DOI: https://doi.org/10.62985/j.huit_ojs.vol24.no5.126

EFFECT OF CULTURE CONDITIONS ON BACTERIA-INDUCED CALCITE PRECIPITATION OF *Bacillus subtilis* QN7 STRAIN

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Received: 7 August 2023; Accepted: 10 November 2023

ABSTRACT

This study was conducted based on the calcium carbonate precipitate from microbially induced calcite precipitation (MICP) to determine the optimal conditions for the highest precipitation. The *Bacillus subtilis* QN7 strain was tested by changing certain parameters, including (1) bacterial density, (2) pH concentration, (3) culture temperature, (4) urea concentration, (5) calcium source, and (6) calcium concentration. From that, the urease activity of this strain was determined. The results showed that the most effective calcium source for calcite precipitation was calcium chloride supplemented with 150-200 mM $CaCl_2$ and 250 mM urea at 40 °C, pH 8; the precipitate formed reached 10.21 g/L and the urease enzyme activity obtained was 703.86 U/L. These initial research results have determined the optimal conditions for the highest calcium precipitation of the *B. subtilis* QN7 strain, which is the premise for further research on potential applications in crack healing, self-healing, and bio-cement production in Vietnam's climatic and environmental conditions.

Keywords: Microbially-induced calcite precipitation (MICP), *Bacillus subtilis* QN7, Self-healing, Crack healing calcium carbonate, Bio-cement.

1. INTRODUCTION

Microbially induced calcium carbonate precipitation (MICP) is a common biologically induced mineralization in nature that plays an important role in some bacteria [1]. During MICP, organisms secrete one or more metabolic products (CO_3^{2-}) that react with ions (Ca^{2+}) in the medium cultures, resulting in the subsequent precipitation of minerals ($CaCO_3$) [2]. Most studies have demonstrated that supplementing MICP into cementitious materials is a cost-effective and eco-friendly method for repairing micro-cracks and for the self-healing process of concrete [3]. Concrete blocks made from cement and other ingredients such as stone, gravel, and sand, after a period of use, will transform in structure. Holes or cracks appear that can reduce the durability of the concrete block. These cracks facilitate the entry of water, gases, salts, acids, and other agents into the concrete's matrix, thus accelerating its degradation, corrosion, and shortening the service life of the structure. Notably, the maintenance of concrete structures significantly impacts community budgets and the environment. Therefore, using MICP's self-healing increases the service life of concrete structures without using costly and/or time-consuming interventions.

This is a continuous process for healing micro-cracks, leading to remarkable improvement in the physico-mechanical properties of bioconcrete [4].

Naturally, bacteria can form calcium carbonate precipitates various such as photosynthesis, urea hydrolysis, sulfate reduction, methane oxidation, through and mechanisms, nitrate reduction [2]. Type is the most widely used method because it is Urea hydrolysis-based easier to operate and known for its high the reaction rate of biogenic urea control, and is hydrolysis is approximately 10^{14} efficiency; times faster than the chemical rate and effectively up to 90% calcite in a short time [5]. Can precipitate generate $CaCO_3$ precipitate in alkaline media produced ammonia leading Bacteria $CaCO_3$ precipitation inside the micro-cracks, thus sealing by biotransforming urea into carbonate and ammonium, with the According and to the research of Álvaro (2018), the process of forming $CaCO_3$ precipitate by microorganisms through urea hydrolysis can be divided into stages: (i) Urea hydrolysis, (ii) Chemical equilibrium: increasing the micro-medium pH, four (iii) Heterogeneous nucleation: calcium formation occurs cell surface, (iv) Successive stratification: nucleation and calcite crystal formation [7]. In the biomineralization process, bacteria serve as nucleation sites through which calcium carbonate precipitates with the bacteria. Therefore, the bacteria must go through a complex metabolic process to generate $CaCO_3$ crystals. This process is greatly influenced by many factors such as pH concentrations, culture temperatures, urea concentrations, calcium sources, calcium concentrations, and the availability of nucleation sites on the bacterial surface [2]. All these parameters greatly affect either the ureolytic activity or $CaCO_3$ crystal formation. The first parameters influence the carbonate ions concentration (CO_3^{2-}), i.e., the saturation state, while the availability of nucleation sites is very important for stable and continuous $CaCO_3$ formation [8]. In the biomineralization process, bacteria serve as nucleation sites, through which $CaCO_3$ precipitates with the bacteria [2]. In recent years, many studies have conducted screening and isolation of $CaCO_3$ precipitation-producing bacteria strains from various sources such as limestone, caves, soils, and seawater, including *Bacillus* spp., *Halomonas eurihalina*, *Helicobacter pylori*, *Kocuria flava CR1*, *Lysinibacilus sphaericus* CH5, *Methylocystis parvum*, *Myxococcus xanthus*, *Proteus mirabilis*, *Pseudomonas denitrificans*, *Sporolactobacillus* spp., and *Sporosarcina ginsengisoli* [9]. Different types of bacteria are able to produce different amounts of urease and $CaCO_3$ precipitate. In fact, the *Bacillus* group is a common bacterium used to produce a high amount of urease and precipitate $CaCO_3$, making it a promising candidate for potential applications in the construction industry [10].

