# DEVELOPING THE BIO-GROUT OF REPAIRING CRACKS IN CONCRETE BASED ON MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION USING *Sporosarcina pasteurii*

**Nguyen Pham Huong Huyen[1*], Nguyen Ngoc Tri Huynh[2], Nguyen Khanh Son[2], Nguyen Thi Nam Phuong[1], Pham Minh Tuan[1]**

[1]*Ho Chi Minh City University of Food Industry*

[2]*Ho Chi Minh City University of Technology, VNU-HCM*

*Email: *nphhuyen@gmail.com*

Received: 11 May 2021; Accepted: 21 July 2021

## ABSTRACT

Recently, there is more research on applying microbiologically induced calcium carbonate precipitation (MICP) to repair the concrete cracks instead of synthetic polymers, which may be harmful to the environment. Ureolytic bacteria, such as *Sporosarcina pasteurii*, have been known to release urease, leading to calcium carbonate precipitation in a calcium-rich environment. Based on this mechanism, the different ratios of building materials and *S. pasteurii* biomass were investigated to obtain the optimized grout to solve the cement cracking problem. Firstly, calcium precipitation condition was optimized at the initial pH of 6 and the concentration of CaCl2 as 250 mM. The optimum bio-grout mixture was obtained with 0.1 mm sand mixed with biomass in ratio of 1:1 with the bacterial density of around 108 CFU/mL. The visual observation and water permeability tests showed that bacterial mortar could repair the cracks and prevent the invasion of liquids and gases compared with the negative control. Besides, SEM and XRD analytical results indicated that the different carbonate polymorphs were formed inside the cracks. Additionally, the plate count method revealed that there were vegetative *S. pasteurii* cells in cracks even after 28 days, which means the bacterial mortar may have a long-lasting effect on the cement.

*Keywords*: MICP, *Sporosarcina pasteurii*, self-healing, calcium carbonate.

## 1. INTRODUCTION

Existing aged concrete shows distress and crack due to mechanical load or environmental effect. This damaged concrete was not designed in the first place to have self-healing capability. Although the commercially available chemical grouts such as cementitious, epoxy, and furan grouts provide a good mechanical property and bonding strength, the adverse environmental effects generated from these materials' production are still in concern [1]. Therefore, it is a demand for ecologically friendly repair materials as MICP based self-healing concrete [2]. Researchers have recently tried to apply MICP and improve the application methods, such as immobilization of bacteria in silica gel [3], diatomite [4] or using high urease producing bacteria, for example: *Bacillus sphaericus* [5], *Bacillus megaterium* [6].

MICP by urea hydrolysis has been used widely because ureolytic bacteria are widespread in the environment and the rate and quantity of the carbonate precipitated can be controlled. In this process, urea is hydrolyzed into ammonium and carbonate under the activity of urease. Then, it gives rise to a pH increase, which shifts the bicarbonate equilibrium, resulting in the

formation of carbonate ions. Urease based MICP has been used for various applications, such as sand consolidation [6], surface treatment of limestone, self-healing concrete [7, 8], bio-bricks [9]. Although precipitating CaCO3 bacteria proved their efficiency, there are many hindering factors such as the survival of bacteria under the highly alkaline pH of concrete, impractical application of soaking cracked cement in bacterial solution. Therefore, to apply the microbial precipitation of calcium carbonate to reality effectively, the type of involved bacteria and the environmental factors have to be considered carefully. Firstly, choosing a bacterial strain that can produce high active urease and act as nucleation sites to initiate precipitation is critical. Besides, among the environmental factors, pH is one of the main factors leading the precipitation; thus, it should be monitored strictly throughout the process. Furthermore, calcium concentration is also an essential variable in the MICP since a certain amount of positively charged calcium ions must interact with the negatively charged bacterial cells [10, 11].

Therefore, in this research, the effect of the pH, the concentration of calcium ions, and nucleation sites' presence to MICP were investigated before crack repairing application. Besides, *Sporosarcina pasteurii* was chosen for investigation because this strain showed a high urease activity and could precipitate a high amount of calcium carbonate [12]. In the previous studies, *S. pasteurii* was investigated mainly for self-healing effects such as mixing directly with cement components to improve strength and durability of concrete [13] or immobilized in polyurethane before mixing to protect from adverse environmental changes [14]. Until now, *S. pasteurii* has not been applied as a bio-grout in the crack closing method which was applied to the existing concrete cracks in the early stage without immersing in carbonate precipitation medium. For this investigation, the bio-grout, the bacteria-based repair mixture, consists of two types of solutions: A, which is composed of bacteria, nutrients, calcium chloride and B containing urea, a main substrate to produce urease enzyme which induces pH increase. Solution A was injected into the crack, then solution B. The crack was formed by three-point bending loading. A water permeability test was conducted to measure the healing efficiency before and after injection. It is necessary to investigate the biomineralization process in the crack to determine the healing agent's effectiveness. This method is more accessible and more practical than previous studies to be commercialized shortly. The bio-grout components are optimized with different sand sizes, ratios of sand, and biomass. The time effect was also investigated to evaluate the ability to repair the cracks of concrete specimens. At the end of the healing period, polished sections of injected specimens are observed with SEM/EDS to analyze and locate bio-minerals.

