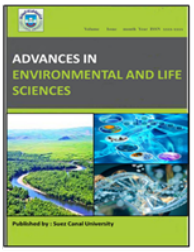
Advances in Environmental and Life Sciences 2 (2) (2022) 32-43

Contents lists available at Egyptian Knowledge Bank

Advances in Environmental and Life Sciences

journal homepage: https://aels.journals.ekb.eg



Factors Affecting Calcite Bio-precipitation via Urea Hydrolyzing Bacterial Consortium

Maha Taha[a,*], Salah Shata[b], Heba Taher[a], Hesham Abdulla[a]

[a]Botany and Microbiology Department Faculty of Science, Suez Canal University, Egypt

[b]Geology Department Faculty of Science, Suez Canal University, Egypt

Abstract

Microbially induced calcite precipitation (MICP) refers to the biomineralization process involving the synthesizing of calcium carbonate by microorganisms. The production of urease by urea-hydrolyzing bacteria, which results in carbonate precipitation, is affected by environmental factors including calcium concentration, bacterial concentration, pH and temperature. This study aims to investigate some factors affecting calcite bio-precipitation via the urea-hydrolyzing consortium. A consortium of three urease-positive, calcite-precipitating strains of Bacillus was selected. XRD analysis of the precipitant confirmed the polymorph type. The studied factors included bacterial cell count, pH, temperature, urea concentration, and calcium concentration. In order to identify the optimum range of each studied factor, the dry weight of the precipitated calcite was measured for all investigated ranges of the studied factors after two weeks of incubation gravimetrically. A multi-level factorial experiment was designed to investigate the impact of interaction between different levels of factors using the statistical software package MINITAB 17. Three main factors were selected, bacterial cell count, urea and calcium concentration. The fourth run, among twelve runs of a multi-level designed experiment, which had the following concentrations (0.3 M urea, 15 g/l $CaCl_2.H_2O$, and low bacterial inoculum (10^5 CFU/50ml)), precipitated 0.43 g of calcite, while the rest of the runs precipitated lower dry weights. It was found that there was no significant increase in the precipitated calcite weight for the performed runs. According to the analysis of factorial design results, the effect of urea concentration as a factor on precipitation rate in the current study was found to be more effective than other studied factors.

Keywords: MICCP, Bio-mineralization, Urease, *Bacillus.*

1. Introduction

Biomineralization is the production of minerals by living organisms as a result of the interaction of their metabolic products with their surroundings **[1].** Mineral production by several bacterial species, such as urea-degrading bacteria has been reported **[2].** The synthesis of calcium carbonate from a supersaturated solution due to the presence of microbial cells and biochemical activity is referred to as MICCP **[3].** During MICCP, organisms can emit one or more metabolic products (CO_3^{2}), which react with ions (Ca^{2+}) in the environment, causing mineral precipitation. **[4].** Among the several bacteria metabolic pathways employed in MICCP, most research have mostly focused on bacterial ureolysis [5-7]**.**

Ureolytic bacteria can catalyze urea to carbonate and ammonium which subsequently raises pH values. When calcium ions exist in the solution with carbonate and high pH, calcium carbonate ($CaCO_3$) is precipitated [7-8]. Biomineralization can result in formation of anhydrous $CaCO_3$ poly-

*Corresponding author.
Email address: maha.012282@science.suez.edu.eg (Maha Taha)

doi 10.21608/AELS.2022.165065.1020

Received: 5 October 2022, Revised:25 October 2022
Accepted: 29 October 2022: Published: 29 October 2022

morphs such as calcite, aragonite and vaterite, as well as hydrated crystalline phases like monohydrocalcite ($CaCO_3 \cdot H_2O$) and hexahydrocalcite or ikaite ($CaCO_3 \cdot 6H_2O$) and amorphous calcium carbonate (ACC) [9]. **C**alcite is the most thermodynamically stable polymorph of $CaCO_3$ and the predominant $CaCO_3$ product in several MICCPs [10-13].

The precipitation of calcium carbonate is regulated by four major factors: calcium ion concentration, the quantity of dissolved inorganic carbon (DIC), the availability of nucleation sites, and pH [14]. The concentration of urease enzymes (i.e., bacteria or urease concentration) and the available substrate (e.g., urea) are the primary determinants of urea hydrolysis, whereas calcite precipitation is related to available Ca^{+2}. [15]. **A** solution that contains equimolar of urea and calcium would provide better conversion to calcite [16].

Bacterial cells served as nucleation sites, therefore, a higher bacterial cell concentration supplied to the substrate would certainly increase the MICCP process [11, 12, 17]. The microbial activity and growth are less sensitive to the temperature within the ranges of 20 to 30 °C. Increment in temperature after 30 °C does not promote the decomposition rate any further [16]. **L**ike all other enzymes, the urease enzyme is only active at a certain range of pH. The optimum pH for the urease enzyme is in the range of 7.5 to 8.0 [11, 18, 19].

The urease-positive bacteria that are suitable for MICCP application belong to the genera Bacillus, Sporosarcina, Spoloactobacilus, Clostridium and Desulfotomaculum [20]. The aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production. Bacillus sp. is the most common bacteria used to precipitate calcium carbonate in their micro-environment through catalytic conversion of urea to ammonia and carbon dioxide [17, 21]. The common species of Bacillus used in previous studies were B. sphaericus [22, 23], B**.** Megaterium [24], and B. Pasteurii [25, 26]. However, the amount of calcite produced in MICCP varied with the types of Bacillus strains [17, 27]. This study aims to investigate some factors that affect calcite bio-precipitation via urea hydrolyzing bacterial consortium.

The MICP technique is an efficient and environmentally safe technology that may be used to solve a variety of environmental issues, such as heavy metals and radionuclide remediation, bioconsolidation, biocement, CO_2 sequestration, and other applications [28]. Bioremediation has been frequently used for contaminant containment or removal; in this situation, containment will suffice. MICP can also be used to enhance the effectiveness of in-situ bioremediation. Urease is an enzyme that promotes enhanced calcite precipitation. However, environmental variables such as calcium content, bacterial concentration, pH, and temperature impede the formation of urease by bacteria and consequently the subsequent carbonate precipitation. MICP can be used to immobilize heavy metals and radionuclides under optimal conditions. However, techniques like bioconsolidation and biocementation demand developments in terms of time and cost [29].

2. Materials & Methods

2.1. Bacterial isolates

Three bacterial strains precipitating calcium carbonate, Bacillus haynesii (OP115674), Bacillus piscis (OP115673) and Bacillus spizizenii (OP115669) were recovered from dumped limestone wall in Al-Mu'izz Street, Cairo, Egypt, were used in this study. In order to compare the calcium carbonate productivity of each strain and to their consortium, an antagonism check was performed before testing their consortium. A dual culture plate assay was used for assessing the ability of bacteria to inhibit each other [30]. The bacterial strains were grown until reaching the stationary phase in a nutrient broth medium and then a fixed inoculum was streaked as a cross-line alternately on Urea-Ca Agar [4].