Currently, many studies have demonstrated that parameters related to medium cultures can directly affect the ability to precipitate $CaCO_3$. However, the selection of the optimal medium for high $CaCO_3$ precipitation has not been extensively studied. Therefore, in this paper, we investigated the optimal conditions for the highest precipitation of Bacillus subtilis QN7 strain by changing certain parameters of cultural media. After that, urease enzyme activity was determined to find out the ideal conditions for culturing this strain. This is an effective approach before being put into large-scale research to produce bio-cements for practical application.

2. MATERIALS AND METHODS

2.1. Microorganisms and culture medium

The bacterium used in this study was *Bacillus subtilis* QN7 strain, which was provided by the Department of Applied Microbiology, Institute of Tropical Biology - Vietnam Academy of Science and Technology, isolated from soil and rock samples in Quang Ninh province. This strain was grown in liquid Luria Bertani medium, which included peptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L), and pH 7.5. The medium was sterilized at 121 °C for 20 min and shaken at 120 rpm at 37 °C. The cell density used in this study was 10^8 cells/mL.

Chemicals used included nutrient broth medium (peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7.5), urea-$CaCl_2$ broth (nutrient broth 3 g/L, $NaHCO_3$ 2.12 g/L, NH_4Cl 10 g/L, $CaCl_2.2H_2O$ 20 g/L, urea 20 g/L, pH 8), NaOH, NaClO, hydrochloric acid, ethanol 96%, NH_4Cl, phenol, sodium nitroprusside, and Milli-Q water.

2.2. Investigation of the effects of cultural parameters on the growth and carbonate precipitation capability of the *Bacillus subtilis* QN7 strain

Nutrient Broth (NB) medium was used to investigate the effects of certain parameters of the cultural media on the growth of the *Bacillus subtilis* QN7 strain, while Urea - $CaCl_2$ (UCB) medium was used to investigate the effects of certain parameters of the cultural media on the generation $CaCO_3$ precipitation ability. Firstly, the *Bacillus subtilis* QN7 strain was enriched in NB medium until a density of 10^8 cells/mL was reached. Then, this strain was subcultured on a liquid UCB medium and incubated at 37 °C. After that, the experiment changed certain parameters including (1) incubation time, (2) bacterial density, (3) pH concentration, (4) culture temperature, (5) urea concentration, (6) calcium source, and (7) calcium concentration according to Table 1.

Table 1. These parameters of the medium cultures affected the growth and precipitation of the *Bacillus subtilis* QN7 strain

	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5
Incubation time	18 hours of culture in 14 days				
Bacterial density	10^6 cell/mL	10^7 cell/mL	10^8 cell/mL		
pH concentration	6	7	8	9	10
Temperature	20 ^oC	30 ^oC	40 ^oC	50 ^oC	
Urea concentration	0 mM	250 mM	500 mM	750 mM	1000 mM
Calcium source	Calcium chloride	Calcium acetate	Calcium lactate	Calcium nitrate	
Calcium concentration	50 mM	100 mM	150 mM	200 mM	250 mM

The effect of parameters on growth of bacteria was determined using colony counting (CFU/mL) and spectroscopic methods. The effect of these parameters on the $CaCO_3$ precipitation activity of bacteria was also evaluated using the method of determining the amount of calcium carbonate formed by Wei *et al* (2015) as follows [11]:

Bacillus subtilis QN7 bacteria were cultured in UCB medium and incubated at 37 °C for 24 hours under shaking conditions (200 rpm) until a concentration of around 108 cells/mL. After that, the whole culture was inoculated into a 50 mL medium which contained different independent variables (see Table 1) and incubated at room temperature for 14 days. The control consisted of an uninoculated liquid calcium carbonate precipitation medium.

