Hindawi
Advances in Materials Science and Engineering
Volume 2022, Article ID 6188680, 13 pages
https://doi.org/10.1155/2022/6188680

*Research Article*

# Study of Concrete Crack Repair using *Bacillus megaterium*

**Wenbo Shi**,[1,2] **Meng Wang**,[1] **Lan Wu**,[1] **Xinhang Xie**,[1] **Miao Wang**,[1] **and Tianjing Lu**[1]

[1]*School of Intelligent Transportation, Xuchang University, Xuchang 461000, China*
[2]*School of Water Resources and Environment, Zhengzhou University, Zhengzhou 450000, China*

Correspondence should be addressed to Wenbo Shi; shwanbe@163.com

Received 25 September 2022; Revised 10 November 2022; Accepted 11 November 2022; Published 22 November 2022

Academic Editor: Shengwen Tang



The low yield of microbial calcium carbonate precipitation due to the strong pH environment has limited the development of biotechnology-based concrete crack repair techniques. In this study, *Bacillus megaterium* was selected to determine the optimal nutrient formulation through orthogonal tests. The growth and reproduction characteristics and urease activity of this strain were analysed by controlling different pH values, and the calcium carbonate precipitation yield under different pH conditions was investigated, and the lower precipitation yield under strong alkaline conditions was improved by using alkaline environment domestication with the addition of urea to the nutrient solution. It has been shown that higher pH results in lower growth and multiplication of *Bacillus megaterium* and weaker urease activity, and a strong alkaline environment can significantly suppress its growth and multiplication and urease activity. At pH 7, the growth and multiplication of *Bacillus megaterium* were the fastest, the urease activity was the strongest, and the precipitation yield was greater. The addition of urea to the nutrient solution to domesticate *Bacillus megaterium* in an alkaline environment can significantly increase the growth rate and precipitation yield, which can effectively solve the problem of insufficient calcium carbonate precipitation under alkaline conditions. Comparing the effectiveness of microbially induced calcium carbonate precipitation (MICP) in repairing concrete after damage before and after microbial domestication shows that the domesticated *Bacillus megaterium* is more effective in reducing the permeability characteristics of concrete and improving its strength.

## 1. Introduction

Concrete is still the most widely used construction material in the world today [1]. However, the relatively low tensile strength of concrete makes it inevitable that cracks will appear under the action of internal and external factors, such as temperature cracks, shrinkage cracks, and later load cracks. The appearance of cracks affects the integrity and functionality of the concrete structure, providing a wide and convenient channel for aggressive media to enter the concrete interior, leading to a decrease in the durability of the concrete structure, and causing huge economic losses and safety hazards. The traditional repair method is mainly manual repair by applying external coating agents such as epoxy, chlorinated rubber, silane, wax, and acrylic resin to the surface of concrete cracks for sealing detected external cracks and pores, but its effectiveness and performance are limited by its poor bonding performance to the concrete matrix and the surface's susceptibility to degradation and delamination with increasing age. More importantly, manual construction is time-consuming and labour-intensive, and the initial microcracks in the concrete cannot be identified and repaired in time, leading to additional cracking.

As a result of the above problems, self-healing concrete has been the focus of research in the engineering community. Self-healing studies of concrete are mainly based on inorganic self-repair and organic self-repair. Some scholars have studied the self-healing ability of concrete after cracking under external forces based on the addition of acetal polymer solutions [2], extremely absorbent polymers (SAP) [3], mineral admixtures, polylactic acid, polystyrene, and activated magnesium oxide to concrete, and Wang's et al. team has carried out more contributions to this [4–6]. However, although the addition of chemical additives has been effective in repairing concrete cracks, their high cost

and environmental impact have limited their use. The alternative biotechnology application of crack repair using microbially induced calcium carbonate precipitation (MICP) has received a lot of attention. This biotechnological approach offers a sustainable, renewable, safe, and permanent solution to serious problems associated with concrete.

The process of calcium carbonate precipitation resulting from the agglomeration of large numbers of microorganisms that can be observed in numerous natural environments is known as biomineralisation. The technique of using some of the microorganisms found in various natural environments, geographical conditions, fresh water, lakes, and soils to react with organic matters such as urea and calcium ions in the reaction environment to precipitate calcium carbonate crystals with superior cementing properties quickly is known as MICP (microbial induced carbonate precipitation) technology [7]. This technique is widely recognized as having the ability to achieve self-healing of concrete cracks [8–11].

This technology uses a class of bacteria capable of producing urease, which catalyzes the hydrolysis of urea and produces carbon dioxide and ammonia. The entire hydrolysis reaction takes place in two stages (equations (1)–(3)): in the first stage, ammonia and carbamic acid are produced; in the second stage, carbamic acid is spontaneously and rapidly hydrolysed to ammonia and carbonic acid. As the reaction proceeds, the urease catabolism reaction increases the pH of its environment.

Urease is able to hydrolyse urea to carbonate and ammonium ions, which can react with calcium ions in the surrounding solution to form calcium carbonate precipitates, in a process where the bacteria simultaneously provide nucleation sites for the deposition of calcium carbonate. Here, the bacterial surface plays a highly significant role in the precipitation of $CaCO_3$ (Equations (4) and (5)). Due to its negative charge, it attracts positively charged calcium ions (Equations (6) and (7)), leading to nonhomogeneous nucleation [12, 13], and the precipitation of calcium carbonate subsequently occurs through successive layering.

The specific reflective equation is

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{bacteria}} NH_2COOH + NH_3, \quad (1)$$

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3, \quad (2)$$

$$NH_3 + H_2O \longrightarrow NH_4 + +OH^-, \quad (3)$$

$$H_2CO_3 \longrightarrow 2H + +2CO_3^{2-}, \quad (4)$$

$$Ca_2 + +CO_3^{2-} \longrightarrow CaCO_3, \quad (5)$$

$$Ca_2 + +Cell \longrightarrow Cell - Ca^{2+}, \quad (6)$$

$$Cell - Ca_2 + +CO_3^{2-} \longrightarrow Cell - CaCO_3, \quad (7)$$

These reactions can help to repair cracks in concrete, but the process is also influenced by a number of additional factors, such as strains of bacteria and the environment. Researchers have carried out experimental studies on various urease-producing bacteria such as *Bacillus sphericus*, *Bacillus pasteurii*, and *Bacillus megaterium* because their spore-forming ability allows them to survive for hundreds of years without nutrients and thus can be buried in concrete for long periods of time [14–18], waiting for microcracks to sprout and repair the cracks through MICP.

Although microorganisms can repair cracks by inducing calcium carbonate precipitation, the reaction process is influenced by a variety of other factors. Wu et al. [19] use *Bacillus cereus* as a repair agent for concrete cracks which was the dominant strain isolated from calcium carbide slag. The optimum concentrations of calcium and urea were 0.9 mol/L and 0.75 mol/L, respectively. The water absorption and chloride ion permeability of the samples were reduced by 12.0% and 10.9%, respectively, healing 100–800 $\mu$m cracks, and the permeability of the healed samples was reduced by about two orders of magnitude. Wang et al. [20] investigated the effect of calcium ion concentration on the effectiveness of the MICP technique for mineralising sandy soils and found that lower calcium ion concentrations (less than 0.5 mol/L) resulted in smaller but more uniformly distributed calcium carbonate crystallite sizes and higher concentrations (greater than 0.5 mol/L) resulted in larger crystallite sizes and uneven distribution. Abo-El-Enein et al. [21] used calcium chloride as the calcium source, and the samples had the lowest water absorption and the highest compressive strength of 1.2 MPa, while calcium acetate and calcium nitrate as calcium sources had mortar strengths of only 1.0 MPa and 0.45 MPa. In addition, the researchers also investigated the effect of various calcium sources such as calcium citrate, calcium oxalate, and calcium oxide on the biomineralisation effect of Bacillus [22–25] and the effects of urea concentration, calcium ion concentration [26, 27], temperature [28, 29], and concrete admixtures on the microorganisms performing MICP. The amount of cement mixed in the concrete mixing process [30] and the mixing method [31] will also have an effect on the effectiveness of microbial repair of cracks at a later stage.