For calcium carbonate productivity, a fixed inoculum of each strain (10^6 CFU/ml) was inoculated in 50 ml Urea-Ca broth in a pre-weighted 100 ml conical flask. For the consortium, equal cell counts of each strain ($3.33*10^5$ CFU) were mixed and inoculated. Two replicates for each inoculum were conducted along with an uninoculated medium as a

control. The cultures were incubated for two weeks at 28 °C and 120 rpm. The produced calcium carbonate crystals were harvested and washed by centrifugation, dried at 30 °C then weighted to evaluate the dry weight. Attached crystals on the flask wall were washed and dried also, and the final dry weights of both the harvested and the attached were recorded.

2.2. X-Ray diffraction (XRD analysis)

The produced calcium carbonate was powdered and analyzed using XRD to identify the carbonate polymorph. This analysis was carried out using a Philips PW1370 X-ray generator fitted with a PW 1390 channel control, a PW1050 vertical goniometer and a digitizer. X-rays were obtained by applying a potential of 40 kv and a current of 30mA on a PW2273/20 copper anode tube. X'Pert High Score version 2 was used to interpret the X-ray diffraction pattern.

2.3. Urease Test

Christensen's medium also called urea agar base (Oxoid) was used for testing urease production. The medium was sterilized by autoclaving at 15 psi for 15 min, while the urea solution was separately sterilized by filtration using a 0.45 μm syringe filter [31]. After cooling the medium to 45 °C, aseptically urea solution was added to a final concentration of 0.4%. The medium was carefully mixed by gentle swirling, then it was distributed into sterile test tubes. The bacterial strains were inoculated on the surface of the medium and then incubated at 37°C for 72 hrs. Urease production was assessed through visual observation for color changes from pale yellow to pink/red.

2.4. Factors affecting calcite bio-precipitation

Six factors that affect calcite precipitation using the three bacterial strains consortium were studied using broth culture in a pre-weighted 100 ml conical flask containing 50 ml of Urea-Ca medium. Bacterial counts from 10^4 to 10^{12} CFU, urea concentrations, 0.15, 0.3, 0.6, 0.9 and 2 Molar, calcium chloride concentrations, 15, 25, 30 and 35 gm/l, initial pHs, 6, 7, 8 and 9, incubation temperatures at 25, 30, 35 and 40 °C, and dynamic and static incubation were tested. Each factor was studied separately; all tests were conducted in duplicate, and the data were averaged. A set of control experiments were carried out under the same conditions as the test without bacteria. The dry weight of the produced calcite was evaluated after incubation for two weeks at 28 °C and 120 rpm. Calcite crystals were washed and harvested by centrifugation and dried at 30^oC. Attached crystals on the flask wall were washed with distilled water and dried also, and the final dry weights of both the harvested and the attached were recorded.

2.5. Multilevel Factorial Design

Multilevel Factorial Design was applied to investigate the main effects and interaction of factors at a different number of levels [32].

Table 1: Runs of theMultilevel Factorial Design

Run Code	urea conc. (M)	$CaCl_2$ (gm/l)	Bacterial Cell Count (CFU)
1	0.3	15	10^4
2	0.3	15	10^4
3	0.3	25	10^8
4	0.3	25	10^4
5	0.15	15	10^8
6	0.15	15	10^4
7	0.15	25	10^8
8	0.15	25	10^4
9	0.6	25	10^8
10	0.6	25	10^4
11	0.6	15	10^8
12	0.6	15	10^4

Three key factors were chosen, bacterial cell count, urea concentration and calcium chloride concentration, which can be controlled on larger scales. In this experiment, the design investigated the effects of the initial bacterial cell count (2 levels: 10^4 and 10^8 CFU), urea concentration (3 levels: 0.15, 0.3 and 0.6 M) and calcium chloride concentration (2 levels: 15 gm/l, 25gm/l) on the final $CaCO_3$ dry weight, using statistical software package MINITAB 17. Twelve runs were tested as shown

in Table (1); all runs were conducted in duplicate, and the data were averaged. A set of control experiments were carried out under the same conditions without bacteria. Dry weights were evaluated as described in section 2.1 and control adjustment.

3. Results and Discussion

3.1. Microbially Induced Calcium Carbonate Precipitation (MICCP)

Microbially Induced Calcium Carbonate Precipitation (MICCP) is applied in a variety of fields, including heavy metal and radionuclide remediation and atmospheric CO_2 sequestration. MICCP has evolved as an effective and environmentally friendly approach to limestone restoration and conservation. Furthermore, the same technique may be utilized to enhance soil and sand quality; MICCP uses are not limited and can be used to develop safe and environmentally stable products **[33- 37].**

In this study, three bacterial strains precipitating calcium carbonate, Bacillus haynesii (OP115674), Bacillus piscis (OP115673) and Bacillus spizizenii (OP115669) were involved. It was recommended that spore-forming bacteria were more suitable for MICCP applications. Many studies used different species of Bacillus to precipitate calcium carbonate [38-40]. The dual culture method was done for assessing the ability of bacteria to inhibit each other [30]. The results showed that there was no antagonism among the tested isolates; hence a consortium of the three isolates is possible to be applied (Photo 1).

Figure (1) shows the differences in the final yield of $CaCO_3$ precipitated by the three bacterial strains in comparison to their consortium. It was found that the consortium precipitated more $CaCO_3$ than each strain separately. B. spizizenii precipitated less than 0.1 g,B. haynesii precipitated 0.27 g while B. piscis and consortium precipitated 0.57 and 0.73 g $CaCO_3$, respectively. Consequentially, the consortium was selected to study factors. **Davis et al., [41]** discovered Bacillus subtillis that produces rhombohedral calcite crystals and Dick et al., [42] **c**ompared B. sphaericus and B. lentus for calcite production.

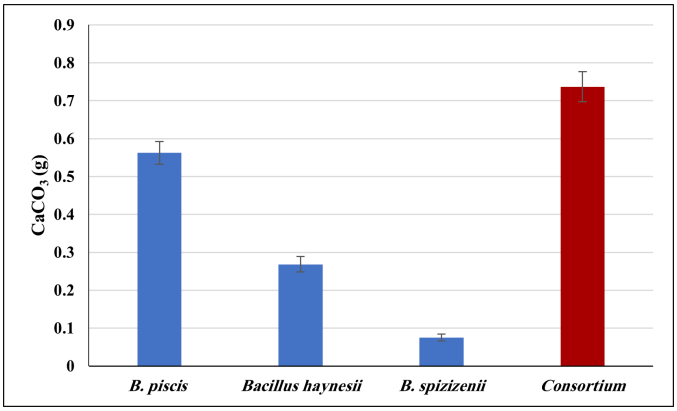


Figure 1: Dry weight of the $CaCO_3$ produced by the three bacterial strains in comparison to their consortium. Error bar indicates standard deviation.