The suspension after 14 was centrifuged at 4 °C, 13,000 rpm for 10 minutes. The pellet, which included calcium carbonate precipitate and bacterial cells, was resuspended in 50 mL of TE buffer (10 mM Tris, 1 mM EDTA pH 8.5). The cell membrane was Lysozyme disrupted with a Sonicator and was added to the cell suspension at a final concentration of 1 mg/mL and incubated at 37°C for 1 hour to hydrolyze the bacterial cell wall. The remaining precipitate was washed with sterile distilled water, then filtered with Whatman filter paper No.2, and dried at 70 °C until the mass was constant. The obtained precipitate was weighed and determined its crystal was shape using SEM analysis technique.

2.3. Determination of urease enzyme activity of *Bacillus subtilis* QN7 strain

To determine the effects of ureolysis and the associated pH change on crystal formation, the activity of the urease enzyme was determined by monitoring the amount of ammonia liberated from urea by the phenol-hypochlorite method [12]. Firstly, bacterial cells were obtained by centrifugation at 6,000 rpm for 10 min at 4 °C, washed twice with piperazine-1-ethanesulfonic acid 50 mM 4-(2-hydroxyethyl) (HEPES, pH 7.5; H3375-25 g). The cells were disrupted at 4 °C for 20 min, then vortexing every 5 mins. The enzyme solution was obtained by centrifuging at 6,000 rpm for 10 min at 4 °C.

An enzymatic catalyzed reaction occurred when 500 µL of culture supernatants were added to 0.5 mL of urease buffer (50 mM HEPES, 25 mM urea) and incubated at 37 °C for 20 minutes. 1.5 mL of Solution A (10 g/L phenol, 50 mg/L sodium nitroprusside) and 1.5 mL of Solution B (5 mg/mL NaOH, 0.044% v/v NaClO) were added to the mixtures and incubated at 37 °C for 30 minutes. The components of the blank sample had all the mixture compounds, except the culture supernatant was replaced by urease buffer. Then, the optical density at 600 nm was measured. The ammonium chloride standard curve was used to determine the amount of ammonia formed in the solution-reaction. The experiments were performed in triplicates.

Urease enzyme activity is determined by the amount of urea used (corresponding to the amount of ammonia formed after the enzymatic reaction). It is the quantity of urease enzyme responsible for producing 1 µmole min^{-1} of NH^{4+} under the experimental conditions (incubation for 10 minutes at 30 °C). The ammonia standard calibration curve was made by preparing serial dilutions of a stock solution of ammonium chloride.

$$1U = 1\ \mu mol\ substrate\ (10^{-6}\ mol)/min.$$

2.4. Statistical methods and data processing

Experiments were carried out with triplicates. The data was recorded and processed using Microsoft Excel 2010 software. Statistical methods, ANOVA analysis of variance, were performed using Minitab v.16 software. Data are presented as mean ± standard deviation with p-value < 0.05.

3. RESULTS AND DISCUSSION

3.1. The effect of incubation time on the growth of *Bacillus subtilis* QN7 strain

Microbial growth was measured continuously for 18 hours of culture. The growth process of the *Bacillus subtilis* QN7 strain was measured and presented as a growth curve, as shown in Figure 1.