In the MICP reaction, pH affects the urease activity of the strain, which in turn affects the calcium carbonate yield and the effectiveness of the MICP technology [32]. Numerous studies have shown that there is a suitable pH range for the strain, above or below which the urease activity of the strain is significantly inhibited. Stocks-Fisher et al. [33] found that the optimum pH range was 7.5 to 9.0. Whiffin et al. [34] concluded that the optimum pH range was 6.0 to 8.5. For pure urease solutions, the effect of pH is greater than the effect on the urease activity of the bacterial solution, probably because for the strain, the urease is present within the cells of the strain and the pH is an indirect effect [35]. Different pH values have a greater effect on the rate of urea decomposition by the strain, while the optimum pH range obtained by different researchers varies, but in general, the activity of urease bacteria ranges from pH 7.0 to 9.0.

The alkaline tolerance of the strain is a prerequisite for the effectiveness of MICP in inducing $CaCO_3$ deposition. pH affects calcite precipitation as the ability of urease to hydrolyse urea is only activated at a specific pH. Most calcite

precipitation occurs under alkaline conditions at pH 8.7 to 9.5, and the carbonate dissolves without forming a precipitate while the pH decreases [36]. As the internal environment of cementitious materials is extremely harsh, with pH values typically above 12, this requires that the microorganisms selected must first be able to resist the highly alkaline environment within the cementitious material.

Therefore, by culturing and domesticating microorganisms to enhance their viability and calcium carbonate yield in highly alkaline environments, it is not only of great significance for biological development but also has far-reaching implications for the application of MICP in concrete self-healing, and the relevant experimental results provide a reference for putting microbial self-healing technology into engineering applications.

*Bacillus megaterium* is a member of the Bacillus family that efficiently converts urea into ammonium carbonate, which in turn forms calcium carbonate precipitates [37]. In this paper, *Bacillus megaterium* is used as an example of a method to enhance the microorganism's ability to perform MICP in a strong alkaline environment through domestication studies.

## 2. Materials and Method

*2.1. Bacteria.* *Bacillus megaterium* is rod-shaped with rounded ends, single or arranged in short chains, 1.2–1.5 × 2.0–4.0 μm long, aerobic, motile, Gram-positive, its bacilli are 1.0–1.2 × 1.5–2.0 μm long, oval, mesophytic or subterminal. Spores vary from oval to elongate and cannot produce acetylmethylmethanol; strains are motile but slow-moving, require free oxygen, and ferment glucose to produce acid; G + C content of DNA is 36%–38%, as shown in Figure 1.

*2.2. Orthogonal Test.* In order to obtain strains with elevated urease activity, experimental studies of *Bacillus megaterium* culture solutions were required. Summarising the results of previous experiments, it was found that the biofilm growth characteristics were used to propose an optimised formulation of the nutrient solution from the nutrient solution constituents. The nutrient solution was improved by studies of absorbance, urease activity, and calcium carbonate yield, and the coarser granular mineralisation effect was used to demonstrate its superiority in aggregating into clusters, offering the possibility of concrete crack repair.

The effect of NaCl, peptone, yeast extract, ammonium nitrate, and sodium lactate on the growth of microbial biofilms and urease activity was investigated based on an orthogonal test. The orthogonal test table is shown in Table 1.

*2.3. Measurement of Optical Density and Enzyme Activity.* The culture broth was placed in an autoclave and autoclaved at 121°C for 20 min, then inoculated on a sterile table, and incubated in a shaking incubator at 35°C and 180 r/min. After the culture was completed, the cell concentration was measured by UV-Vis spectrophotometer at 600 nm, and the results were recorded as $OD_{600}$. The absorbance ($OD_{600}$) was used to study the change in cell concentration of the strain [38].

In addition to the growth and reproduction characteristics of the strain, urease activity is also an essential indicator to study. After a certain period of time, the organic matter contained in the yeast extract has broken down and reached an equilibrium state. Therefore, without adding fresh nutrients, the concentration of ions in the bacterial solution will remain constant, and the conductivity of the solution will remain at a stable value. If fresh nutrients are added (e.g., a certain concentration of urea solution), the urease in the bacterial solution can rapidly catalyze the hydrolysis of these urea ions, and the newly produced carbonate and ammonium ions will increase the conductivity of the solution, and the amount of hydrolysis is proportional to the change in conductivity of the solution during urea hydrolysis [34]. The conductivity method was used to detect urease activity in the absence of $Ca^{2+}$. The value of the change in conductivity per unit time can be measured quickly and easily with a conductivity meter (INESA DDSJ-319). The bacterial urease activity is calculated as shown in equation (8), and the unit urease activity is calculated as shown in equation (9), dividing the urease activity by the bacterial concentration $OD_{600}$ to obtain the units of urease activity, which reflects the ability of the bacterial solution to hydrolyse urea per unit $OD_{600}$ value [39].

During the test, 12 ml of the bacterial solution to be tested was mixed with 108 ml of urea solution at room temperature, and then, the conductivity change was monitored over 15 min, using a conductivity meter, and the average change per minute was converted to the amount of hydrolysed urea per minute in three parallel groups each.

$$\begin{pmatrix}\text{Bacterial urease activity}\\ \left(m\text{Mureahydrolysed}\bullet\text{min}^{-1}\right)\end{pmatrix} = \begin{pmatrix}\text{Value of conductivity change per minute}\\ \left(ms\bullet\text{min}^{-1}\right)\end{pmatrix} \times 10 \times 11.11, \tag{8}$$

$$\begin{pmatrix}\text{Unit urease activity}\\ \left(m\text{Mureahydrolysed}\bullet\text{min}^{-1}\bullet OD^{-1}\right)\end{pmatrix} = \frac{\text{Bacterial urease activity}\left(m\text{Mureahydrolyse d}\bullet\text{min}^{-1}\right)}{\text{Bacterial concentration}\left(OD_{600}\right)}. \tag{9}$$

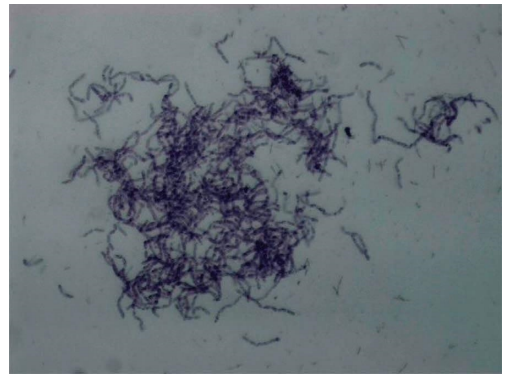

Figure 1: *Bacillus megaterium* under a 40x microscope.

Table 1: Orthogonal test ratios.

| No. | NaCl (M) | Peptone (g/L) | Yeast extract (g/L) | Ammonium nitrate (g/L) | Sodium lactate (g/L) |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 5 | 3 | 3 | 3 |
| 3 | 0 | 10 | 6 | 6 | 6 |
| 4 | 0 | 15 | 9 | 9 | 9 |
| 5 | 5 | 0 | 3 | 6 | 9 |
| 6 | 5 | 5 | 0 | 9 | 6 |
| 7 | 5 | 10 | 9 | 0 | 3 |
| 8 | 5 | 15 | 6 | 3 | 0 |
| 9 | 10 | 0 | 6 | 9 | 3 |
| 10 | 10 | 5 | 9 | 6 | 0 |
| 11 | 10 | 10 | 0 | 3 | 9 |
| 12 | 10 | 15 | 3 | 0 | 6 |
| 13 | 15 | 0 | 9 | 3 | 6 |
| 14 | 15 | 5 | 6 | 0 | 9 |
| 15 | 15 | 10 | 3 | 9 | 0 |
| 16 | 15 | 15 | 0 | 6 | 3 |

*2.4. Calcium Carbonate Yield Tests.* The MICP calcification test was performed in a conical flask by preparing a 50 mL gelling solution, in which urea and calcium chloride were used in equal amounts, set at 1 M and 2 M, respectively. The gelling solution was mixed with 50 mL of bacterial solution at a concentration of $OD_{600}$ of 1.7. Considering that the nutrient solution might be depleted after 48 hours of incubation, 50 ml of the nutrient solution was added to some of the test groups. The nutrient solution is given in Table 2. After mixing, the initial pH was 7, and the reaction temperature was 25°C. Calcium carbonate yields were calculated after two and four days, respectively.