3.2. X-Ray Diffraction (XRD analysis)

Bacterial cells provide nucleation sites (heterogeneous nucleation) that affect the specific types of minerals formed **[4].** The results of XRD analysis determined the polymorph of the precipitated calcium carbonate. The consortium of the three bacterial strains was confirmed to precipitate pure calcite. Figure (2) showed XRD patterns of pure calcite and Table (2) showed identified patterns of the XRD patterns. Despite extensive studies on bacterial carbonatogenesis, little is known about what the cause(s) of polymorph selection is during bacterial calcium carbonate mineralization. It has also been suggested that the phase and morphology of calcium carbonate are bacterial (or strain)-specific [9, 43- 45]. Ercole et al . [46], showed that EPS isolated from B. firmus and B. sphaericus induce the precipitation of calcite. Tourney and Ngwenya [47], indicated that dissolved organic carbon (DOC) released from EPS produced by B. liqueniformis complexes Ca ions and favors calcite precipitation over vaterite.

3.3. Urease Test

Calcium carbonate bio-precipitation has a different mechanism; the urease test was done as a confirmatory test on the mechanism of precipitation. The selected bacterial strains were tested for urease production. After incubation for 3 days at 28 °C, the three strains were able to change the Christensen's media slants from yellow to a pink color indicating urease production (Photo 2).

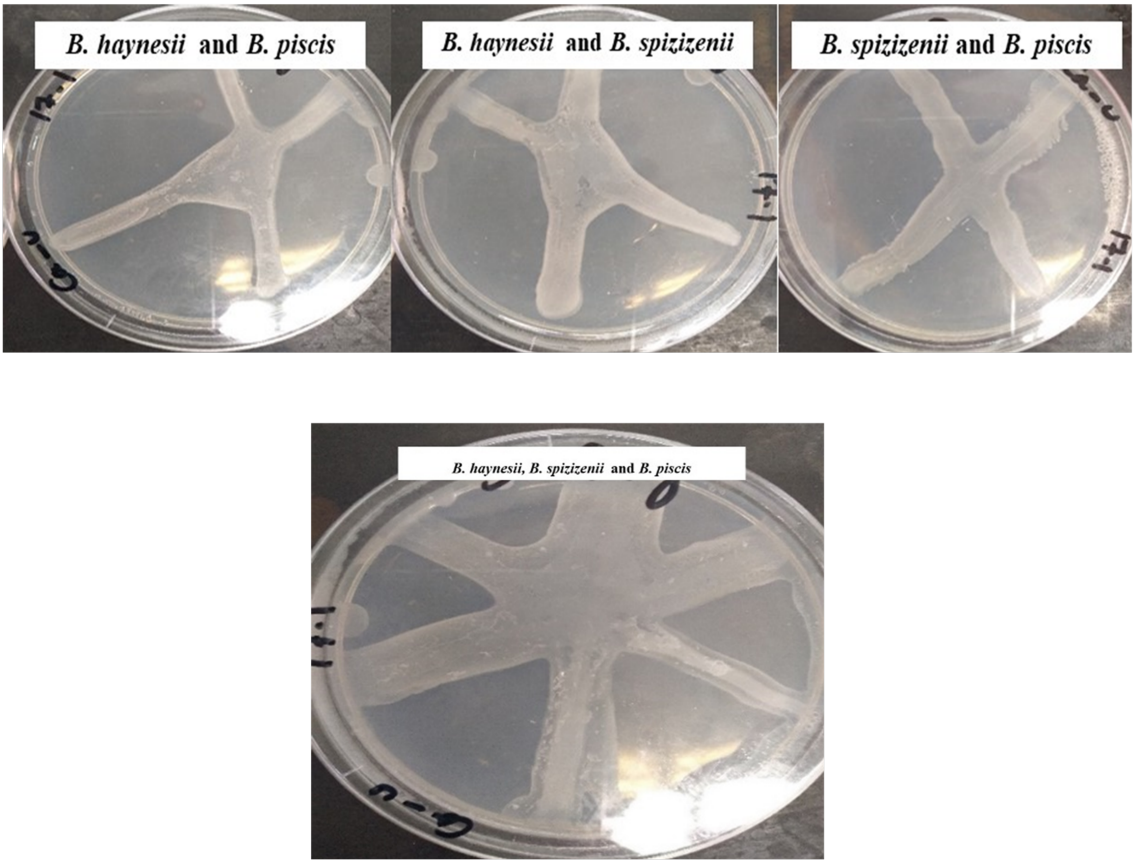


Photo 1: Antagonism test of the three bacterial strains on Urea-Ca-agar medium.

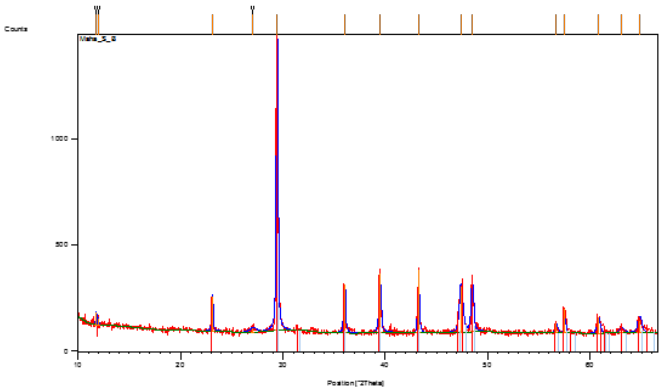


Figure 2: XRD pattern of pure calcite the selected bacterial consortium

For calcium carbonate precipitation in a medium containing urea, a specific concentration of urea acts as a nitrogen source to support the growth of the urease-producing isolates [48, 49]. One mole of urea is hydrolyzed to produce one mole of ammonia and one mole of carbamate, which is then spontaneously hydrolyzed to produce another mole of ammonia and carbonic acid [11]. In water, these two products (NH_3 and H_2CO_3) are further equilibrated to generate bicarbonate, two moles of ammonium, and two moles of hydroxide ions. The hydroxide ions raise the pH, which can cause the bicarbonate equilibrium to change, resulting in the creation of carbonate

Table 2: Identifiedpatterns of the XRD patterns.

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	01-086-2334	88	Calcite	-0.032	0.975	$Ca\ (CO_3)_2$

ions[21].

