Y. designed the study and prepared for the manuscript.

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References

1. Mitchell, J.K.; Santamarina, J.C. Biological considerations in geotechnical engineering. *J. Geotech. Geoenviron. Eng.* **2005**, *131*, 1222–1233. [\[CrossRef\]](#)
2. Paul, E.; Clark, F. *Soil Microbiology and Biochemistry*; Academic Press: New York, NY, USA, 1996; p. 254.

3. Chu, J.; Ivanov, V.; Naeimi, M.; Stabnikov, V.; Liu, H.L. Optimization of calcium-based bioclogging and biocementation of sand. *Acta Geotech.* **2014**, *9*, 277–285. [[CrossRef](#)]
4. Harkes, M.P.; van Paassen, L.A.; Booster, J.L.; Whiffin, V.S.; van Loosdrecht, M. Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecol. Eng.* **2010**, *36*, 112–117. [[CrossRef](#)]
5. Achal, V.; Pan, X. Influence of calcium sources on microbially induced calcium carbonate precipitation by *Bacillus* sp. CR2. *Appl. Biochem. Biotechnol.* **2014**, *173*, 307–317. [[CrossRef](#)] [[PubMed](#)]
6. Shahrokhi-Shahraki, R.; Zomorodian, S.M.A.; Niazi, A.; O’Kelly, B.C. Improving sand with microbial-induced carbonate precipitation. *Proc. Inst. Civ. Eng. Improv.* **2015**, *168*, 217–230. [[CrossRef](#)]
7. Chang, I.; Im, J.; Cho, G.C. Introduction of microbial biopolymers in soil treatment for future environmentally-friendly and sustainable geotechnical engineering. *Sustainability* **2016**, *8*, 251. [[CrossRef](#)]
8. Al Qabany, A.; Soga, K.; Santamarina, C. Factors affecting efficiency of microbially induced calcite precipitation. *J. Geotech. Geoenviron. Eng.* **2011**, *138*, 992–1001. [[CrossRef](#)]
9. Ng, W.S.; Lee, M.L.; Hii, S.L. An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement. *World Acad. Sci. Eng. Technol.* **2012**, *62*, 723–729.
10. Mujah, D.; Shahin, M.A.; Cheng, L. State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. *Geomicrobiol. J.* **2017**, *34*, 524–537. [[CrossRef](#)]
11. Wang, Z.; Zhang, N.; Cai, G.; Jin, Y.; Ding, N.; Shen, D. Review of ground improvement using microbial induced carbonate precipitation (MICP). *Mar. Georesour. Geotechnol.* **2017**, *35*, 1135–1146. [[CrossRef](#)]
12. Cheng, L.; Shahin, M.; Mujah, D. Influence of key environmental conditions on microbially induced cementation for soil stabilization. *J. Geotech. Geoenviron. Eng.* **2017**, *143*, 04016083. [[CrossRef](#)]