## 2. MATERIALS AND METHODS

### 2.1. Bacteria and culture medium

The bacterium used in this study was *Sporosarcina pasteurii* DSM-33 from German Collection of Microorganisms and Cell Cultures (DSMZ). *S. pasteurii* were grown in Tryptone Soya medium containing (per Liter) 20 g urea, 15 g peptone from casein, 5 g peptone from soymeal, 5 g NaCl at 30 °C for 24h. The bacteria cells in vegetative form with a concentration of around 108 cells/mL determined by plate count and spectroscopic methods, were extracted by centrifugation at 5000 rpm for 15 minutes. *S. pasteurii* was further tested for the urease activity. This was determined in the media according to the phenol hypochlorite assay method [15].

### 2.2. Investigation of the pH and substrate concentration effects to the carbonate precipitation capability of *S. pasteurii*

Different values of pH and the concentration of calcium chloride as the main substrate were investigated to evaluate the potential to induce calcium carbonate precipitation in

different environments. pH ranged from 6 to 10 at the calcium chloride concentration of 100 mM. Calcium chloride concentration was examined from 25 mM to 500 mM at the optimum pH obtained from the previous investigation. The carbonate precipitation capability was determined by using calcium carbonate precipitation (CCP) media containing (per Liter) 20 g urea, 2.12 g NaHCO3, 10 g NH4Cl, 11.1 g CaCl2, 3 g nutrient broth, with the investigating ranges of pH and CaCl2 concentration (modified from [16]). The plates were then incubated at 120 rpm, 30 °C for 7 days.

Then, the culture was centrifuged at 5000 rpm for 15 min. After removing the supernatant, the pellet including precipitated calcium carbonate and bacterial cells were placed in a buffer of TE 50 mL (10mM Tris, 1mM EDTA pH 8.5). Then, lysozyme was added at a concentration of 1 mg/mL and incubated at 37 °C for 1 hour to hydrolyze the bacterial cell wall. The rest of the bacterial cell was removed by centrifugation. The precipitate was washed with sterile distilled water, then dried at 37 °C until the mass was constant. The obtained precipitate was weighed and determined its crystal shape by SEM analysis technique.

### 2.3. Concrete samples and crack preparation

For all the investigations, concrete samples were made by using ordinary Ha Tien cement, white sand and tap water. The building material sand, cement and water were weighed according to the ratio: 3:1:0.5 by weight, then molded to the artificial concrete samples size 4 cm × 4 cm × 16 cm [4].

Closely realistic cracks were obtained by performing three-point bending loading. After annealing, the concrete sample was put in a cement bending compressor (Matest–E183N) to create cracks with strength of 5,530 - 7,382 MPa.

### 2.4. Investigation of bio-grout components

In this research, sand size, biomass percentage, and curing time were chosen to investigate the effect of bacterial mortar to healing the crack and preventing water permeability. White sand was selected in different sizes of 0.1 mm, 0.3 mm, and 0.7 mm and mixed with biomass in ratio of 1:1 (w/w), the negative control is the cracked concrete without any treatment. The biomass of *S. pasteurii* in the mixture ranged from 0 to 100%. Concrete samples were cured for 7, 14, 21 and 28 days.

To investigate the crack repair effectiveness, the bio-grout, the bacteria-based repair mixture consists of two types of solutions namely A which is composed of bacteria, optimum CCP media without urea and B containing urea, a main substrate to promote massive calcium carbonate precipitation. Solution A was injected into the artificial concrete crack, then solution B. The total volume of 2 solutions were 2 mL for each sample. During the investigation, all the samples were fixed with tape around the crack to avoid the loss of bio-grout to the outside environment. To determine the effectiveness of the healing agent, it was necessary to investigate bio-mineralization process taking place in the crack by water permeability test, the viability of *S. pasteurii* bacteria and SEM/EDS technique.