3.4. Factors influencing MICP efficiency

The activity of urease and the amount of calcite precipitation are based on several environmental factors. Indeed, many factors affect urease activity and calcite precipitation, including bacteria type, bacteria cell concentrations, pH, temperature, urea, and calcium concentrations [14, 15, 50, 51]. The following results show the effect of several factors on calcite precipitation using the selected consortium.

3.4.1. Bacterial cell count:

Bacterial cells served as nucleation sites for $CaCO_3$ precipitation; also urea hydrolysis has a direct relationship with bacterial cell counts [11]. Inoculation of different bacterial counts (CFU) showed variations in the precipitated calcite weight (g). High and very low cell concentrations caused lower calcite precipitation. Results showed that 10^8 CFU/ 50 ml was the optimum cell concentration that precipitated the highest calcite up to 0.74 g as shown in Figure (3). Increasing the concentrations of bacterial cells from 10^4 to 10^8 cells increased the amount of calcite precipitation via increases in the urease concentration for urea hydrolysis [12, 52]. Therefore, urea hydrolysis has a direct relationship with bacterial cell concentrations [50]. Increasing the concentrations of bacterial cells to more than 10^8 cells didn't increase calcite precipitation. The increase in the number of cells releases a high amount of ammonia due to urea hydrolysis, which is detrimental to most bacterial cells [53].

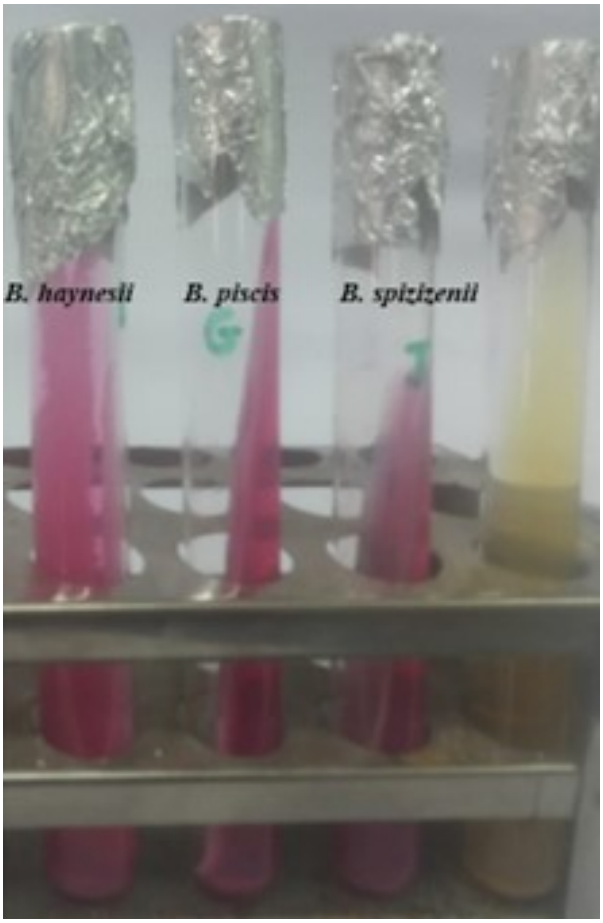


Photo 2: Urease production of the selected bacterial strains on Christensen's medium.

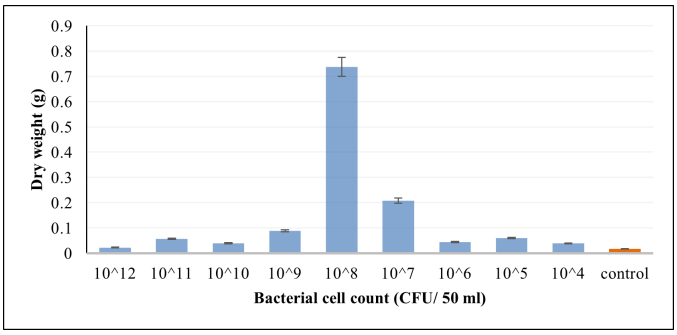


Figure 3: Dry weight of the precipitated calcite by different bacterial cell counts (CFU). Error bar indicates standard deviation.

3.4.2. Urea Concentration

The hydrolysis of urea by urease not only increases the pH but also uses it as a nitrogen and energy source [11, 37, 48]. Urea is considered the substrate for urease enzyme so the hydrolysis of urea mainly depends on the urea concentration. Increasing the urea concentration resulted in increases in calcite precipitation from 0.9 M urea to 0.67 g. It was noticed that a further increase in urea concentration of more than 0.9 M showed a reduction in precipitated calcite. It is assumed that an increase in urea concentration requires an increase in calcium ion concentration. The same assumption was obtained by Nemati et al., [16] who concluded that a solution containing equimolars of both reactants would provide better conversion to calcite. Compared with the control (uninoculated),

the inoculated consortium precipitated higher calcite by increasing the urea concentration except for 2M urea (Figure 4).

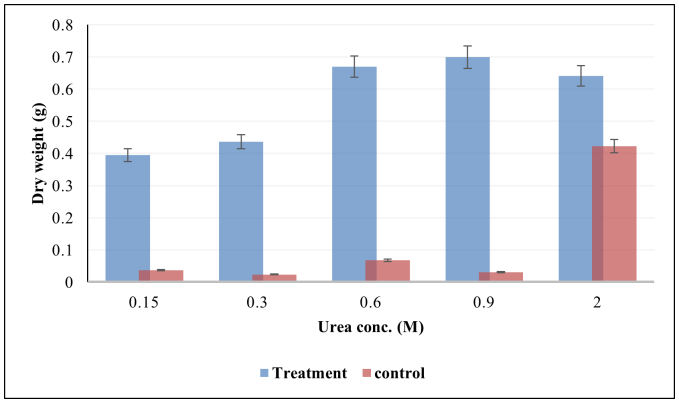


Figure 4: The precipitated calcite by the tested consortium at different urea concentrations (M). Error bar indicates standard deviation.

3.4.3. *Calcium concentration*

Calcite precipitation is dependent on the concentration of Ca^{2+} and $CO_3{}^{2-}$ in the solution. Ca^{2+} is not likely utilized by metabolic processes but accumulates outside the cell, where it is readily available for $CaCO_3$ precipitation **[3].** Calcium chloride was used as the calcium source in this study, The effect of different concentrations of calcium salt studied on the bio-precipitation rate of calcite is shown in Figure (5). High and low concentrations of calcium chloride cause a low precipitation rate. The concentration of 25 g/l shows the optimum calcium salt weight that resulted in the best productivity of calcite (0.66 g). Silver et al. [54], **p**roved that treatment containing 25 g/l of calcium chloride was better than 30 and 35 g/l. The same results were obtained by Okwadha and Li, [52] and De Muynck et al. [33] reported that the best urea and CaCl concentrations for calcite precipitation were 0.5 and 0.25 M, respectively.