The calcium carbonate yields were calculated as follows [40]:

(1) The reaction solution was filtered, and the conical flask was filtered twice more with water, and then, the conical flask and the filter paper were dried in an oven at 110°C. After 24 h, the conical flask and the filter paper were weighed to obtain the mass $M_1$

(2) The conical flask and the filter paper were acid-washed with a prepared hydrochloric acid solution (2 M) and rinsed twice with water, and then, the conical flask and the filter paper were again dried in an oven at 110°C, and after 24 h, the conical flask and the filter paper were weighed again to obtain the mass $M_2$

(3) The difference between the two masses $M_1 - M_2$ is the actual mass of calcium carbonate produced, while the theoretical mass of calcium carbonate is obtained by multiplying the ionic concentration $m$ used in the gelling solution with the amount $V$

(4) The final calcium carbonate yield is the actual amount of calcium carbonate produced divided by the theoretical amount of calcium carbonate generated

*2.5. pH Domestication Test.* As the urease component is a protein, it is an amphoteric electrolyte, and the environmental pH also has a significant effect on the urease activity of the strain [41, 42]. To investigate the effect of pH on bacterial urease activity, the pH of the nutrient solution was controlled using NaOH at 6, 7, 8, 9, 10, and 11. The variation of $OD_{600}$ and unit urease activity with pH of the bacterial solution is shown in Figure 2.

As shown in Figure 2, the optimum pH range for *Bacillus megaterium* was between 6 and 8. The concentration and urease activity were higher at pH 7, which is similar to the results of Whiffin's study [34] on the effect of pH on the

Table 2: Calcium carbonate yield experimental programme.

| No. | *Bacillus megaterium* suspension (ml) | Culture fluid (ml) | Gelling solution | Cultivation time (D) |
|---|---|---|---|---|
| 1 | 50 | 0 | 1 M urea and calcium chloride 50 ml | 2 |
| 2 | 50 | 50 | 1 M urea and calcium chloride 50 ml | 2 |
| 3 | 50 | 0 | 2 M urea and calcium chloride 50 ml | 2 |
| 4 | 50 | 50 | 2 M urea and calcium chloride 50 ml | 2 |
| 5 | 50 | 0 | 1 M urea and calcium chloride 50 ml | 4 |
| 6 | 50 | 50 | 1 M urea and calcium chloride 50 ml | 4 |
| 7 | 50 | 0 | 2 M urea and calcium chloride 50 ml | 4 |
| 8 | 50 | 50 | 2 M urea and calcium chloride 50 ml | 4 |

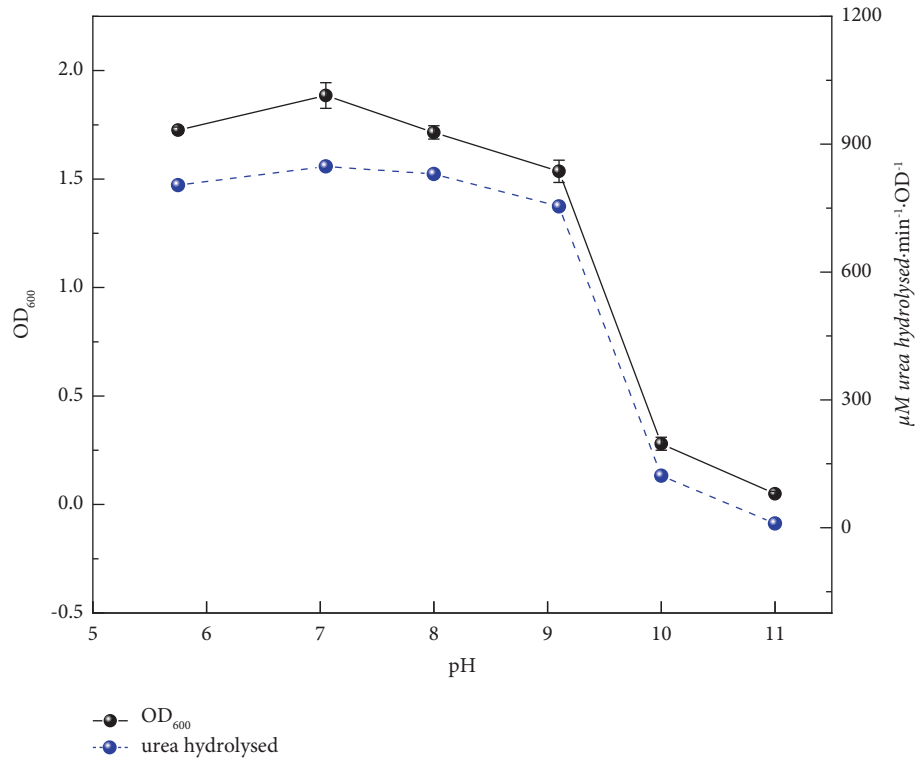


Figure 2: Effect of pH on the concentration and urease activity of the bacterial solution.

growth and reproduction of the strain. Bacterial solution urease activity was best in the pH range of 6.0 to 8, where the effect of pH was negligible. At pH values greater than 8, both the urease activity and the concentration of the bacterial solution decrease significantly with increasing pH.

The experiment was carried out by domesticating *Bacillus megaterium* with strong alkalis in order to improve the survival and activity of the strain in strong alkaline environments. The pH was set from low to high in four gradients: 9, 9.5, 10, and 10.5. Firstly, the pH was set to 9, and the selected *Bacillus megaterium* was incubated at 5% v/v every 2 days for a period of 6 days. At the end of this period, the pH was raised to 9.5, again with 5% v/v inoculation every 2 days, again for 6 days. Later, the pH was raised to 10 in the same manner. After domestication, *Bacillus megaterium* was subjected to a comparison test under different pH conditions, i.e., reinoculated at 1% v/v for 48 h. The $OD_{600}$ and urease activity of the domesticated bacteria were measured at different pH conditions.

*2.6. Concrete Crack Repair Test.* Concrete materials and proportions are as follows:

Cement: P.O. 42.5, apparent density 3.1 g/cm$^3$

Fly ash: class II fly ash, apparent density 2.2 g/cm$^3$

Fine aggregate: river sand, zone 2 medium sand, apparent density 2.61 g/cm$^3$

Coarse aggregate: crushed stone, 5–25 mm, continuous aggregate, apparent density 2.7 g/cm$^3$

Additive: polycarboxylic acid high-efficiency water reducing agent

Mixer: DT2000ZBW vibrational mixer was used. The mixer is capable of applying vibrations during the mixing process. The addition of vibration energy is able to break the hydration product wrapping film of cement agglomerated particles, releasing cement particles to allow full hydration reactions to occur, increasing the proportion of cement slurry, and reducing the impact

on results of cement particles that are not completely reflected during later tests.

Concrete proportioning is shown in Table 3.

In total, eight concrete blocks were prepared in the above proportions and placed under standard curing conditions for seven days before strength testing. Four specimens that did not break significantly after damage were selected for MICP testing, and the seven-day age compressive strengths of the four specimens were 44.3 MPa, 37.8 MPa, 43.4 MPa, and 37.5 MPa, respectively.

The penetration characteristics of the concrete after yielding were assessed using the penetration apparatus shown in Figure 3. The test block was placed on top of the lower support body with a sleeve nested above it and sealed around the block with a waterproof sealant, after which water was poured in and the water level was maintained at 18 cm, and recorded the time it takes for the water above to finish penetrating.

The concrete was filled with the bacterial and gelling solutions using the filling device as shown in Figure 4.

The test blocks were placed individually in acrylic boxes and filled with the bacteriological solution above the support of the test blocks. The bacterial solution slowly percolated through the concrete and surrounding crevices into the storage space below. Two hoses were used, one to transport the bacterial solution from the beaker to the top of the test block via a peristaltic pump (shown as the black line in Figure 4) and the other to pump the bacterial solution that had penetrated from the concrete test block into the lower storage space back into the beaker via a peristaltic pump (shown as the blue line in Figure 4), the whole process lasting 4 hours. The completed test blocks were placed in a 0.1 M solution of calcium chloride and urea for 10 minutes for microbial fixation. Afterwards, the bacteriological solution in the device was replaced with a 0.6 M calcium chloride and urea solution, and the gelling solution was infused in cycles for 18 hours according to the method of infusion of the bacteriological solution, after which the test was completed and taken out and left to stand at room temperature for 24 hours. This test procedure was repeated six times, after which the permeation characteristics and residual strength tests were carried out.

## 3. Results and Discussion

*3.1. Determining the Optimal Culture Solution.* An orthogonal test was designed to investigate the most suitable culture solution for *Bacillus megaterium* for MICP, and the results of the orthogonal test are shown in Figure 5.