### 2.5. Water permeability

The efficiency of the different bio-grout components was investigated by measuring the water permeability rate of the cracked concrete specimens. The used test method is a modified version of the low-pressure water permeability test described by [3]. Concrete samples were placed into the water permeability determination system which is illustrated in Figure 1. Water pressure was generated by adding 500 mL of water to the top of the samples, and by following

the descent of the water column in time, rate of water permeability could be determined by the equation below. The system is closed to prevent water evaporation to the outside environment.

$$WPR = \frac{V}{t}$$

WPR: Water permeability rate (mL/min)

V: Water volume (mL)

t: Time for water volume passing from part 1 to part 2 of the model (mins)

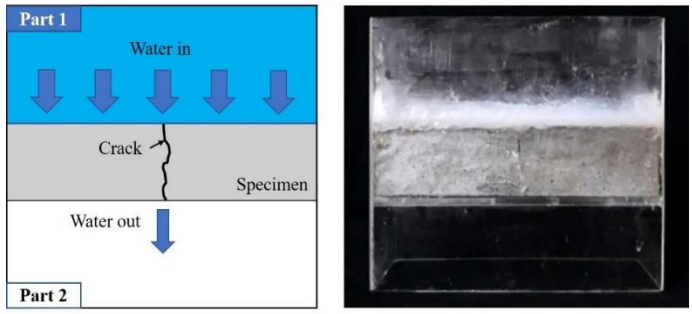



*Fig. 1.* Water permeability test setup.

### 2.6. Evaluating the viability of *S. pasteurii* bacteria on concrete samples

The bio-grout of 7, 14, 21 and 28-day cured bacteria-containing geopolymer specimens were used to investigate the viability of the high pH enduring bacteria, i.e. *S. pasteurii*, by the plate count method. 0.1 gram of bio-grout was added into 10 mL of sterile saline (8.5 g/L NaCl) in a falcon tube. Ten-fold serial dilution (three dilutions in total) was made from this solution. After that, 100 µL suspension was taken from 10-2 and 10-3 dilution and was spread onto Tryptone Soya Agar homogeneously. All agar plates were incubated upside down at static condition at 30 ℃. Colonies on the agar plates were examined after 24h-48h. All of data treatments were done with Excel software.

### 2.7. Characterization of carbonate crystals

Morphology of calcium carbonate precipitation was observed by scanning electron microscopy (SEM Hitachi S-450). The collected carbonate crystals were mounted directly into the SEM stubs and sputter-coated with a platinum mixture (Hitachi HUS-5GB coating unit). Scanning was performed by JSM-IT200 Jeol (Tokyo, Japan) under the condition of accelerating voltage at 25 kV.

X-ray diffraction (XRD) was used to determine the mineralogy of calcium carbonate precipitation induced by *S. pasteurii*. The collected dry bio-grout after 28 days of incubation was analyzed using Bruker D8 Advance (US).

## 3. RESULTS AND DISCUSSION

### 3.1. Effects of pH and calcium chloride concentration to the precipitation

In this research, *S. pasteurii* DMS-33 shows high urease activity 450.40 ± 8.6 U/mL after 48h, which was proved to induce the carbonate precipitation. This result is similar to urease activity of this strain investigated at 48 hours by Chahal *et al*. [13]. This parameter is important in determining the carbonate precipitation ability under urea hydrolysis mechanism.

The results of investigating pH and calcium concentration effects to precipitated carbonate mass are illustrated in Figure 2 and Figure 3. Calcium carbonate precipitation occurs when calcium ion is combined with either carbonate or bicarbonate ions. To understand the effect of environmental factors, patterns of CaCO3 precipitation under chemical induction in the absence of microorganisms were also examined with changes in pH in CCP media. The total carbonate precipitates increased with the increase of pH values from 6 to 10. However, compared with the chemically induced samples (without bacteria inoculation) at each pH, biologically induced carbonate precipitates decreased when initial pH was above 6 and in balance with chemically induced mass at pH was 8. Although *S. pasteurii* DMS-33 was investigated to show that it could endure and grow well under high pH above 8 (data not shown), when the initial pH was more than 6, there was premature $CaCO_3$ precipitation caused by chemically induction process under high pH. It is important to prevent premature precipitation because calcium is required to be present in positively charged ions to interact with the negatively charged bacterial cells to connect the bio-grout material during the incubation. On the other side, when pH is low (less than 6), the total mass of precipitated $CaCO_3$ was decreased. It can be explained that under low pH, urea was not degraded bio-chemically fast enough to increase pH, induce carbonate precipitation. In general, *S. pasteurii* should be firstly grown in a high pH medium to obtain high concentration of cells which could endure high pH. Then the biomass was inoculated in a precipitation medium, urea is used as a substrate for calcium metabolism under the effect of the urease enzyme. Therefore, the precipitation medium's initial pH was set to be 6 for all the following investigations.