3.4.4. *PH*

Calcite precipitation is influenced by pH because the urease enzyme will only be active at pH values specific for urea hydrolysis. The initial pH value of the growth medium is considered a key factor, as shown in Figure (6). pH at 6, 7, and 8 showed a linear relationship and gradual increase

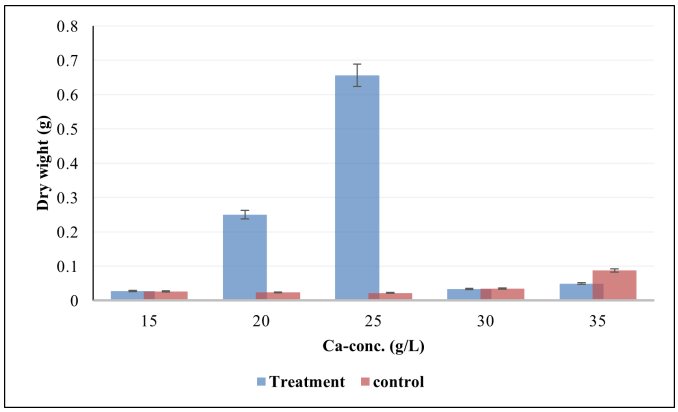


Figure 5: Dry weight of the precipitated calcite by the tested consortium at different calcium concentrations (g). Error bar indicates standard deviation

in the final dry weight in comparison to their controls but pH 9 showed the highest elevation in the treatment and control (uninoculated) as well. A high pH is very important for ammonia production by urea hydrolysis. At pH 9, both treatment and control precipitated approximately the same dry weight of calcite indicating the chemical precipitation was not enzymatically. Many investigators have reported that the optimum pH for urease is 8.0, above which the enzyme activity decreases [11, 53]. At lower pH than 7, the carbonate will tend to dissolve rather than precipitate [11], [56-58]. In contrast, Mobley et al., [59] and Stabnikov et al . [60], found that the optimum pH was nearly neutral.

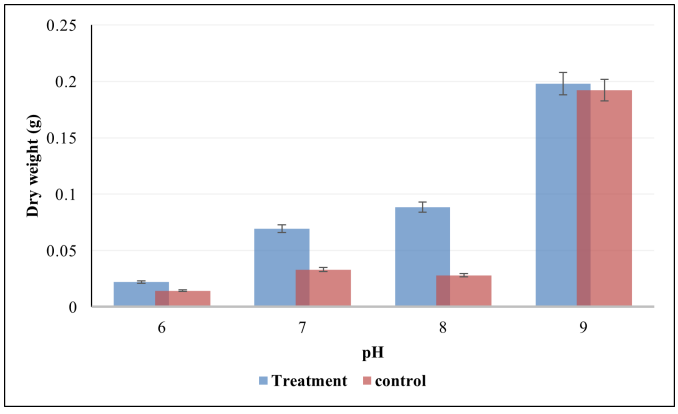


Figure 6: Dry weight of the precipitated calcite by the tested consortium at different pH values. Error bar indicates standard deviation

3.4.5. Temperature

Temperature is the main factor affecting enzymatic activity, like other enzymatic reactions, The catalysis of urea by urease is temperature dependent. The effect of five temperatures has been examined. Figure (7) showed the optimum precipitation rate at room temperature, which was ranging between 17 to 23°C during the experiment; it precipitated 0.34 g calcite. While a lower rate of precipitation (lower than 0.05 g) was obtained at lower and higher temperatures. These results are in line with most of the reported optimum temperatures for urease activity, which ranged between 20 and 37 °C [52]. Mitchel and Ferris[34] reported that the urease activity increased by about 5 and 10 times when the temperature increased from 15 to 20 °C and 10 to 20 °C, respectively.

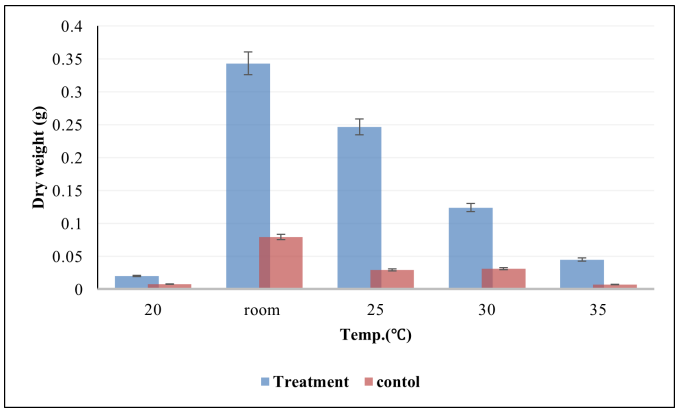


Figure 7: Dry weight of the precipitated calcite by the tested consortium at different temperature values (°C).

3.4.6. Static and dynamic incubation

Dynamic incubation improves the distribution of oxygen and nutrients and makes the growth homogenous. Static incubation is costly, and effective while shaking conditions are not applicable on larger scales. To compare the two conditions, two sets were conducted, one using shaking at 120 rpm and the other statically incubated. Dynamic incubation showed a slight difference in the final dry weight than the static incubation (Figure 8). The dry weight of the precipitated calcite was 0.3 and 0.25 from dynamic and static incubation, respectively.

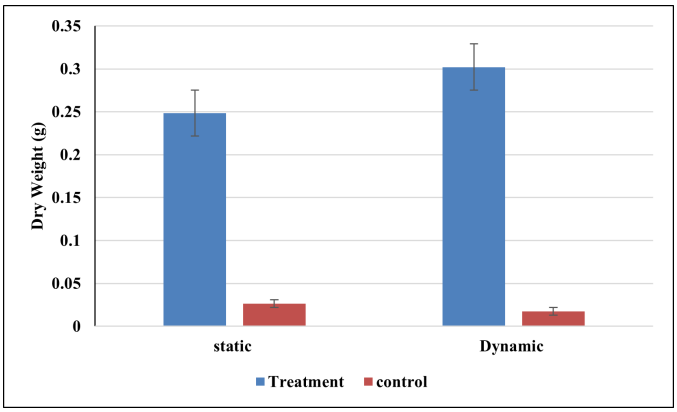


Figure 8: Dry weight of the precipitated calcite resulted from static versus dynamic incubation at 120 rpm.