The bar plot shows OD600, and the line plot shows the deceleration activity in Figure 5. As shown in the graph, the best results were achieved with the No. 7 ration, with an $OD_{600}$ of 1.73 and a urease activity of 0.8. This ration was compared to other scholarly ration studies as shown in Figure 6.

As can be seen from Figure 6, comparing the culture trials conducted by Sun et al. [29] using LB nutrient solution and the improved nutrient solution adopted by Attri et al. [37] for *Bacillus megaterium*, the No. 7 nutrient solution used in this thesis reached an absorbance $OD_{600}$ of 1.2 after 24 h and 1.7 after 48 h, which was 2.1 times higher than LB nutrient solution and 1.7 times higher than ATTRI nutrient solution, respectively. Therefore, the nutrient solution with the No. 7 ratio was able to obtain a higher number of strains of bacteria with the same time addition. As the urease-catalyzed urea hydrolysis reaction occurs, the originally nonconductive urea is hydrolysed to conductive $NH^{4+}$ and $CO_3^{2-}$, and the efficiency of urea hydrolysis is directly related to the cell concentration [41], the higher the cell concentration, the more $CO_3^{2-}$ is catalyzed, and the more $CaCO_3$ precipitates are produced when $Ca^{2+}$ is added; therefore, the nutrient solution configuration using the No. 7 ratio will be used in subsequent trials.

*3.2. Determining the Optimum Gelling Solution and Maintenance Method.* The effect of the urea and calcium chloride ratios, maintenance time, and the addition of nutrient solution required by *Bacillus megaterium* to carry out MICP was investigated by means of a calcification test. The test results are shown in Figure 7.

As can be seen from the graph, the longer the reaction time, the higher the rate of calcium carbonate production. The longer time allows for a more adequate urease reaction and sufficient time for $Ca^{2+}$ to combine with $CO_3^{2-}$ ions to form a precipitate. After 48 hours of incubation with an $OD_{600}$ of 1.7, the nutrients in the bacterial broth were already depleted. Fresh bacterial cells are produced when fresh nutrients are added to the calcification reaction, and doubling the nutrient solution increases the reproduction effect of the bacteria and thus the rate of calcium carbonate production. The mixture of 2 M calcium chloride and 2 M urea solution compared to the 1 M calcium chloride and urea solution did not yield additional calcium carbonate precipitation, which is consistent with the findings of Chen et al. [43] and Gowthaman et al. [44]. The main reasons for this result could be several: 1. the concentration of urea was overly elevated, and the ionic concentration of the decomposed urea was greater, along with the higher alkalinity, both of which inhibited urease activity and thus reduced calcium carbonate precipitation; 2. the higher calcium chloride concentration inhibited the hydrolysis of urea [45]; 3. the high concentration of urea tended to cause the disruption of the hydrophobic sway between protein molecules and inhibited the urease activity of the bacteria [46].

*3.3. Effect of Alkaline Domestication on the Viability and Urease Activity of Bacillus megaterium.* The test results showed that domestication on top of the original nutrient solution did not significantly improve the viability of *B. megaterium* in strong alkaline environments (as shown in CONTROL in Figures 8 and 9).

Sun [47] found that the addition of urea significantly improved the activity and calcium production of *Bacillus megaterium* in low-temperature environments through domestication. In this study, the effect of urea addition on the viability and activity of *B. megaterium* was investigated

Table 3: Proportioning of C40 concrete.

| Cement (kg/m$^3$) | Silica sand (kg/m$^3$) | Coarse aggregate (kg/m$^3$) | | Water (kg/m$^3$) | Additive (kg/m$^3$) |
|---|---|---|---|---|---|
| | | 5–10 mm | 10–25 mm | | |
| 510 | 787 | 375 | 549 | 165 | 13.3 |

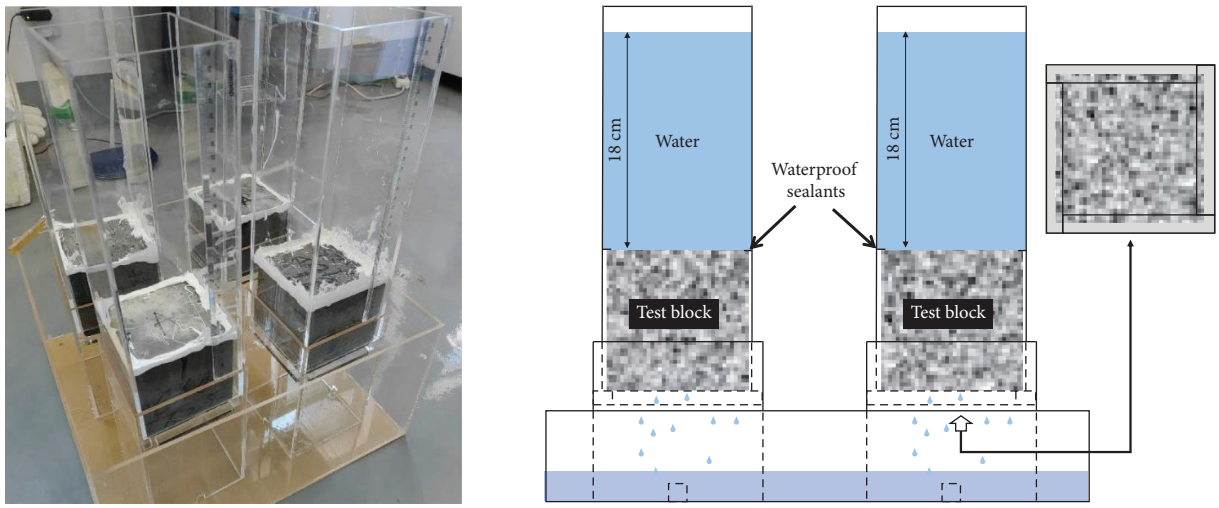


Figure 3: Equipment for assessing the permeability properties of concrete.

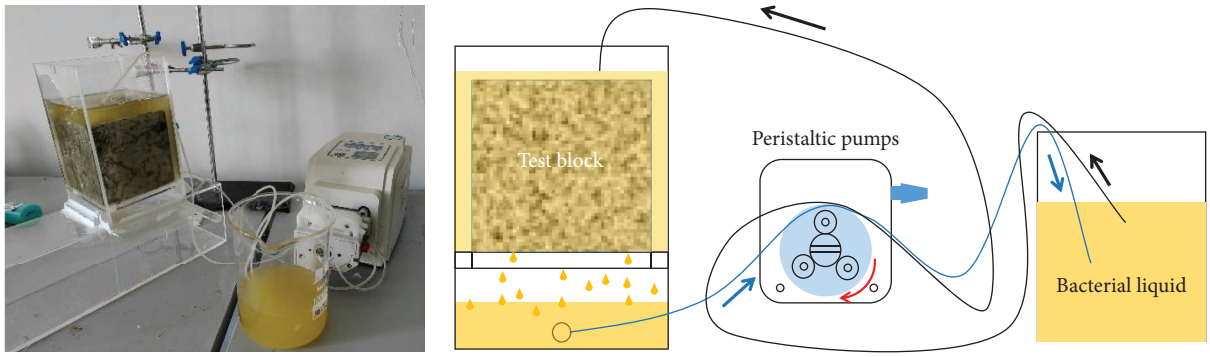


Figure 4: Method of performing MICP on concrete and the device used.

by adding 0.2 M, 0.4 M, and 0.6 M urea during the domestication phase as shown in Figures 8 and 9.

It can be seen from Figures 8 and 9 that the addition of urea during the domestication process significantly increases the reproductive characteristics and urease activity of *Bacillus megaterium*. The domestication process with the addition of 0.6 M urea was able to increase the $OD_{600}$ of the bacteria by 60% and the urease activity by 130% in an alkaline environment at pH = 10. The increase in urease activity sets the stage for an increase in calcium production rates. Calcium production rates of *Bacillus megaterium* in different pH environments after domestication are shown in Figure 10.

As shown in Figure 10, the microbially induced calcium carbonate precipitation yield increased with the addition of urea after the strain was domesticated in an alkaline environment. The most significant increase in calcium carbonate precipitation yield was achieved when urea was added at 0.6 M. The calcium carbonate yield of *Bacillus megaterium* was about 80% higher than that without urea, showing that this method was effective in solving the problem of insufficient calcium carbonate precipitation under strong alkaline conditions. The addition of urea helped *Bacillus megaterium* to adapt more quickly to the alkaline environment and to multiply, thus increasing the yield of calcium carbonate precipitation.