Different concentrations of calcium chloride were investigated, and the highest mass of carbonate precipitate was obtained at 250 mM. When calcium chloride concentration varied from 25 to 250 mM, the total mass of precipitated carbonate changed significantly. However, when the calcium chloride concentration increased above 250 mM, the total mass seems to be stable; it can be inferred that higher calcium chloride concentration does not mean the higher precipitated carbonate mass. These investigated results of pH and carbonate concentration are following the suggestion of [17] about a typical carbonate precipitation environment which comprises high extracellular calcium concentration (compared to intracellular) and low extracellular compared to intracellular proton concentrations (as a result of alkaline pH regimes).

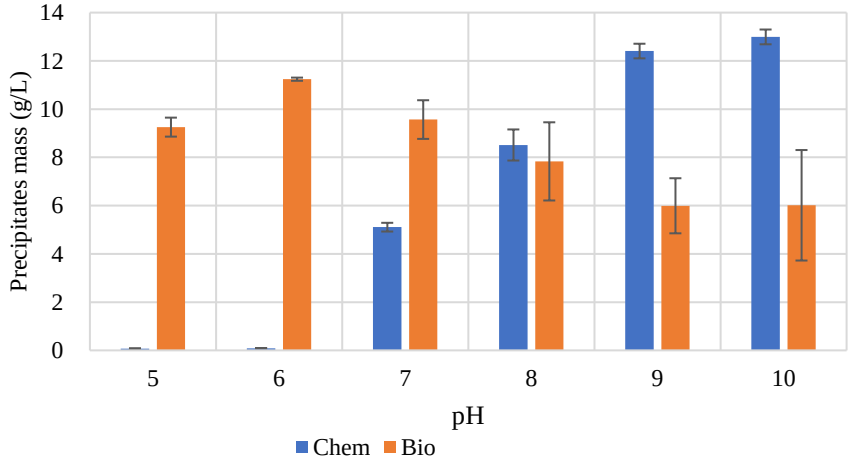



*Fig. 2.* The change of precipitated carbonate under the effect of pH.
Chem: chemically induced carbonate precipitation,
Bio: biologically induced carbonate precipitation.

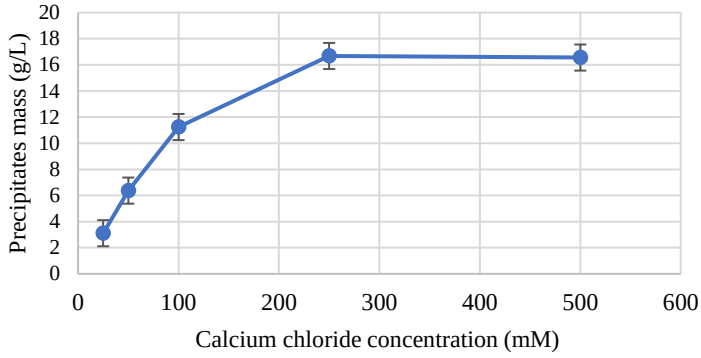



*Fig. 3.* The change of precipitated carbonate under the effect of calcium chloride concentration.

### 3.2. Bio-grout mixture proportions

According to Figure 4, after 14 days of incubation, the water permeability rate decreased with the decrease of sand size and the highest reduction of more than 93% was obtained when 0.1 mm sand was used. This result shows that bio-grout helped to improve the water-resistance of the crack. Therefore, the 0.1 mm sand was chosen for the following experiments. It is noted that the larger sand grains may cause weak bonding via low-strength bio-cementation under MICP.

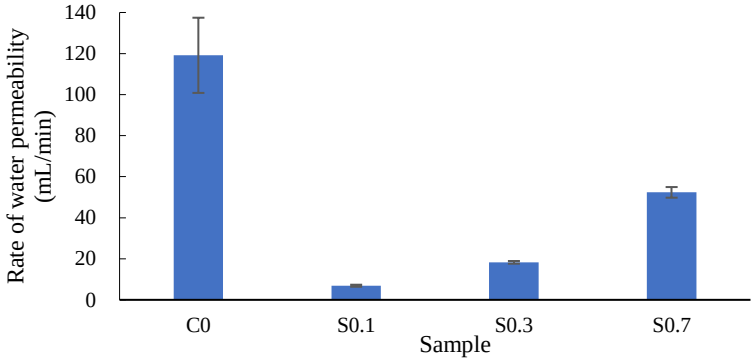



*Fig. 4.* The water permeability rate of different bio-grout components.
C0: Control (the cracked concrete without any treatment)
S0.1, S0.3, S0.7: Sands with diameter ranges from 0.1 to 0.7 mm