3.5. Multilevel Factorial Design

Three main factors that can be controlled on larger scales, bacterial cell concentration, urea concentration, and calcium salt concentration, were chosen to investigate the interaction of the factors. Dry weights of the precipitated calcite resulting from the interaction between the tested factors are illustrated in Figure (9). As displayed in the results, the increase in urea and $CaCl_2$ concentrations to a certain limit led to an increase in calcite precipitation. On the other hand, high bacterial concentration retarded the effect on precipitation. According to the obtained results, only run 4, which consisted of 0.3 M urea, 15 g $CaCl_2.H_2O$ and 10^4 CFU bacterial cell count-precipitated 0.43 g calcite, while the rest runs precipitated lower weights. Interaction between factors has been studied also by Al Qabany et al., [51] who obtained similar results.

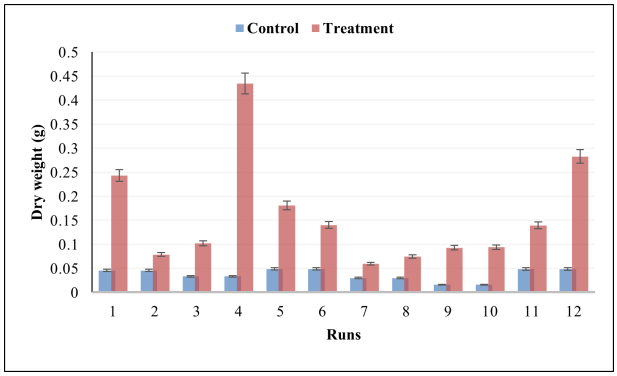


Figure 9: Dry weight (g) of the precipitated calcite resulted from 12 multi-Factorial design runs.

Table 3: Response Surface Regression: Treatment vs urea concentration(M), $CaCl_2$ (g/l), Inoculum Log (CFU)

Factors	**Coef.**	**t-value**	**p-value**
Urea Conc	0.0212	0.42	0.697
$CaCl_2$	-0.0183	-0.44	0.684
Bact. Cell count	-0.0253	-0.6	0.578
Urea Conc.*$CaCl_2$	-0.0147	-0.3	0.782
Urea Conc.* Bact. Cell Count	-0.0174	-0.35	0.745
$CaCl_2$ * Bact. Cell count	-0.0333	-0.8	0.467

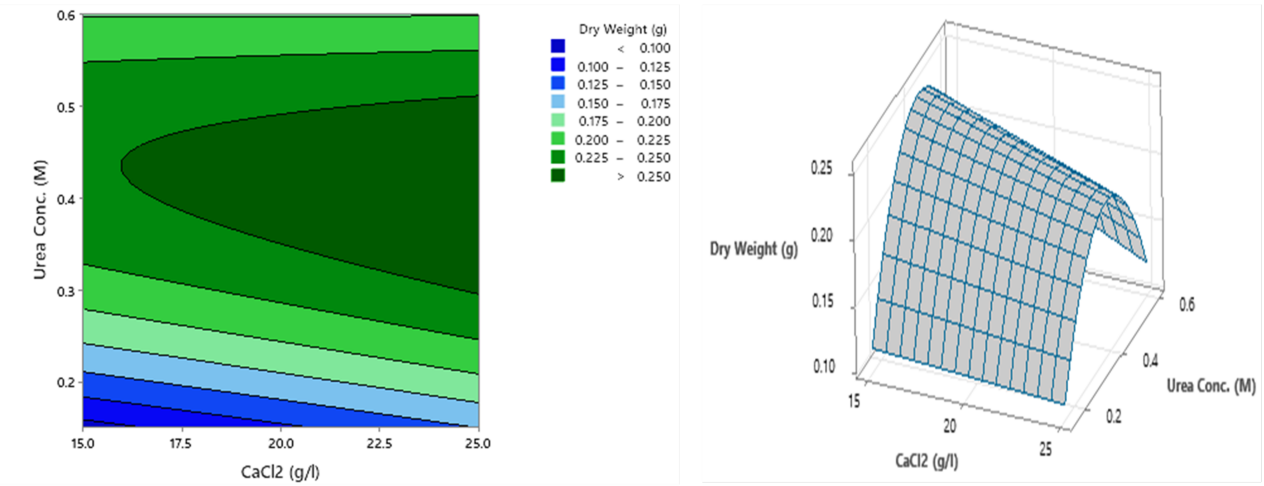


Figure 10: Contour Plot and Surface Plot of Dry weight (g) vs $CaCl_2$ (g/l), urea concentration (M) and hold continuous variable of bacterial cell count at 10^5 CFU.

Higher concentrations not only result in thicker calcite matrices but possibly also give a faster decline in bacterial activity because the urea becomes less available to the encapsulated microbial cells to hydrolyze. As displayed in Figures (10) the increase in urea and $CaCl_2$ concentration increased calcite precipitation. On the other hand, urea concentration higher than 0.5 M has retarded effect on precipitation even with a high amount of $CaCl_2$. Table (3) demonstrated the Response Surface Regression of the precipitated calcite versus urea conc. (M), CaCl2 (g/l), bacterial inoculum. The statistical analysis illustrated that there was no significant increase in precipitated calcite weight for the performed runs. The highest p-value was 0.782 for (urea concentration and calcium chloride concentration), and 0.745 for (urea concentration and bacterial inoculum) respectively, confirmed that the effect of urea concentration on the precipitated dry weight is higher than that of the other two tested $facto_r$s.

4. Conclusion

In conclusion the Microbially Induced Calcium Carbonate Precipitation (MICCP) technology is used in various fields of application for solving various environmental problems such as heavy metal removal, radionuclide immobilization, bioconsolidation, soil grouting, and CO_2 sequestration; the promising, effective results and the facility of application made it the technology of choice for such environmental purposes. However, the production of urease by bacteria and thus the resulting carbonate precipitation is inhibited by environmental factors including calcium concentration, bacterial concentration, pH, and temperature. Studying the factors affecting MICP for optimization and for better understanding the interaction between

different levels of studied factors on the selected strains of Bacillus, the spore-forming bacteria are suitable for MICCP applications in different fields. The tested bacterial consortium was found to be more effective in calcite precipitation than each strain separately, which enhances their compatibility and applicability in nature. Urea hydrolysis is an easy, efficient, and applicable mechanism for calcium carbonate precipitation. In the present study, bacterial cell count, urea concentration, and calcium concentration can be controlled on a larger scale as key factors affecting the MICP process. According to the analysis of factorial design results, the effect of urea concentration as a factor on precipitation rate in the current study was found to be more effective than other studied factors.

References

[1] S. M. Abdullah, K. Kolo, K. O. Konhauser, M. Pirouei, Microbial Domains and Their Role in the Formation of Minerals, in: Mineral Formation by Microorganisms, Springer, 2022, pp. 1–39.

[2] S. Joshi, S. Goyal, A. Mukherjee, M. S. Reddy, Microbial healing of cracks in concrete: a review, Journal of Industrial Microbiology and Biotechnology 44 (11) (2017) 1511–1525.