*3.4. Research on Concrete Cracks Repair by MICP.* As the concrete broke differently on different faces during the strength test, the penetration of each face was recorded separately by the device shown in Figure 3 as shown in Table 4.

The blocks used for testing were subjected to MICP according to the method shown in Figure 4. The test divided the four blocks into two groups, one with untamed *Bacillus megaterium* and one with tamed *Bacillus megaterium*. After MICP-based concrete crack repair tests, the 18 cm waterhead penetration times for each face of concrete are shown in Table 5.

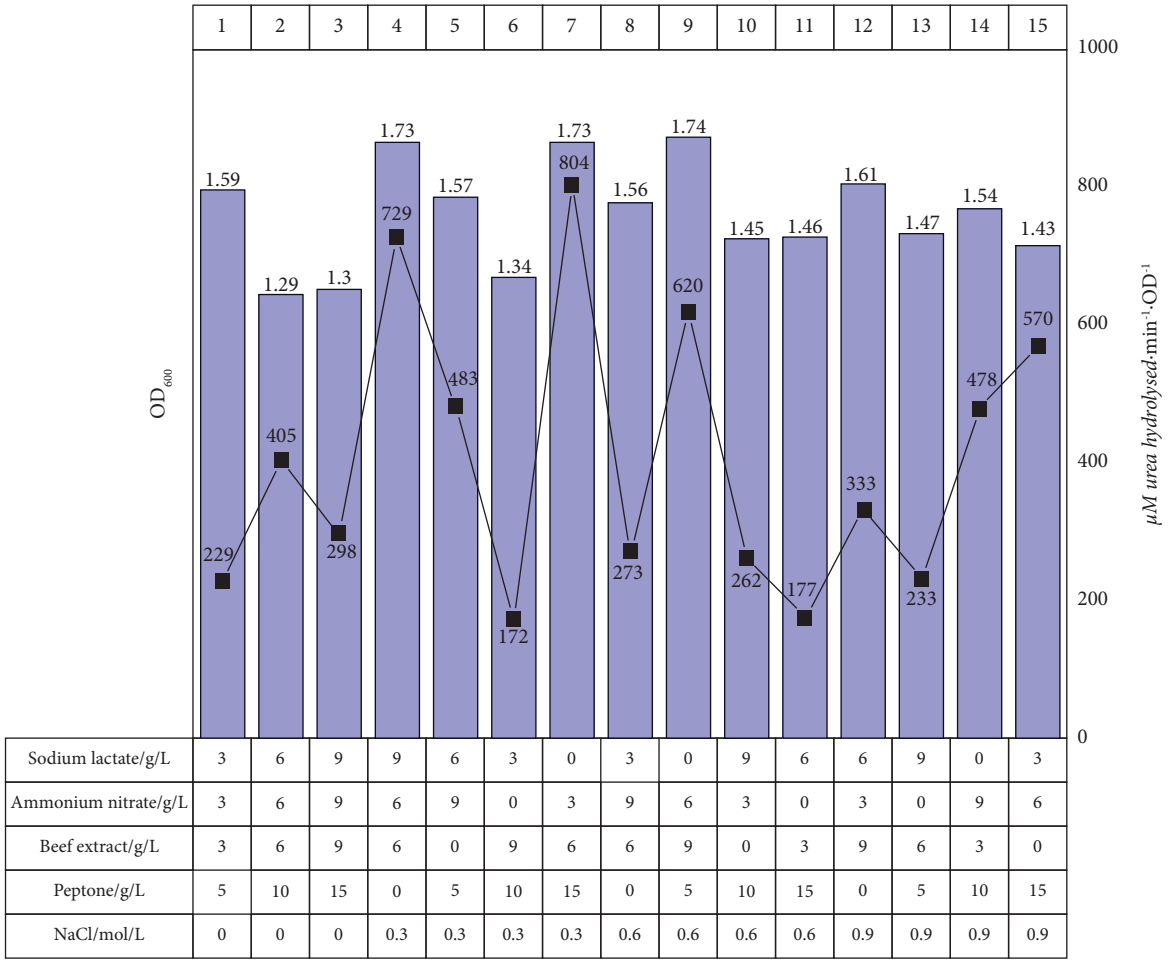


Figure 5: The results of the orthogonal test.

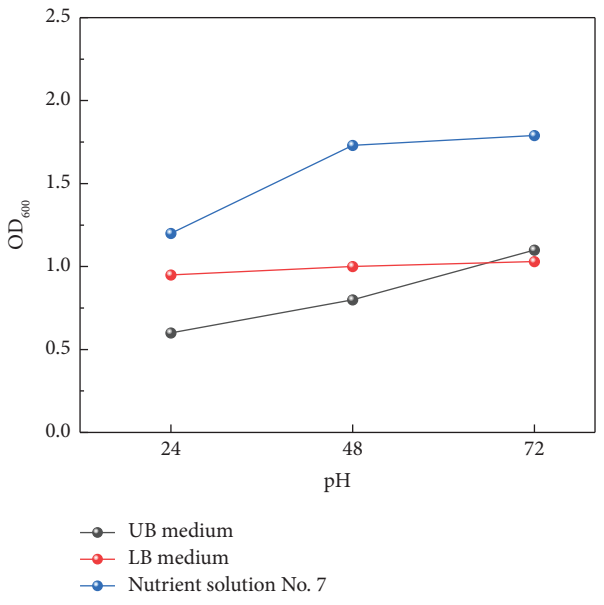


Figure 6: Comparison of common nutrient solution formulations.

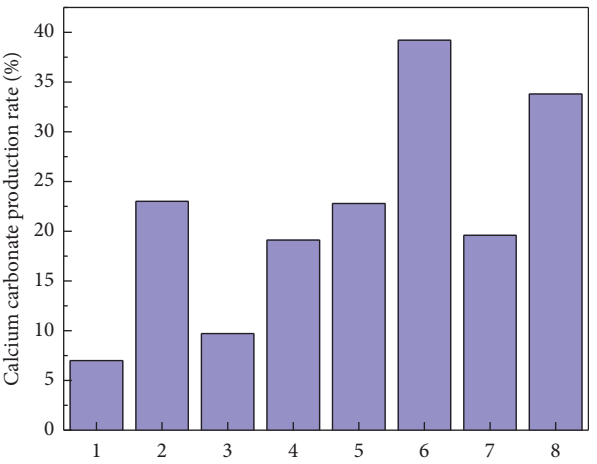


Figure 7: Calcium carbonate yield test results.

Table 5 shows the time taken for 18 cm of water to penetrate through the damaged concrete after MICP, and the water penetration characteristics of the concrete were

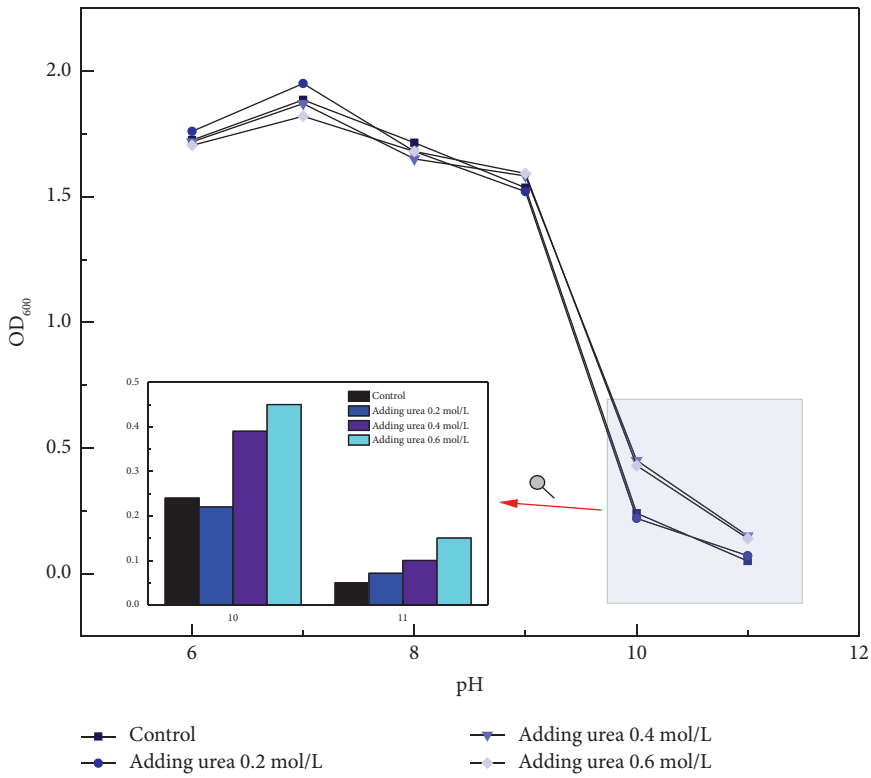


Figure 8: Domestication effect of adding different urea ($OD_{600}$).