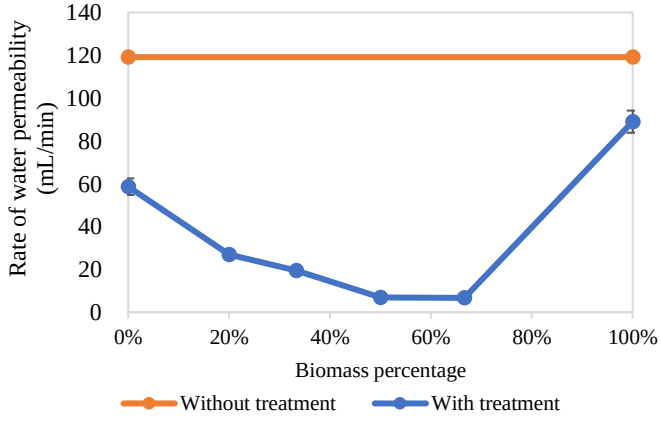



*Fig. 5.* The water permeability rate of different biomass percentage.

According to Fig. 5, in the 0% sample (only sands and CCP medium, without biomass), rate of water permeability was decreased compared with the cracked sample without treatment because the urea can be hydrolyzed in aqueous condition, that leads to the chemical carbonate precipitation to connect the sands. In the findings of [18], under suitable condition and in the appropriate media, most bacteria can form $CaCO_3$ crystals. This experiment was conducted in the outside environment; thus, other microorganisms also grew and induced calcium carbonate precipitation after a period of time. However, with *S. pasteurii*, a high urease-producing bacterium, MICP was induced faster, thus decreasing the rate of water permeability. When the biomass percentage increased, the water permeability rate also decreased until the percentage reached 50%. After this value, although the biomass percentage increased, the rate did not change significantly. It is cleared with results of bio-grout containing 100% biomass; although it could hinder water permeability, the effect was not so significant because the carbonate bridge was not strong enough to fill in the crack. These findings are in agreement with [5] who measured the water permeability in terms of the depth of water penetration into mortar sample and the water tightness of cracked mortar was improved nearly 80% with $CaCO_3$ formation. Therefore, the bio-grout composed of 0.1 mm sand and *S.pasteurii* biomass with a ratio of 1:1 (w/w) was chosen for the following experiments.

### 3.3. Evaluation of crack healing over curing time

All of the previous experiments were done in 14 days. This experiment was to test the calcium carbonate precipitation process in the crack versus time. After 7 days of incubation, $CaCO_3$ bridges were not enough for fill in all the void, thus water could permeate the crack rapidly. After 21 days, cured cement exhibited significant permeability reduction as it can been from Fig.6, where precipitation of bio-minerals mostly blocks the crack path to prevent water to flow through in water permeability test. A gradually decreasing water permeability rate that means an improvement of water sealing of cracked samples could be seen over curing time. The positive effect of longer curing time to water absorption was also investigated by [19] with cement mortar covered with bacteria-based coating. After 28 days, this rate seems to reach a stable value. Therefore, it is suggested that after 21 days, biomass and CCP medium should be added to promote the carbonate precipitation in the crack.

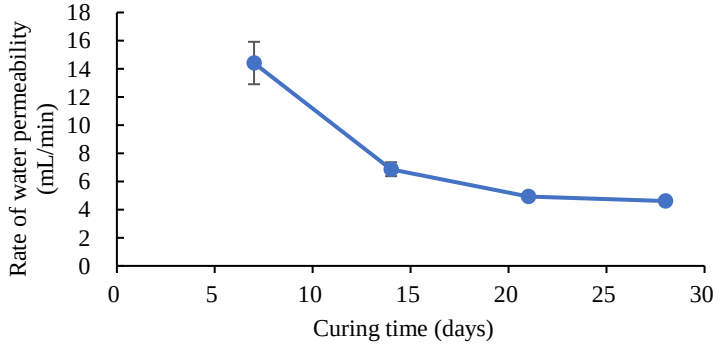



*Fig. 6.* The water permeability rate of the optimum mixture components as a function of curing time.