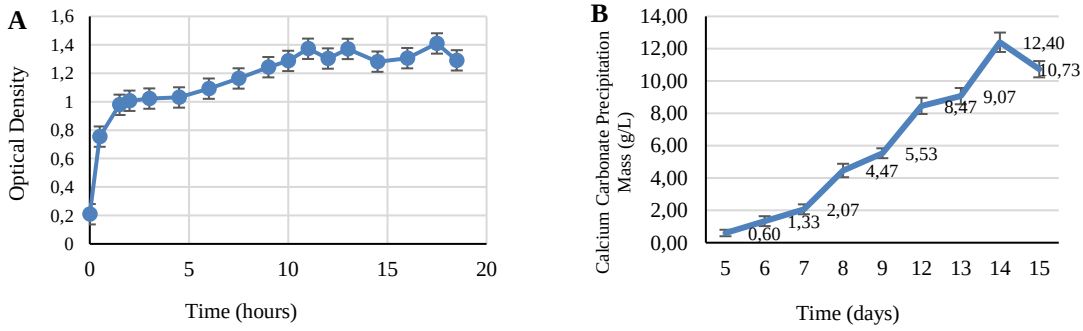

Figure 1. The growth curve of the *Bacillus subtilis* QN7 strain (A) in 18 hours of culture; (B) 14 days of cultures

Figure 1A showed that the *B. subtilis* QN7 strain had a growth phase (log phase) lasting up to 11 hours. At this time, the bacteria entered the stable phase. Thus, this strain of bacteria grew

to the optimal concentration at 11 hours. However, to facilitate the investigation and arrangement of survey experiments for the bacterial strain, the selected time point for the next inoculations was from 16 hours to 18 hours (at this time, the bacteria were still in the stability phase). Bacteria started to generate precipitate calcium carbonate on day 5 (Figure 1B) with the amount of precipitate obtained as 0.6 g/L. From the 7th to the 12th day, the precipitation obtained ranged from 2.07 to 8.47 g/L calcium carbonate, which quadrupled and peaked at 14 days of culture with the precipitate weight of 12.4 g/L. Thus, the 14th day was selected to obtain the precipitate and determine the ability to create precipitate in the next experiments.

3.2. The effect of bacterial density, pH, temperature, and urea concentration on the growth and generation of calcium carbonate precipitate of *Bacillus subtilis* QN7 strain

The activity of urease and the amount of $CaCO_3$ precipitation are based on several environmental factors, including the input bacterial density (10^6, 10^7, 10^8); pH (6, 7, 8, 9, 10); temperature (20 °C, 30 °C, 40 °C, 50 °C) and urea concentration (0 mM, 250 mM, 500 mM, 750 mM, 1000 mM), on the growth and calcium carbonate precipitate ability of *Bacillus subtilis* QN7. The results were shown in Figure 2.

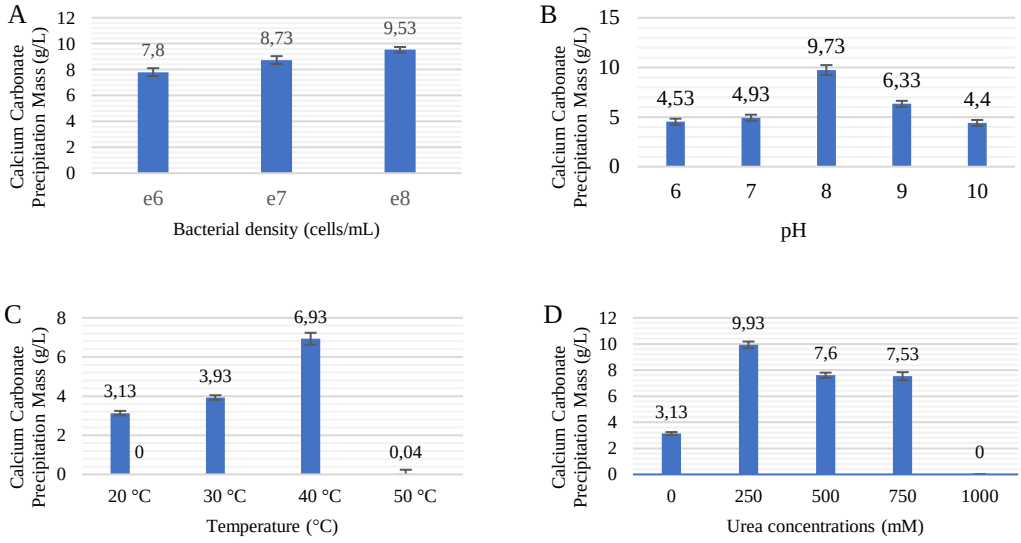

Figure 2. Effects of some culture parameters on the growth and generation of calcium carbonate precipitate of *Bacillus subtilis* QN7 strain: A) Bacterial density; B) Ambient pH; C) Temperature; and D) Urea