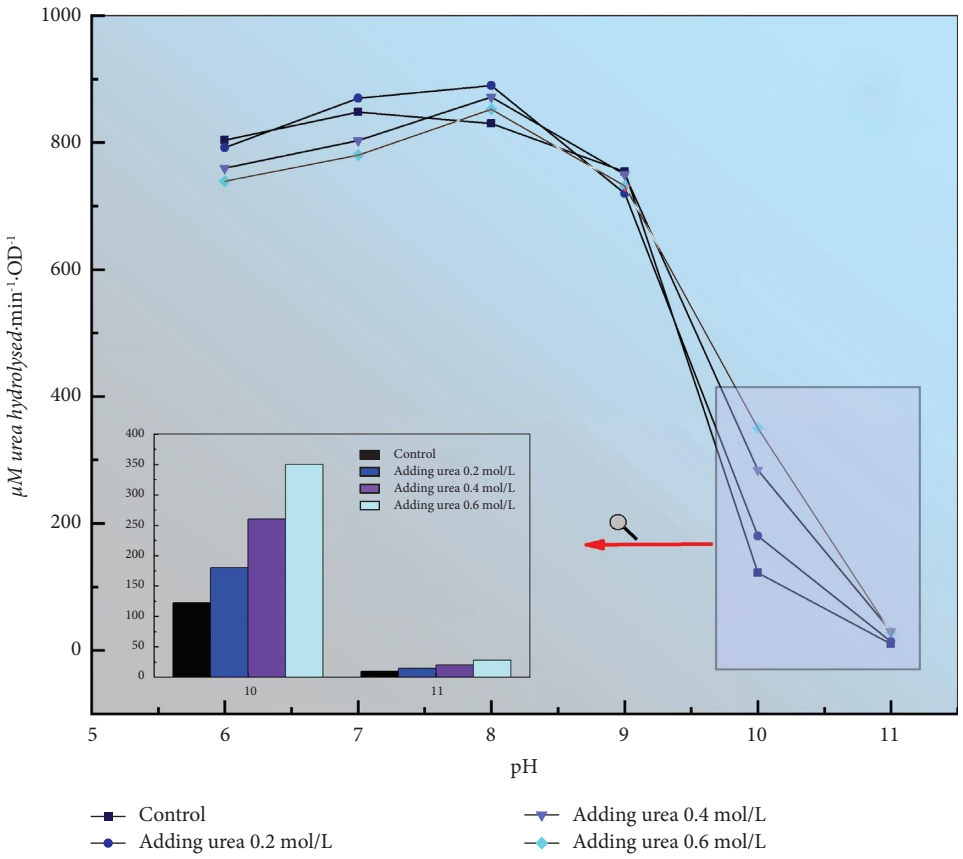


Figure 9: Domestication effect of adding different urea (urease activity).

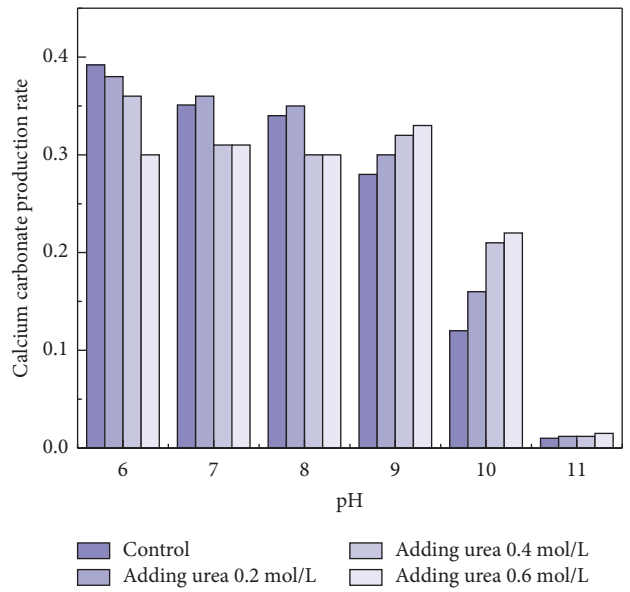


Figure 10: Calcium production rates of microorganisms with different urea additions.

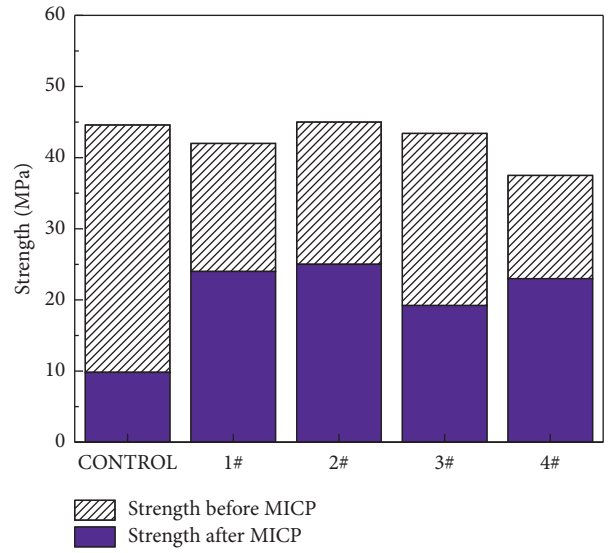


Figure 11: Plot of concrete strength change after MICP using domesticated and untamed *Bacillus megaterium*, respectively.

Table 4: Time taken for water penetration on each side of concrete before MICP.

| Test block number | *A*-side | *B*-side | *C*-side | *D*-side | *E*-side | *F*-side |
|---|---|---|---|---|---|---|
| 1# | — | 12 | — | — | 50 | 260 |
| 2# | 160 | — | 220 | — | 16 | 11 |
| 3# | 200 | 170 | 2 | — | 10 | 15 |
| 4# | 10 | 14 | 280 | 200 | — | — |

*Note.* Penetration times over 360 min are marked as "—."

Table 5: Time taken for water penetration on each side of concrete after MICP.

| Test block number | *A*-side | *B*-side | *C*-side | *D*-side | *E*-side | *F*-side |
|---|---|---|---|---|---|---|
| 1# | — | 40 | — | — | 215 | — |
| 2# | 240 | — | — | — | 23 | 18 |
| 3# | — | 410 | 5 | — | 42 | 50 |
| 4# | — | 210 | — | — | — | — |

*Note.* Penetration times over 360 min are marked as "—."

reduced after MICP with both bacterial solutions. In general, the microorganisms domesticated by strong alkali were better able to repair the concrete cracks by MICP, especially in test block 4 where only the original maximum fracture surface was water infiltrated after 210 min, while all other surfaces were infiltrated for more than 6 h. The *C* side of test block 3 was not as effective as expected due to the large width of the cracks, while for all other surfaces, the repair effect was better.

The repaired concrete was repositioned on a pressure test machine to test the strength of the test blocks, and the results are shown in Figure 11.

As can be seen in Figure 11, the strength of the concrete was increased after MICP, with the use of the alkaline domesticated microorganisms being 2.1 times more effective in repairing the strength of the concrete than the unmoderated microorganisms (CONTROL).

The above tests show that MICP is more effective at repairing microcracks in concrete that can be accessed by liquid penetration but less effective at repairing wider cracks. The alkaline domestication of *Bacillus megaterium* improved the viability and urease activity of the strain in the concrete, allowing for more efficient MICP repair of cracks. The residual strength of the concrete was significantly improved after the cracks were repaired.

At the end of the compressive strength test, the postdamage specimens were observed, and the fracture surface was carried out along the fracture surface produced by the first compressive strength. The fracture surfaces of the postdamage specimens all had white precipitated material on them, which was significantly different from the grey concrete sections. When dilute hydrochloric acid was added dropwise to the concrete material, there was no obvious reaction, whereas when it was added to the white part, a large number of bubbles were generated and the white material dissolved, as shown in Figure 12. Thus, it can be basically judged that the white precipitate is a calcium carbonate precipitate produced by the MICP repair.