13. Soon, N.W.; Lee, L.M.; Khun, T.C.; Ling, H.S. Factors affecting improvement in engineering properties of residual soil through microbial-induced calcite precipitation. *J. Geotech. Geoenviron. Eng.* **2014**, *140*, 04014006. [[CrossRef](#)]
14. Umar, M.; Kassim, K.A.; Chiet, K.T.P. Biological process of soil improvement in civil engineering: A review. *J. Rock Mech. Geotech. Eng.* **2016**, *8*, 767–774. [[CrossRef](#)]
15. Mahawish, A.; Bouazza, A.; Gates, W.P. Factors affecting the bio-cementing process of coarse sand. *Proc. Inst. Civ. Eng. Improv.* **2018**, 1–45. [[CrossRef](#)]
16. Zhao, Q.; Li, L.; Li, C.; Li, M.; Amini, F.; Zhang, H. Factors affecting improvement of engineering properties of MICP-treated soil catalyzed by bacteria and urease. *J. Mater. Civ. Eng.* **2014**, *26*, 04014094. [[CrossRef](#)]
17. Wang, R.; Qian, C.; Wang, J. Study on microbiological precipitation of CaCO_3 . *J. Southeast Univ.* **2005**, *35*, 95–191.
18. Castanier, S.; Le Métayer-Levrel, G.; Perthuisot, J.P. Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view. *Sediment. Geol.* **1999**, *126*, 9–23. [[CrossRef](#)]
19. Sahrawat, K. Effects of temperature and moisture on urease activity in semi-arid tropical soils. *Plant Soil* **1984**, *78*, 401–408. [[CrossRef](#)]
20. Liang, Z.P.; Feng, Y.Q.; Meng, S.X.; Liang, Z.Y. Preparation and properties of urease immobilized onto glutaraldehyde cross-linked chitosan beads. *Chin. Chem. Lett.* **2005**, *16*, 135–138.
21. Nemati, M.; Greene, E.; Voordouw, G. Permeability profile modification using bacterially formed calcium carbonate: comparison with enzymic option. *Process Biochem.* **2005**, *40*, 925–933. [[CrossRef](#)]
22. Nemati, M.; Voordouw, G. Modification of porous media permeability, using calcium carbonate produced enzymatically in situ. *Enzyme Microb. Technol.* **2003**, *33*, 635–642. [[CrossRef](#)]
23. Rebata-Landa, V. Microbial Activity in Sediments: Effects on Soil Behavior. Ph.D. Thesis, Georgia Institute of Technology, Atlanta, GA, USA, 2007.
24. Cheng, L.; Shahin, M.; Cord-Ruwisch, R.; Addis, M.; Hartanto, T.; Elms, C. In Soil stabilisation by microbial-induced calcite precipitation (micp): Investigation into some physical and environmental aspects. In Proceedings of the 7th International Congress on Environmental Geotechnics, Melbourne, Australia, 10–14 November 2014; p. 1105.
25. Jones, T.; Detwiler, R. In Fracture-aperture alteration induced by calcite precipitation. In Proceedings of the 49th US Rock Mechanics/Geomechanics Symposium, San Francisco, CA, USA, 28 June–1 July 2015.