The results in Figure 2A showed that when the input bacterial density reached 10^8 cells/mL, the bacteria would develop for a high calcium carbonate yield of 9.53 g/L. At concentrations of 10^6 and 10^7 cells/mL, calcium carbonate yields were lower, lower 7.8 g/L and 8.73 g/L, respectively. Therefore, the input bacterial density of $\leq 10^8$ cells/mL was used to conduct the survey in the next experiments.

In Figure 2B, this bacterium can grow and generate calcium carbonate precipitates at all investigated pH ranges. High alkaline pH conditions (pH 10) lead to reduced precipitation, proving that the pH affects the activity of enzymes involved in the precipitation of the bacterial strain, as the urease enzyme is only active at pH values specific for urea hydrolysis. According to Stabnikov *et al.* (2013), both Halophiles and Alkaliphiles urease-producing bacteria are active at high concentrations of inorganic salts and at pH above 8.5 [13]. However, most microorganisms cannot survive in a medium with a pH value greater than 10 [14]. Thus, the

pH parameters have a significant effect on the $CaCO_3$ precipitation ability of the *B. subtilis* QN7 strain, with pH 8 obtaining a higher precipitate than the precipitate at the remaining pH. This may explain the inhibition of growth by excess of substrate (urea) or catabolic repression or increase of pH in the microenvironment of the cell.

In Figure 2C, the ambient temperatures of 20 °C and 30 °C have the lowest precipitation (3.13 g/L and 3.93 g/L, respectively). The *B. subtilis* QN7 strain generates a high $CaCO_3$ precipitate at 40 °C with a precipitated mass of 6.93 g/L. During 24 hours of culture, the bacteria produced a negligible amount of precipitate at 50 °C. Therefore, in this investigation, 35 °C is the optimal condition for enzyme activity.

Besides, urea concentration is an important parameter affecting the precipitation process, especially for bacterial strains that precipitate $CaCO_3$ by urea hydrolysis, such as *B. subtilis* QN7 strain. Experiments were conducted to investigate the concentration of urea in the medium cultures, changing the range from 0 mM to 250 mM, 500 mM, 750 mM, and 1000 mM, as shown in Figure 2D. According to the results, it was found that when urea is added, the precipitate volume increases sharply. The highest amount of $CaCO_3$ obtained at 250 mM urea was 9.93 g/L. There was no precipitation at 1000 mM urea concentration, which indicates that too high a concentration of urea will inhibit the ability of the bacterial strain to generate the calcium carbonate precipitate. Thus, the urea concentration of 250 mM (15 g/L) will be selected as the appropriate concentration for this strain to induce $CaCO_3$ precipitation.

3.3. The effect of calcium sources and concentrations on the growth and generation of calcium carbonate precipitate of *Bacillus subtilis* QN7 strain

Providing calcium sources and concentrations plays a key role as a substrate for calcium carbonate generation. The addition of different calcium sources to the culture medium can stimulate bacterial precipitation, as shown in *Figure 3.*

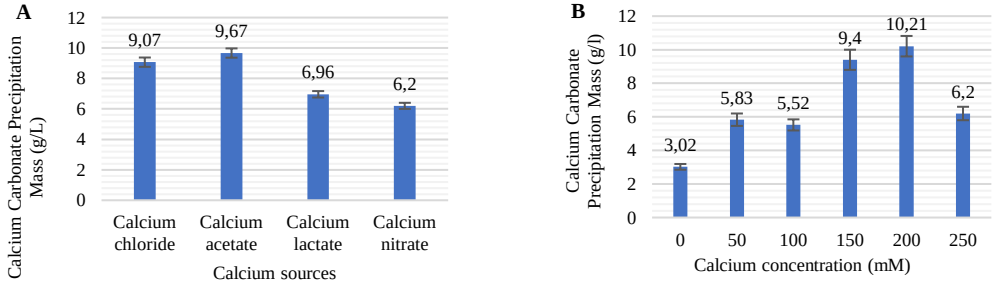

Figure 3. Effect of different calcium sources (A) and calcium concentrations (B) on growth and calcium carbonate precipitate of *B. subtilis* QN7 strain