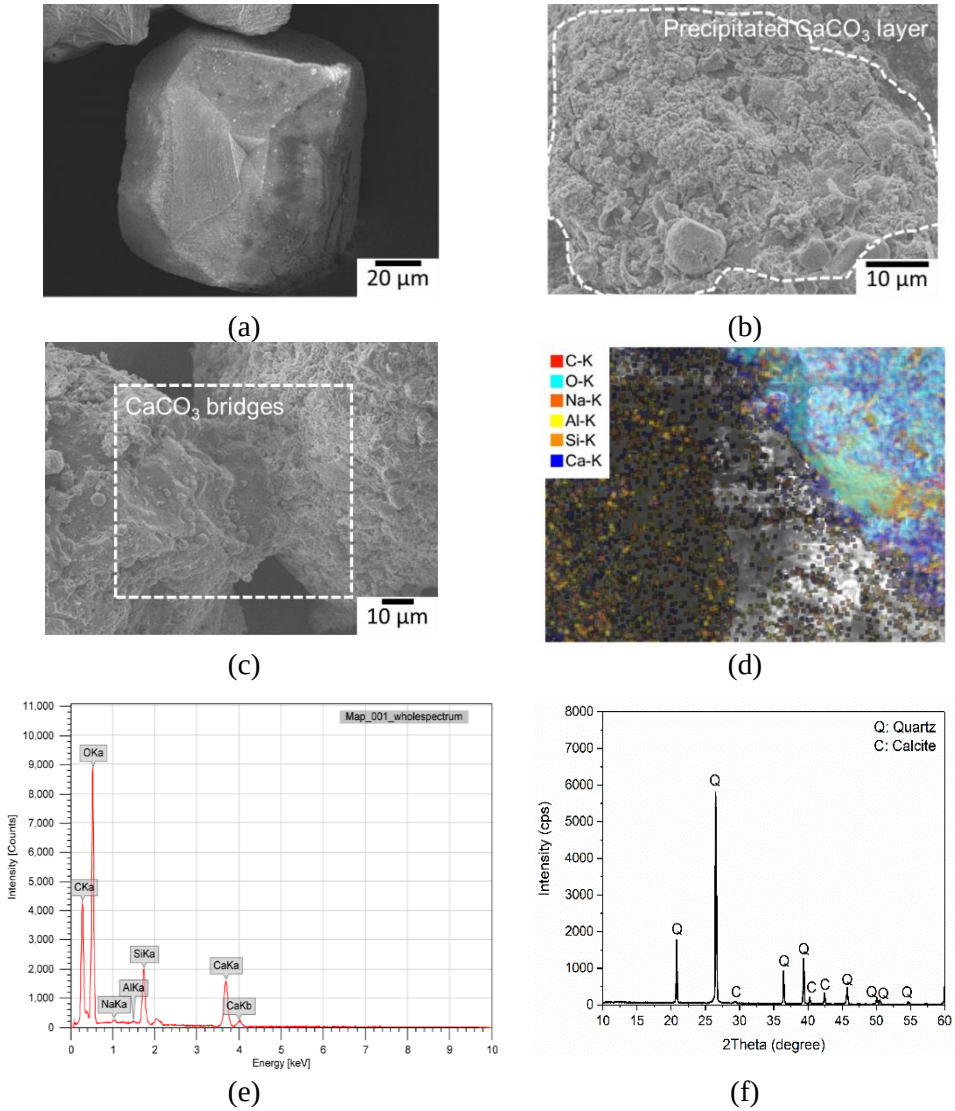
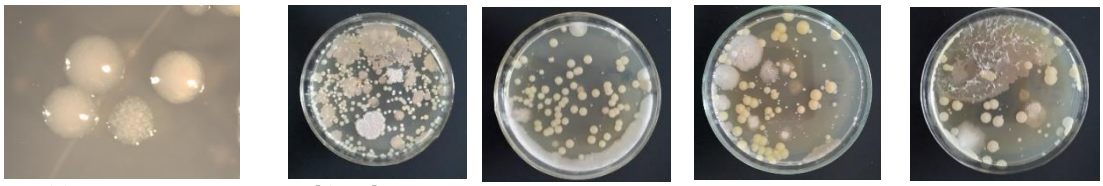



*Fig. 7.* SEM, EDS and XRD results of calcium carbonate precipitates in liquid culture and cracked mortar specimens: (a) SEM micrograph of CaCO3 precipitates in CCP medium; (b) SEM micrograph of precipitated CaCO3 layer on sand; (c) SEM micrograph of CaCO3 bridges between sands; (d) EDS mapping of elemental distribution analysis for CaCO3 bridges between sands; (e) EDS Spectra CaCO3 bridges between sands; (f) XRD pattern of the precipitated CaCO3.