In summary, cracks in concrete can be repaired by MICP to help restore some of the strength and impermeability of the damaged concrete. However, it is important to note that ammonia is produced during the MICP process, which can have an environmental impact. There are three general approaches to this problem: 1. ammonia is reduced from the reaction process by using porous materials with adsorption capabilities, such as zeolites, to adsorb ammonia [48]. 2. By adding $HPO_4^{2-}$ and $Mg^{2+}$ instead of $Ca^{2+}$ to the bacterial suspension (BS), $NH^{4+}$ precipitates as $MgNH_4PO_4{\cdot}6H_2O$. Like the carbonate from MICP, the phosphate precipitate can also be used as a cement, but the resulting cured sand has a lower strength compared to MICP [48]. 3. Some alternative MICP routes can avoid ammonia production, such as nitrate

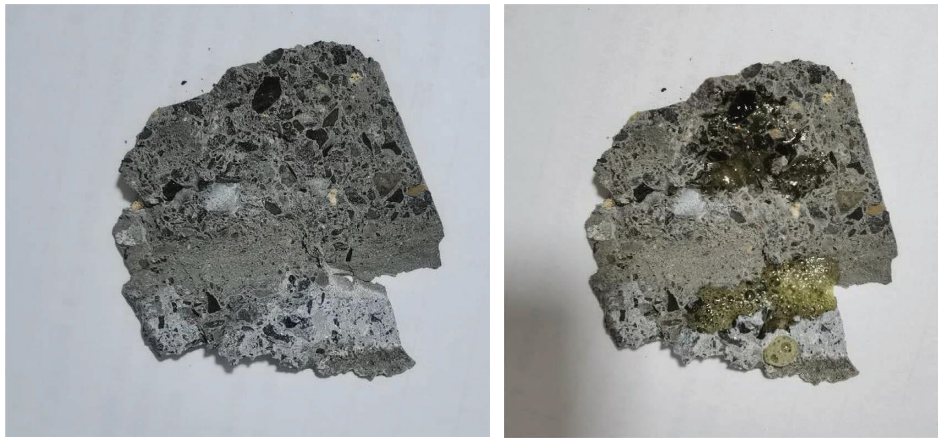

Figure 12: Cracking before and after the addition of dilute hydrochloric acid drops.

reduction, sulphate reduction, iron reduction, and methoxide reduction [32, 49–52]. In contrast to urea hydrolysis, these pathways are carried out by anaerobic microorganisms, which indirectly produce MICP at a lower rate and require more stringent conditions to maintain activity.

## 4. Summary

The application of microorganisms in concrete crack repair has been widely recognized in the engineering community, but the alkaline environment in concrete has become a bottleneck preventing the promotion of this technology. In this paper, extensive experiments were carried out to improve the growth properties of *Bacillus megaterium* at different values of pH, as well as the reproduction properties and urease activity of the strain in alkaline environments, by means of strong alkaline domestication.

(1) The optimal nutrient formula for *Bacillus megaterium* was determined by orthogonal testing, which improved the reproductive performance of the microorganism compared to normal media.

(2) Higher values of pH are associated with slower growth and reproduction of *Bacillus megaterium* and lower urease activity, and the high pH environment could significantly inhibit the growth and reproduction of *Bacillus megaterium* and urease activity. In the environment of pH 7, the growth and multiplication of *Bacillus megaterium* were the fastest, and the urease activity was the strongest.

(3) In the process of alkaline domestication of *Bacillus megaterium*, the increase of urea can significantly improve the reproduction characteristics, urease activity, and calcium production rate of microorganisms in an alkaline environment, and this method can effectively solve the problem of insufficient calcium carbonate precipitation under strong alkaline conditions.

(4) The alkaline domesticated *Bacillus megaterium* was able to reduce the water penetration characteristics of the concrete better than the untamed *Bacillus megaterium*, increasing the strength repair effect by a factor of 2.1.

## Data Availability

All data generated or analysed during this study are included in this published article.

## Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

## Consent

Consent is not applicable.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

WS made a contribution to conduct the experimental test, analysed the test results, and drafted the manuscript. MW made a contribution to the conception, to design of the work, and to revise the manuscript. LW, XX, MW, and TL made a contribution to conduct the experimental test.

## Acknowledgments

This investigation was supported by the Henan Youth Science Fund Project (20230041034), National Key R&D Program of China (2018YFB1600700), and National Natural Science Foundation of China (51578147). The authors thank the Henan Youth Science Fund Project for designing the study and writing the manuscript and the National Natural Science Foundation of China for the collection of data.

## References

[1] A. R. Suleiman, A. J. Nelson, and M. L. Nehdi, "Visualization and quantification of crack self-healing in cement-based materials incorporating different minerals," *Cement and Concrete Composites*, vol. 103, pp. 49–58, 2019.

[2] C. Dry, "KL-2 factors affecting self repairing of composites," *The Proceedings of the JSME Materials and Processing Conference (M&P)*, vol. 10.1, pp. 343–351, 2002.

[3] G. Lefever, D. Snoeck, D. G. Aggelis, N. De Belie, S. Van Vlierberghe, and D. Van Hemelrijck, "Evaluation of the self-healing ability of mortar mixtures containing superabsorbent polymers and nanosilica," *Materials*, vol. 13, no. 2, p. 380, 2020.

[4] L. Wang, G. Li, X. Li et al., "Influence of reactivity and dosage of MgO expansive agent on shrinkage and crack resistance of face slab concrete," *Cement and Concrete Composites*, vol. 126, Article ID 104333, 2022.

[5] L. Wang, T. He, Y. Zhou et al., "The influence of fiber type and length on the cracking resistance, durability and pore structure of face slab concrete," *Construction and Building Materials*, vol. 282, Article ID 122706, 2021.

[6] L. Wang, Z. Yu, B. Liu, F. Zhao, S. Tang, and M. Jin, "Effects of fly ash dosage on shrinkage, crack resistance and fractal characteristics of face slab concrete," *Fractal and Fractional*, vol. 6, no. 6, p. 335, 2022.

[7] F. G. Ferris, L. G. Stehmeier, A. Kantzas, and F. M. Mourits, "Bacteriogenic mineral plugging," *Journal of Canadian Petroleum Technology*, vol. 35, no. 08, 1996.

[8] Y. Wu, J. B. Ajo-Franklin, N. Spycher et al., "Geophysical monitoring and reactive transport modeling of ureolytically-driven calcium carbonate precipitation," *Geochemical Transactions*, vol. 12, no. 1, pp. 7–20, 2011.

[9] J. Jiang, X. Zheng, S. Wu, Z. Liu, and Q. Zheng, "Nondestructive experimental characterization and numerical simulation on self-healing and chloride ion transport in cracked ultra-high performance concrete," *Construction and Building Materials*, vol. 198, pp. 696–709, 2019.

[10] N. K. Dhami, M. S. Reddy, and A. Mukherjee, "*Bacillus megaterium* mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials," *World Journal of Microbiology and Biotechnology*, vol. 29, no. 12, pp. 2397–2406, 2013.

[11] M. Bagga, C. Hamley-Bennett, A. Alex et al., "Advancements in bacteria based self-healing concrete and the promise of modelling," *Construction and Building Materials*, vol. 358, Article ID 129412, 2022.

[12] E. Bäuerlein, "Biomineralization of unicellular organisms: an unusual membrane biochemistry for the production of inorganic nano-and microstructures," *Angewandte Chemie International Edition*, vol. 42, no. 6, pp. 614–641, 2003.

[13] S. Douglas and T. J. Beveridge, "Mineral formation by bacteria in natural microbial communities," *FEMS Microbiology Ecology*, vol. 26, no. 2, pp. 79–88, 1998.

[14] V. Achal, A. Mukherjee, and M. Sudhakara Reddy, "Microbial Concrete way to enhance the durability of building structures," *Cell*, vol. 2, p. 2, 2011.

[15] W. De Muynck, D. Debrouwer, N. De Belie, and W. Verstraete, "Bacterial carbonate precipitation improves the durability of cementitious materials," *Cement and Concrete Research*, vol. 38, no. 7, pp. 1005–1014, 2008.

[16] N. Chahal, R. Siddique, and A. Rajor, "Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete," *Construction and Building Materials*, vol. 28, no. 1, pp. 351–356, 2012.