Figure 3A shows that the bacterial strain can grow in different calcium sources, with the highest precipitate being from calcium acetate source at 9.67 g/L. According to the results of ANOVA analysis, the bacteria supplemented with calcium acetate source did not differ from the bacteria supplemented with calcium chloride. However, calcium chloride is cheaper than calcium acetate, so calcium chloride will be selected as the calcium source to supplement the precipitation of *B. subtilis* QN7. For calcium concentrations in Figure 3B, the amount of precipitation between the groups is relatively uniform. For the group that did not add calcium, the precipitate was quite small (3.02 g/L). The amount of precipitate obtained was quite high at a calcium concentration of 150 mM (9.40 g/L), and there was no difference with a concentration of 200 mM (10.21 g/L). Therefore, the concentration of calcium in the range of 150 - 200 mM will be selected as the optimal condition for the *B. subtilis* QN7 strain to induce $CaCO_3$ precipitation. Similar results were reported for *Oceanobacillus* sp., isolated from soil

and rock samples in Kien Giang province [15], Their result showed that $CaCl_2$ was the best Ca^{2+} source for calcite precipitation, the oder environmental factors of 50 g/L $CaCl_2$ was suitable condition for calcite precipitation with the highest $CaCO_3$ concentration of 52,05 g/L determined in the production medium.

3.4. SEM analysis and determination of urease enzyme activity of the *Bacillus subtilis* QN7 strain

To determine if the observed crystals were indeed the desired mineral calcium carbonate, SEM analysis was used to visualize bio-cementation or bio-precipitation on inorganic soil due to MICP. A sample of the crystals was subjected to scanning electron microscopy (SEM), and the amount of ammonia in the enzyme solution was determined based on the ammonium chloride standard curve.

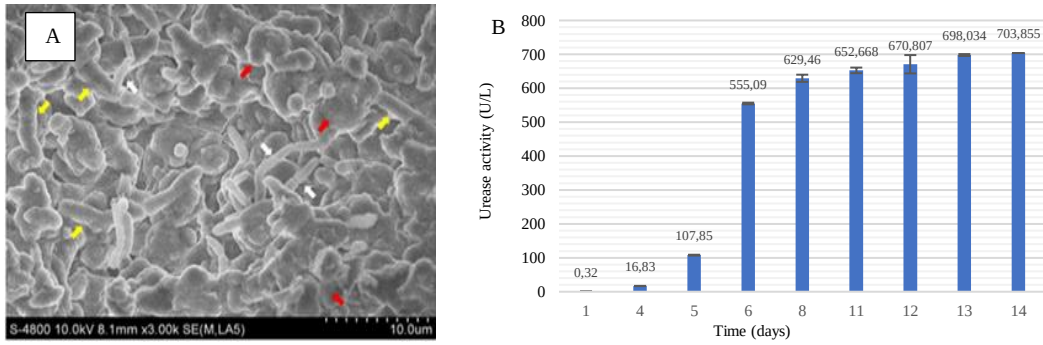

Figure 4. SEM imaging of the calcite carbonate precipitate obtained from strain *B. subtilis* QN7 after 14 days of culture at 3,000x magnification (A) and the amount of urease enzyme obtained after 14 days of culture of B. subtilis QN7 strain (B)

SEM imaging in Figure 4A revealed that *B. subtilis* QN7 bacterial cells were rod-shaped, 0.8 - 1.0 µm × 2.5 - 3.5 µm, existing individually (white arrow). Some cells showed calcium carbonate precipitate formation around the surface (yellow arrow). Furthermore, the amount of precipitate accumulated at the cell surface was increasing and forming clusters of mineral crystals (red arrow). This proved that the *B. subtilis* QN7 strain could generate calcium carbonate precipitates on the surface of concrete mortar and had the potential to be applied to bio-cement production for construction works.