The microstructures analysis confirmed that $CaCO_3$ could be produced without continuous addition of bacterial solution. X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDS) analysis of the precipitated calcium carbonate powder and bio-grout are given in Fig. 7. The $CaCO_3$ zones (or treated zones) is composed of calcite and vaterite crystals but in aqueous condition, the predominant precipitate was calcite with a rhombohedral crystalline structure; in contrast, in bio-grout, the predominant one is vaterite with a round structure with a size of approximately 5-10 µm (Fig. 7b). The EDS (Fig. 7e) peaks of bio-grout show that the elemental composition of the precipitate is composed of carbon (C), oxygen (O) and calcium (Ca). The EDS analyses confirmed that the particles observed in the SEM were calcium carbonate precipitation. The elemental mapping of the samples allowed visualization of the regions where calcium carbonate particles had formed (Fig. 7d). This is a further evidence to show that the microbially generated carbonate precipitate

is responsible for clogging the sand pore spaces, thereby restricting flow of water and decreasing the permeability of the cement crack.



(a) *S. pasteurii* colonies (b) 7 days (c) 14 days (d) 21 days (e) 28 days

*Fig. 8.* Evaluation of *S. pasteurii* viability in cracked mortar specimens vs. curing time.
(a) *S. pasteurii* colonies provided by the supplier;
(b) to (e) Tryptone Soya Agar plates of bio-grout samples after 7, 14, 21 and 28 days.

The viability of *S. pasteurii* was evaluated by plate count method using Tryptone Soya Agar. *S. pasteurii* colonies were identified based on the supplier's description (Fig. 8a) and Gram-staining confirmation. Although this medium contains high concentration of urea, there were other microorganism which can endure high pH; thus, it was unable to determine the exact number of viable cells. However, Fig. 8b to 8e showed that density of viable *S. pasteurii* cells decreased with curing time. This observation is in accordance with the decrease of water permeability rate vs. time (Fig. 6), that means calcium precipitation gradually happened with *S. pasteurii* cells as nucleation sites. The appearance of *S.pasteurii* colonies shows that there were still vegetative cells in bio-grout even after 28 days; thus, it is suggested that calcification can be activated with addition of CCP medium.

## 4. CONCLUSIONS

*Sporosarcina pasteurii* was optimized in the suitable carbonate precipitation medium with the initial pH as 6 and high calcium chloride to obtain the high mass of precipitates without medium addition vs. time. Based on these results, bio-grout using *Sporosarcina pasteurii* mixed with 0.1 mm sand in ratio of 1:1 (w/w) shows positive effects on $CaCO_3$ precipitation and preventing water flow through cracks compared with the cracked specimen without treatments. The bacterial-based repair solution, firstly designed for concrete surface crack repair in Vietnam, showed promising potential as a healing agent through the effectiveness of crack sealing and liquid tightness.

**Acknowledgments:** This work was funded by Ho Chi Minh City University of Food Industry (Contract number 54/HD-DCT dated September 9, 2020). The authors acknowledge Mr. Son Pham and Mr. Tu Tran for their support.

## REFERENCES

1. Jonkers H.M. - Self healing concrete: a biological approach, In: Self-healing materials, Springer, 195–204, Switzerland (2007).
2. Jonkers H.M., Schlangen E. - Development of a bacteria-based self-healing concrete, Proceedings of the International FIB Symposium, Netherlands (2008) 425–430.
3. Van Tittelboom K., De Belie N., De Muynck W., Verstraete W. - Use of bacteria to repair cracks in concrete, Cement and Concrete Research **40** (2010) 157–166.
4. Huynh N.N.T., Phuong N.M., Toan N.P.A., Son N.K. - Bacillus subtilis HU58 Immobilized in micropores of diatomite for using in self-healing concrete, Procedia engineering **171** (2017) 598–605.