[3] V. Achal, X. Pan, Influence of calcium sources on microbially induced calcium carbonate precipitation by Bacillus sp, CR2. Applied Biochemistry and Biotechnology 173 (1) (2014) 307–317.

[4] N. Kaur, M. S. Reddy, A. Mukherjee, Biomineralization of calcium carbonate polymorphs by the bacterial strains isolated from calcareous sites, Journal of Microbiology and Biotechnology 23 (5) (2013) 707–714.

[5] V. S. Whiffin, L. A. V. Paassen, M. P. Harkes, Microbial Carbonate Precipitation as a Soil Improvement Technique, Geomicrobiology Journal 24 (5) (2007) 417–423.

[6] M. B. Burbank, T. J. Weaver, B. C. Williams, Crawford, R. L, Urease activity of ureolytic bacteria isolated from six soils in which calcite was precipitated by indigenous bacteria, Geomicrobiology Journal 29 (4) (2012) 389–395.

[7] J. T. Dejong, B. M. Mortensen, B. C. Martinez, D. C. Nelson, Bio-mediated soil improvement, Ecological Engineering 36 (2) (2010) 197–210.

[8] Y. Fujita, J. Taylor, L. Wendt, D. Reed, R. Smith, Evaluating the potential of native ureolytic microbes to remediate 90Sr contaminated environment, Environ Sci Technol 44 (2010) 7652–7658.

[9] S. Shougrakpam, Engineering Begaviour Of Microbially Cemented Soils Using Sporosarcina Pasteurii (Doctoral Dissertation) (2021).

[10] N. Spanos, P. G. Koutsoukos, The transformation of vaterite to calcite: Effect of the conditions of the solutions in contact with the mineral phase, Journal of Crystal Growth 191 (4) (1998) 783–790.

[11] S. Stocks-Fischer, J. K. Galinat, S. S. Bang, Microbiological precipitation of CaCO3, Soil Biology and Biochemistry 31 (11) (1999) 1563–1571.

[12] W. Li, L. P. Liu, P. P. Zhou, L. Cao, L. J. Yu, S. Y. Jiang, Calcite precipitation induced by bacteria and bacterially produced carbonic anhydrase, Current Science 100 (4) (2011) 502–508.

[13] G. Ganendra, W. D. Muynck, A. Ho, E. C. Arvaniti, B. Hosseinkhani, J. A. Ramos, . . Boon, N, Formate oxidation-driven calcium carbonate precipitation by Methylocystis parvus OBBP, Applied and environmental microbiology 80 (15) (2014) 4659–4667.

[14] X. Chen, D. Zhang, S. L. Larson, J. H. Ballard, H. M. Knotek-Smith, J. Nie, . . Han, F. X, Microbially Induced Carbonate Precipitation Techniques for the Remediation of Heavy Metal and Trace Element-Polluted Soils and Water, Water, Air, & Soil Pollution 232 (7) (2021) 1–15.

[15] B. M. Mortensen, M. J. Haber, J. T. Dejong, L. F. Caslake, D. C. Nelson, Effects of environmental factors on microbial induced calcium carbonate precipitation, Journal of applied microbiology 111 (2) (2011) 338–349.

[16] M. Nemati, E. A. Greene, G. Voordouw, Permeability profile modification using bacterially formed calcium carbonate: comparison with enzymic option, Process Biochemistry 40 (2) (2005) 925–933.

[17] W.-S. Ng, M.-L. Lee, S.-L. Hii, An Overview of the Factors Affecting Microbial-Induced Calcite Precipitation and its Potential Application in Soil Improvement, International Journal of Civil and Environmental Engineering 6 (2) (2012) 188–194.

[18] D. J. Evans, D. G. Evans, S. S. Kirkpatrick, D. Y. Graham, Characterization of the Helicobacter pylori urease and purification of its subunits, Microbial Pathogenesis 10 (1) (1991) 15–26.

[19] K. D. Arunachalam, K. S. Sathyanarayanan, B. S. Darshan, R. B. Raja, Studies on the characterisation of Biosealant properties of Bacillus sphaericus, International Journal of Engineering Science and Technology 2 (3) (2010) 270–277.

[20] C. I. Noshi, J. J. Schubert, Self-healing biocement and its potential applications in cementing and sand-consolidation jobs: A review targeted at the oil and gas industry, Society of Petroleum Engineers - SPE Liquids-Rich Basins Conference - North America 1 (2018) 1–19.

[21] P. Anbu, C. H. Kang, Y. J. Shin, J. S. So, Formations of calcium carbonate minerals by bacteria and its multiple applications. Springer Plus, 5(1) (2016), 1–26.

[22] K. V. Tittelboom, N. D. Belie, W. D. Muynck, W. Verstraete Use of bacteria to repair cracks in concrete. Cement and Concrete Research, 40(1) (2010), 157–166.(2010).

[23] H. E. Enshasy, D. J. Dailin, R. A. Malek, N. Z. Nordin, H. C. Ramdas, R. Lalloo, Y. Korany, H. C. Eyahmalay, J. Ramchuran, S, J. N. Fitzner, B, Biocement: A novel approach Keat, J. Eyahmalay, S. Ramchuran, J. N. C. Ghong, V. M.

in the restoration of construction materials, Molecules 5 (2) (2020) 177–198.

[24] R. Andalib, M. Z. Majid, M. W. Hussin, M. Ponraj, A. Keyvanfar, J. Mirza, H. S. Lee, Optimum concentration of Bacillus megaterium for strengthening structural concrete, Construction and Building Materials 118 (2016) 180–193.

[25] S. Maheswaran, S. S. Dasuru, A. R. C. Murthy, B. Bhuvaneshwari, R. Kumar, V. Palani, G. S. Iyer, N. R. Krishnamoorthy, S. Sandhya, S, Strength improvement studies using new type wild strain bacillus cereus on cement mortar, Current Science 106 (1) (2014) 50–57.

[26] S. Mar, O. Cabestrero, C. Demergasso, S. Olivares, V. Zetola, S. Mar, An indigenous bacterium with enhanced performance of microbially-induced Ca-carbonate biomineralization under extreme alkaline conditions for concrete and soil-improvement industries, Acta Biomaterialia (2020).

[27] E. F. Haney, M. J. Trimble, R. E. Hancock, Microtiter plate assays to assess antibiofilm activity against bacteria, Nature Protocols 16 (5) (2021) 2615–2632.

[28] P. Anbu, C. H. Kang, Y. J. Shin, Formations of calcium carbonate minerals by bacteria and its multiple applications, SpringerPlus 5 (2016) 250–250.