The urease enzyme activity of the *B. subtilis* QN7 strain was shown in *Figure 4B,* tending from day 5 to rapidly day to start to increase 6. By day 14, the urease enzyme activity was obtained at 703.86 U/L, corresponding to the catalytic ability to convert 703.86 µmol of substrate to 1 liter after 1 minute at standard conditions. Similar results were reported for the urease activity was used to evaluate the effective of MICP [16]. Finally, optimal conditions for this strain culture were determined, including cell density (10^8 cells/mL), pH 8, temperature (40 ^oC), and urea concentration (250 mM). A calcium chloride source with a concentration of 150 - 200 mM was used to determine the urease enzyme of the bacterial strain in 14 days of culture.

4. CONCLUSION

The study optimized culture conditions to obtain a high amount of calcium carbonate precipitate from the *Bacillus subtilis* QN7 strain. The individual single-parameters for determination included bacterial density, pH concentration, culture temperature, urea concentration, calcium source, and calcium concentration. The results showed that calcium chloride was the most effective calcium source under the conditions of pH 8, 150 - 200 mM $CaCl_2$, and 250 mM urea. Culture temperature at 40 °C showed the highest precipitation with a

mass of 10.21 g/L. Additionally, the article determined that the obtained urease enzyme activity reached 703.86 U/L, corresponding to the catalytic ability to convert 703.86 µmol of substrate to 1 liter after 1 minute under standard conditions. These results initially contribute to a fuller understanding of the potential for research applications of *B. subtilis* QN7 strain in Vietnam's climate and environmental conditions; this is the premise for further studies in developing a large-scale production process to create environmentally friendly bio-cement products.

Acknowledgments: This research is funded by the National Science and Technology Project (Project Number: ĐTĐL.CN-116/21).

REFERENCES

1. Liu Y., Ali A., Su J.F., Li K., Hu R.Z., Wang Z. - Microbial-induced calcium carbonate precipitation: Influencing factors, nucleation pathways, and application in wastewater remediation. The Science of the Total Environment **860** (2023) 160439. https://doi.org/10.1016/j.scitotenv.2022.160439.
2. Anbu P., Kang C. H., Shin, Y. J., So J. S. - Formations of calcium carbonate minerals by bacteria and its multiple applications. SpringerPlus **5** (250) (2016). https://doi.org/10.1186/s40064-016-1869-2.
3. Tang Y., Xu J. - Application of microbial precipitation in self-healing concrete: A review on the protection strategies for bacteria. Construction and Building Materials **306** (2021) 124950. https://doi.org/10.1016/j.conbuildmat.2021.124950.
4. Pungrasmi W., Intarasoontron J., Jongvivatsakul P., Likitlersuang S. - Evaluation of microencapsulation techniques for MICP bacterial spores applied in self-healing concrete. Scientific Reports **9** (12484) (2019). https://doi.org/10.1038/s41598-019-49002-6.
5. Xu J., Wang X., Yao W. - Coupled effects of carbonation and bio-deposition in concrete surface treatment. Cement and Concrete Composites **104** (2019) 103358. https://doi.org/10.1016/j.cemconcomp.2019.103358.
6. Nasser A. A., Sorour N. M., Saafan M. A., Abbas R. N. - Microbially-Induced-Calcite-Precipitation (MICP): A biotechnological approach to enhance the durability of concrete using *Bacillus pasteurii* and *Bacillus sphaericus*. Heliyon **8** (7) (2022) E09879. https://doi.org/10.1016/j.heliyon.2022.e09879.
7. Torres-Aravena A. E., Duarte-Nass C., Azócar L., Mella-Herrera R., Rivas M., Jeison D. - Can microbially induced calcite precipitation (MICP) through a ureolytic pathway be successfully applied for removing heavy metals from wastewaters? *Crystals* **8 (11)** (2018) 438. https://doi.org/10.3390/cryst8110438.
8. Phillips A. J., Gerlach R., Lauchnor E., Mitchell A. C., Cunningham A. B., Spangler L. - Engineered applications of ureolytic biomineralization: a review. Biofouling **29** (6) (2013) 715-733. https://doi.org/10.1080/08927014.2013.796550.
9. Ferris F. G., Phoenix V., Fujita Y., Smith R.W. - Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20°C in Artificial groundwater. Geochimica et Cosmochimica Acta **68** (8) (2004) 1701-1710. https://doi.org/10.1016/S0016-7037(03)00503-9.
10. Tepe M., Arslan Ş., Koralay T., Doğan N. M. - Precipitation and characterization of $CaCO_3$ of *Bacillus amyloliquefaciens* U17 strain producing urease and carbonic anhydrase. Turkish Journal of Biology **43** (3) (2019) 198-208. https://doi.org/10.3906/biy-1901-56.
11. Wei S., Cui H., Jiang Z., Liu H., He H., Fang N. - Biomineralization processes of calcite induced by bacteria isolated from marine sediments. Brazilian Journal of Microbiology **46** (2) (2015) 455-464. https://doi.org/10.1590/S1517-838246220140533.