26. Ferris, F.; Stehmeier, L.; Kantzas, A.; Mourits, F. Bacteriogenic mineral plugging. *J. Can. Pet. Technol.* **1996**, *35*, [CrossRef]
27. Stocks-Fischer, S.; Galinat, J.K.; Bang, S.S. Microbiological precipitation of CaCO₃. *Soil Biol. Biochem.* **1999**, *31*, 1563–1571. [CrossRef]
28. Evans, D.; Evans, D.G.; Takemura, T.; Nakano, H.; Lampert, H.C.; Graham, D.Y.; Granger, D.N.; Kvietys, P.R. Characterization of a *Helicobacter pylori* neutrophil-activating protein. *Infect. Immun.* **1995**, *63*, 2213–2220. [PubMed]
29. Arunachalam, K.D.; Sathyanarayanan, K.; Darshan, B.; Raja, R.B. Studies on the characterisation of Biosealant properties of *Bacillus sphaericus*. *Int. J. Eng. Sci. Technol.* **2010**, *2*, 270–277.
30. Mobley, H.; Island, M.D.; Hausinger, R.P. Molecular biology of microbial ureases. *Microbiol. Rev.* **1995**, *59*, 451–480. [PubMed]
31. Ciurli, S.; Marzadori, C.; Benini, S.; Deiana, S.; Gessa, C. Urease from the soil bacterium *Bacillus pasteurii*: immobilization on Ca-polygalacturonate. *Soil Biol. Biochem.* **1996**, *28*, 811–817. [CrossRef]
32. Dupraz, S.; Ménez, B.; Gouze, P.; Leprovost, R.; Bénézech, P.; Pokrovsky, O.S.; Guyot, F. Experimental approach of CO₂ biomineralization in deep saline aquifers. *Chem. Geol.* **2009**, *265*, 54–62. [CrossRef]
33. Fujita, Y.; Redden, G.D.; Ingram, J.C.; Cortez, M.M.; Ferris, F.G.; Smith, R.W. Strontium incorporation into calcite generated by bacterial ureolysis. *Geochim. Cosmochim. Acta* **2004**, *68*, 3261–3270. [CrossRef]
34. Ferris, F.; Phoenix, V.; Fujita, Y.; Smith, R. Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20 °C in artificial groundwater. *Geochim. Cosmochim. Acta* **2004**, *68*, 1701–1710. [CrossRef]
35. Khan, J.A. Biodegradation of azo dye by moderately halotolerant *Bacillus megaterium* and study of enzyme azoreductase involved in degradation. *Adv. Biotech.* **2011**, *10*, 21–27.
36. DeJong, J.T.; Mortensen, B.M.; Martinez, B.C.; Nelson, D.C. Bio-mediated soil improvement. *Ecol. Eng.* **2010**, *36*, 197–210. [CrossRef]
37. Ehrlich, H.L. Geomicrobiology: its significance for geology. *Earth Sci. Rev.* **1998**, *45*, 45–60. [CrossRef]
38. Akiyama, M.; Kawasaki, S. Microbially mediated sand solidification using calcium phosphate compounds. *Eng. Geol.* **2012**, *137*, 29–39. [CrossRef]
39. Stabnikov, V.; Jian, C.; Ivanov, V.; Li, Y. Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand. *World J. Microbiol. Biotechnol.* **2013**, *29*, 1453–1460. [CrossRef] [PubMed]
40. Mortensen, B.; DeJong, J. Strength and stiffness of MICP treated sand subjected to various stress paths. In Proceedings of the Geo-Frontiers 2011, Advances in Geotechnical Engineering, Dallas, TX, USA, 13–16 March 2011; pp. 4012–4020.
41. Kim, G.; Youn, H. Microbially induced calcite precipitation employing environmental isolates. *Materials* **2016**, *9*, 468. [CrossRef] [PubMed]
42. ASTM-D4373. *Standard Test Method for Rapid Determination of Carbonate Content of Soils*; ASTM International: West Conshohocken, PA, USA, 2014.
43. Frankenberger, W.; Dick, W. Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Sci. Soc. Am. J.* **1983**, *47*, 945–951. [CrossRef]
44. Mobley, H.; Hausinger, R. Microbial ureases: Significance, regulation, and molecular characterization. *Microbiol. Rev.* **1989**, *53*, 85–108. [PubMed]
45. Lauchnor, E.G.; Topp, D.; Parker, A.; Gerlach, R. Whole cell kinetics of ureolysis by *Sporosarcina pasteurii*. *J. Appl. Microbiol.* **2015**, *118*, 1321–1332. [CrossRef] [PubMed]
46. Bang, S.S.; Galinat, J.K.; Ramakrishnan, V. Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*. *Enzyme Microb. Technol.* **2001**, *28*, 404–409. [CrossRef]
47. Whiffin, V.S. Microbial CaCO₃ Precipitation for the Production of Biocement. Ph.D. Thesis, Murdoch University, Murdoch, Australia, 2004.
48. Dhami, N.K.; Reddy, M.S.; Mukherjee, A. Synergistic role of bacterial urease and carbonic anhydrase in carbonate mineralization. *Appl. Biochem. Biotechnol.* **2014**, *172*, 2552–2561. [CrossRef] [PubMed]

49. Warth, A. Relationship between the heat resistance of spores and the optimum and maximum growth temperatures of *Bacillus* species. *J. Bacteriol.* **1978**, *134*, 699–705. [[PubMed](#)]
50. Balan, S.S.; Fathima, F.; Jayalakshmi, S. Characterization of urease enzyme from marine bacterium *Klebsiella* species. *Afr. J. Microbiol. Res.* **2012**, *6*, 5914–5923.

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