[29] K. M. N. Wani, B. A. Mir, Application of Bio-Engineering for Marginal Soil Improvement: An Eco-Friendly Ground Improvement Technique, Indian Geotechnical Journal (2022) 1–19.

[30] A. M. Skidmore, C. H. Dickinson, Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi, Transactions of the British Mycological Society 66 (1) (1976) 57–64.

[31] W. B. Christensen, Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types, Journal of bacteriology 52 (4) (1946) 461–466.

[32] P. T. Teo, S. K. Zakaria, N. M. Sharif, A. A. Seman, M. A. A. Taib, J. J. Mohamed, . . Masri, M. N, Application of general full factorial statistical experimental design's approach for the development of sustainable clay-based ceramics incorporated with malaysia's electric arc furnace steel slag waste, Crystals 11 (4) (2021) 442–442.

[33] W. D. Muynck, K. Verbeken, N. D. Belie, W. Verstraete, Influence of urea and calcium dosage on the effectiveness of bacterially induced carbonate precipitation on limestone, Ecological Engineering 36 (2) (2010) 99–111.

[34] A. C. Mitchell, K. Dideriksen, L. H. Spangler, A. B. Cunningham, R. Gerlach, Microbially enhanced carbon capture and storage by mineral-trapping and solubility-trapping, Environ Sci Technol 44 (2010) 5270–5276.

[35] N. Yoshida, E. Higashimura, Y. Saeki, Catalytic biomineralization of fluorescent calcite by the thermophilic bacterium Geobacillus thermoglucosidasius, Appl Environ Microbiol 76 (2010) 7322–7327.

[36] N. Hamdan, E. Kavazanjian, B. E. Rittmann, Sequestration of radionuclides and metal contaminants through microbially induced carbonate precipitation, Pan-Am CGS Geotechnical conference (2011).

[37] V. Achal, X. Pan, D. Zhang, Bioremediation of strontium (Sr) contaminated aquifer quartz sand based on carbonate precipitation induced by Sr resistant Halomonas sp, Chemosphere 89 (6) (2012) 764–768.

[38] H. M. Jonkers, E. Schlangen Development of a bacteria-based self healing concrete. Tailor Made Concrete Structures, 1(2008), 425-430.

[39] V. Wiktor, H. M. Jonkers, Quantification of crack-heal-. ing in novel bacteria-based self-healing concrete Cement Concrete Composites 33(7) (2011), 763-770.

[40] T. Sharma, M. Alazhari, A. Heath, K. Paine, Alkaliphilic Bacillus species show potential application in concrete crack repair by virtue of rapid spore production and germination then extracellular calcite formation, Journal of Applied Microbiology 122 (5) (2017).

[41] B. J. Davis, D. E. Lennard, C. A. Lee, H. F. Tiano, S. G. Morham, W. C. Wetsel, R. Langenbach, Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin 1β, Endocrinology 140 (6) (1999) 2685–2695.

[42] J. Dick, W. D. Windt, B. D. Graef, H. Saveyn, P. V. D. Meeren, N. D. Belie, W. Verstraete, Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species, Biodegradation 17 (4) (2006) 357–367.

[43] C. Rodriguez-Navarro, C. Jimenez-Lopez, A. Rodriguez-Navarro, M. T. Gonzalez-Muñoz, M. Rodriguez-Gallego, Bacterially mediated mineralization of vaterite, Geochimica et Cosmochimica Acta 71 (5) (2007) 1197–1213.

[44] N. K. Dhami, M. S. Reddy, A. Mukherjee, Biomineralization of calcium carbonate polymorphs by the bacterial strains isolated from calcareous sites, Journal of microbiology and biotechnology 23 (5) (2013) 707–714.

[45] N. K. Dhami, M. S. Reddy, A. Mukherjee, Application of calcifying bacteria for remediation of stones and cultural heritages, Frontiers in Microbiology 5 (2014) 304–304.

[46] C. Ercole, P. Cacchio, A. L. Botta, V. Centi, A. Lepidi, Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides, Microscopy and Microanalysis 13 (1) (2007) 42–50.

[47] J. Tourney, B. T. Ngwenya, Bacterial extracellular polymeric substances (EPS) mediate CaCO3 morphology and polymorphism, Chemical Geology 262 (3-4) (2009) 138–146.

[48] H. L. Mobley, R. P. Hausinger, Microbial ureases: significance, regulation, and molecular characterization, Microbiological reviews 53 (1) (1989) 85–108.

[49] V. Achal, A. Mukherjee, P. C. Basu, M. S. Reddy, Strain improvement of Sporosarcina pasteurii for enhanced urease and calcite production, Journal of industrial Microbiology and Biotechnology 36 (7) (2009) 981–988.

[50] W. S. Ng, M. L. Lee, S. L. Hii, An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement, International Journal of Civil and Environmental Engineering 6 (2) (2012) 188-194.

[51] A. A. Qabany, K. Soga, C. Santamarina, Factors affecting efficiency of microbially induced calcite precipitation, J Geotech Geoenviron Eng 138 (2012) 992–1001.

[52] G. D. Okwadha, Li, J, Optimum conditions for microbial carbonate precipitation, Chemosphere 81 (9) (2010) 1143–1148.

[53] L. Cheng, R. Cord-Ruwisch, M. A. Shahin, Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation, Canadian Geotechnical Journal 50 (1) (2013) 81–90.

[54] I. A. Silver, Measurement of pH and ionic composition of pericellular sites, Philosophical Transactions of the Royal Society of London. B 271 (1975) 261–272.

[55] C. M. Gorospe, S. H. Han, S. G. Kim, J. Y. Park, C. H. Kang, J. H. Jeong, J. S. So, Effects of different calcium salts on calcium carbonate crystal formation by Sporosarcina pasteurii KCTC 3558, Biotechnology and bioprocess engineering 18 (5) (2013) 903–908.

[56] R. E. Loewenthal, G. Marais, Carbonate chemistry of aquatic systems: theory and application, Ann Arbor Science, Ann Arbor, 1978.

[57] F. G. Ferris, V. Phoenix, Y. Fujita, R. W. Smith, Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20 ◦C in artificial groundwater, Geochim Cosmochim Acta 67 (2003) 1701–1710.

[58] S. Dupraz, B. Ménez, P. Gouze, R. Leprovost, P. Bénézeth, O. S. Pokrovsky, F. Guyot, Experimental approach of CO2 biomineralization in deep saline aquifers, Chemical Geology 265 (1-2) (2009) 54–62.

[59] H. L. Mobley, M. D. Island, R. P. Hausinger, Molecular biology of microbial ureases, Microbiol Rev 59 (1995) 451–580.

[60] V. Stabnikov, C. Jian, V. Ivanov, Li, Y, Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand, World Journal of Microbiology and Biotechnology 29 (8) (2013) 1453–1460.