12. Bibi S., Oualha M., Ashfaq M. Y., Suleiman M. T., Zouari N. - Isolation, differentiation and biodiversity of ureolytic bacteria of Qatari soil and their potential in microbially induced calcite precipitation (MICP) for soil stabilization. RSC Advances **8** (2018) 5854-5863. https://doi.org/10.1039/C7RA12758H.
13. 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 Journal of Microbiology and Biotechnology **29** (8) (2013) 1453-1460. https://doi.org/10.1007/s11274-013-1309-1
14. Shanmugam R., Gowsalya M., Gowshalya P., Ramathi J., Bhavithra R. - Optimization of fault in structure by using *Bacillus subtilis*. Materials Today: Proceedings **68** (5) (2022) 1408-1414. https://doi.org/10.1016/j.matpr.2022.06.463.
15. Pham Anh Vu,Vu Thi Tuyet Nhung, Nguyen Hoang Dung, Tran Trung Kien, Le Quynh Loan, Huynh Thi Diep, Tran Thi My Ngoc, Le Tan Hung - Investigate influence of some culture conditions of *Oceanobacillus* sp. on calcite precipitation and concrete binding ability. Journal of Science Technology & Food - Ho Chi Minh City University of Food Industry **21** (2) (2021) 89-99.
16. Ibtisam A.H., Fatma N.T., Ahmed E.Z. - Urease activity and induction of calcium carbonate precipitation by *Sporosarcina pasteuri* NCIMB 8841. Journal of Applied Sciences Research **9** (3) (2013) 1525-1533.

TÓM TẮT

ĐÁNH GIÁ ẢNH HƯỞNG CỦA MỘT SỐ ĐIỀU KIỆN NUÔI CẤY ĐẾN SỰ HÌNH THÀNH KẾT TỦA CALCIUM CARBONATE CỦA CHỦNG VI KHUẨN *Bacillus subtilis* QN7

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Nghiên cứu này nhằm thu lượng kết tủa calcium carbonate cao nhất dựa trên hoạt động của vi khuẩn cảm ứng sự hình thành kết tủa canxit (MICP). Cụ thể, để xác định điều kiện tối ưu nhằm thu lượng kết tủa cao nhất của chủng *Bacillus subtilis* QN7, thí nghiệm tiến hành thay đổi một số thông số bao gồm (1) mật độ vi khuẩn, (2) độ pH, (3) nhiệt độ nuôi cấy, (4) nồng độ urea, (5) nguồn calcium và (6) nồng độ calcium, từ đó xác định hoạt tính urease của chủng này. Kết quả cho thấy nguồn calcium hiệu quả nhất thu hàm lượng kết tủa cao là calcium clorua bổ sung 150 - 200 mM $CaCl_2$ và 250 mM urea ở 40 ^oC, pH 8, hàm lượng kết tủa đạt 10,21 g/L, hoạt độ enzyme urease thu được là 703,86 U/L. Kết quả nghiên cứu bước đầu đã xác định được điều kiện tối ưu để chủng *B. subtilis* QN7 tạo kết tủa $CaCO_3$ tốt nhất. Đây là tiền đề cho các nghiên cứu tiếp theo về tiềm năng ứng dụng chủng *B. subtilis* QN7 trong việc hàn gắn vết nứt, khả năng tự phục hồi và sản xuất xi măng sinh học trong điều kiện khí hậu và môi trường Việt Nam.

Keywords: Kết tủa calcite do vi sinh vật (MICP), *Bacillus subtilis* QN7, tự liền, chữa vết nứt canxi carbonate, xi măng sinh học.