5. Jongvivatsakul P., Janprasit K., Nuaklong P., Pungrasmi W., Likitlersuang S. - Investigation of the crack healing performance in mortar using microbially induced calcium carbonate precipitation (MICP) method, Construction and Building Materials **212** (2019) 737–744.
6. Nguyen-Pham H.H., Nhi V.Y., Thuy N.T.T., Tuan P.M. - Investigation of applying wild Bacillus species for sand stiffening, Vietnam Journal of Science and Technology **57** (2019) 143.
7. Huynh N.N.T., Imamoto K., Kiyohara C. - A Study on Biomineralization using Bacillus Subtilis Natto for Repeatability of Self-Healing Concrete and Strength Improvement, Journal of Advanced Concrete Technology 17 (2019) 700–714.
8. Huynh N.N.T., Imamoto K., Kiyohara C. - Compressive Strength Improvement and Water Permeability of Self-Healing Concrete Using Bacillus Subtilis Natto, XV International Conference on Durability of Building Materials and Components, Spain (2020).
9. Lambert S.E., Randall D.G. - Manufacturing bio-bricks using microbial induced calcium carbonate precipitation and human urine, Water research **160** (2019) 158–166.
10. De Muynck W., De Belie N., Verstraete W. - Microbial carbonate precipitation in construction materials: a review, Ecological Engineering **36** (2010) 118–136.
11. Joshi S., Goyal S., Mukherjee A., Reddy M.S. - Microbial healing of cracks in concrete: a review, Journal of industrial microbiology and biotechnology **44** (2017) 1511–1525.
12. Hammes F., Boon N., de Villiers J., Verstraete W., Siciliano S.D. - Strain-specific ureolytic microbial calcium carbonate precipitation, Applied and Environmental Microbiology **69** (2003) 4901–4909.
13. Chahal N., Siddique R., Rajor A. - Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of concrete incorporating silica fume, Construction and Building Materials **37** (2012) 645–651.
14. Bang S.S., Galinat J.K., Ramakrishnan V. - Calcite precipitation induced by polyurethane immobilized Bacillus pasteurii, Enzyme and Microbial Technology **28** (2001) 404–409.
15. Natarajan K.R. - Kinetic study of the enzyme urease from Dolichos biflorus, Journal of chemical education **72** (1995) 556.
16. 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** (2015) 455–464.
17. Hammes F., Verstraete W. - Key roles of pH and calcium metabolism in microbial carbonate precipitation, Reviews in environmental Science and Biotechnology **1** (2002) 3–7.
18. Boquet E., Boronat A., Ramos-Cormenzana A. - Production of calcite (calcium carbonate) crystals by soil bacteria is a general phenomenon, Nature **246** (1973) 527–529.
19. Huynh N.N.T., Nhu N.Q., Son N.K. - Developing the solution of microbially induced CaCO3 precipitation coating for cement concrete, IOP Conference Series: Materials Science and Engineering, Malaysia (2018).

## TÓM TẮT

# PHÁT TRIỂN VỮA SINH HỌC LÀM LIỀN VẾT NỨT BÊ-TÔNG DỰA TRÊN SỰ KẾT TỦA CALCIUM CARBONATE DO VI SINH VẬT GÂY RA BẰNG CÁCH SỬ DỤNG *Sporosarcina pasteurii*

Nguyễn Phạm Hương Huyền[1*], Nguyễn Ngọc Trí Huỳnh[2],
Nguyễn Khánh Sơn[2], Nguyễn Thị Nam Phương[1], Phạm Minh Tuấn[1]
[1]*Trường Đại học Công nghiệp Thực phẩm TP. Hồ Chí Minh*
[2]*Trường Đại học Bách Khoa - Đại học Quốc gia TP.HCM*
*Email: *nphhuyen@gmail.com*

Gần đây, có nhiều nghiên cứu hơn về việc ứng dụng quá trình tạo tủa calcium carbonate dưới tác động của vi sinh vật (MICP) để sửa chữa các vết nứt bê tông thay vì polymer tổng hợp, chất có thể gây hại cho môi trường. Vi khuẩn mang tính ureolytic, chẳng hạn như *Sporosarcina pasteurii*, được biết có tiết ra enzyme urease, dẫn đến tạo tủa calcium carbonate trong một môi trường giàu calcium. Dựa trên cơ chế này, các tỷ lệ khác nhau của vật liệu xây dựng và sinh khối *S. pasteurii* đã được khảo sát để thu được loại vữa tối ưu nhằm giải quyết vấn đề nứt bê tông. Đầu tiên, điều kiện tạo tủa calcium được tối ưu ở pH ban đầu là 6 và nồng độ $CaCl_2$ là 250 mM. Hỗn hợp vữa sinh học tối ưu thu được với cát kích thước 0,1 mm trộn với sinh khối theo tỷ lệ 1:1 với mật độ vi khuẩn là khoảng $10^8$ CFU/mL. Quan sát bằng mắt thường và kiểm tra độ thấm nước cho thấy vữa vi khuẩn có thể sửa chữa các vết nứt và ngăn chặn sự xâm nhập của chất lỏng và khí so sánh với đối chứng âm. Bên cạnh đó, kết quả phân tích SEM và XRD chỉ ra rằng các cấu trúc đa hình carbonate khác nhau được hình thành bên trong các vết nứt. Ngoài ra, phương pháp đổ đĩa cho thấy có các tế bào *S. pasteurii* sinh dưỡng tồn tại trong các vết nứt ngay cả sau 28 ngày, điều này có nghĩa là vữa vi khuẩn có thể có tác dụng lâu dài trên xi măng.

*Từ khóa:* MICP, *Sporosarcina pasteurii*, tự liền, calcium carbonate.