[17] J. Y. Wang, H. Soens, W. Verstraete, and N. De Belie, "Self-healing concrete by use of microencapsulated bacterial spores," *Cement and Concrete Research*, vol. 56, pp. 139–152, 2014.

[18] R. Andalib, M. Z. Abd Majid, M. W. Hussin et al., "Optimum concentration of Bacillus megaterium for strengthening structural concrete," *Construction and Building Materials*, vol. 118, pp. 180–193, 2016.

[19] M. Wu, X. Hu, Q. Zhang, D. Xue, and Y. Zhao, "Growth environment optimization for inducing bacterial mineralization and its application in concrete healing," *Construction and Building Materials*, vol. 209, pp. 631–643, 2019.

[20] X. M. Wang, W. Guo, and F. Yu, "Experimental study of effect of nutrient concentration on physico-mechanical properties of cemented sand," *Rock and Soil Mechanics*, vol. 37, no. S2, pp. 363–368, 2016.

[21] S. A. Abo-El-Enein, A. H. Ali, F. N. Talkhan, and H. A. Abdel-Gawwad, "Utilization of microbial induced calcite precipitation for sand consolidation and mortar crack remediation," *HBRC Journal*, vol. 8, no. 3, pp. 185–192, 2012.

[22] V. Wiktor and H. M. Jonkers, "Quantification of crack-healing in novel bacteria-based self-healing concrete," *Cement and Concrete Composites*, vol. 33, no. 7, pp. 763–770, 2011.

[23] J. Xu, W. Yao, and Z. Jiang, "Non-ureolytic bacterial carbonate precipitation as a surface treatment strategy on cementitious materials," *Journal of Materials in Civil Engineering*, vol. 26, no. 5, pp. 983–991, 2014.

[24] V. Achal and X. Pan, "Influence of calcium sources on microbially induced calcium carbonate precipitation by *Bacillus* sp," *Applied Biochemistry and Biotechnology*, vol. 173, no. 1, pp. 307–317, 2014.

[25] J. Zhang, Y. Liu, T. Feng et al., "Immobilizing bacteria in expanded perlite for the crack self-healing in concrete," *Construction and Building Materials*, vol. 148, pp. 610–617, 2017.

[26] C. Rodriguez-Navarro, F. Jroundi, M. Schiro, E. Ruiz-Agudo, and M. T. González-Muñoz, "Influence of substrate mineralogy on bacterial mineralization of calcium carbonate: implications for stone conservation," *Applied and Environmental Microbiology*, vol. 78, no. 11, pp. 4017–4029, 2012.

[27] L. Chaurasia, V. Bisht, L. P. Singh, and S. Gupta, "A novel approach of biomineralization for improving micro and macro-properties of concrete," *Construction and Building Materials*, vol. 195, pp. 340–351, 2019.

[28] W. De Muynck, K. Verbeken, N. De Belie, and W. Verstraete, "Influence of temperature on the effectiveness of a biogenic carbonate surface treatment for limestone conservation," *Applied Microbiology and Biotechnology*, vol. 97, no. 3, pp. 1335–1347, 2013.

[29] X. Sun, L. Miao, L. Wu, and R. Chen, "Improvement of biocementation at low temperature based on Bacillus megaterium," *Applied Microbiology and Biotechnology*, vol. 103, no. 17, pp. 7191–7202, 2019.

[30] Y. N. Wang, B. X. Zhang, and B. S. Jiang, "Study of solidification technology of marine sludge by MICP combined with portland cement," in *Belt and Road Webinar Series on Geotechnics, Energy And Environment*, pp. 258–270, Springer, Singapore, 2021, March.

[31] L. Jiang, W. Lu, W. Wang, Y. Zhang, Q. Han, and Z. Li, "Mechanical properties and frost resistance of self-healing concrete based on expended perlite immobilized bacteria," *Construction and Building Materials*, vol. 348, Article ID 128652, 2022.

[32] F. Su and Y. Y. Yang, "Microbially induced carbonate precipitation via methanogenesis pathway by a microbial consortium enriched from activated anaerobic sludge," *Journal of Applied Microbiology*, vol. 131, no. 1, pp. 236–256, 2021.

[33] S. Stocks-Fischer, J. K. Galinat, and S. S. Bang, "Microbiological precipitation of CaCO3," *Soil Biology and Biochemistry*, vol. 31, no. 11, pp. 1563–1571, 1999.

[34] V. S. Whiffin, L. A. Van Paassen, and M. P. Harkes, "Microbial carbonate precipitation as a soil improvement technique," *Geomicrobiology Journal*, vol. 24, no. 5, pp. 417–423, 2007.

[35] L. B. Van Paassen, *Ground Improvement by Microbially Induced Carbonate Precipitation*, Delft University of Technology, Delft, Netherlands, 2009.

[36] B. C. Martinez, J. T. DeJong, T. R. Ginn et al., "Experimental optimization of microbial-induced carbonate precipitation for soil improvement," *Journal of Geotechnical and Geoenvironmental Engineering*, vol. 139, no. 4, pp. 587–598, 2013.

[37] R. Attri, A. Shukla, V. Sharma, and A. Thakur, "Influence of Bacillus megaterium on crack he*aling and mechanica*l properties of concrete," in *Proceedings of the International Conference on Innovative Technologies For Clean And Sustainable Development*, pp. 139–155, Springer, Cham, 2020, February.

[38] J. K. Fredrickson and M. Fletcher, *Subsurface Microbiology and Biogeochemistry*, Wiley & Sons, New York, NY, 2001.

[39] A. I. Omoregie, G. Khoshdelnezamiha, N. Senian, D. E. L. Ong, and P. M. Nissom, "Experimental optimisation of various cultural conditions on urease activity for isolated *Sporosarcina pasteurii s*trains and evaluation of their biocement potentials," *Ecological Engineering*, vol. 109, pp. 65–75, 2017.

[40] Y. Wang, C. Konstantinou, K. Soga, G. Biscontin, and A. J. Kabla, "Use of microfluidic experiments to optimize MICP treatment protocols for effective strength enhancement of MICP-treated sandy soils," *Acta Geotechnica*, vol. 17, no. 9, pp. 3817–3838, 2022.

[41] Q. Zhao, "Experimental study on soil improvement using microbial induced calcite precipitation (MICP)," China Univ. of Geosciences, China, 2014.

[42] W. S. Ng, M. L. Lee, and S. L. Hii, "An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement," *International Journal of Civil & Environmental Engineering*, vol. 6, no. 2, pp. 188–194, 2012.

[43] M. Chen, S. Gowthaman, K. Nakashima, and S. Kawasaki, "Evaluatingmechanical strength of peat soil treated by fiber incorporated BIO-cementation," *International Journal of GEOMATE*, vol. 20, no. 78, pp. 121–127, 2021.

[44] S. Gowthaman, M. Chen, K. Nakashima, and S. Kawasaki, "Effect of scallop powder addition on MICP treatment of amorphous peat," *Frontiers in Environmental Science*, vol. 9, 2021.

[45] H. Yamaguchi and M. Miyazaki, "Refolding techniques for recovering biologically active recombinant proteins from inclusion bodies," *Biomolecules*, vol. 4, no. 1, pp. 235–251, 2014.

[46] M. U. Safdar, M. Mavroulidou, M. J. Gunn, J. Garelick, I. Payne, and D. Purchase, "Innovative methods of ground improvement for railway embankment peat fens foundation soil," *Géotechnique*, vol. 71, no. 11, pp. 985–998, 2021.

[47] X. Sun, *Study on mechanism of microbial mineralization and application of it to repair concrete cracks. Chinese Doctor's Thesis*, Southeast University, Dhaka, Bangladesh, 2018.

[48] X. Yu, Q. Zhan, C. Qian, J. Ma, and Y. Liang, "The optimal formulation of bio-carbonate and bio-magnesium phosphate cement to reduce ammonia emission," *Journal of Cleaner Production*, vol. 240, Article ID 118156, 2019.

[49] O. Braissant, A. W. Decho, C. Dupraz, C. Glunk, K. M. Przekop, and P. T. Visscher, "Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals," *Geobiology*, vol. 5, no. 4, pp. 401–411, 2007.

[50] V. Stabnikov and V. Ivanov, "Biotechnological production of biogrout from iron ore and cellulose," *Journal of Chemical Technology and Biotechnology*, vol. 92, no. 1, pp. 180–187, 2017.

[51] L. A. Van Paassen, C. M. Daza, M. Staal, D. Y. Sorokin, W. van der Zon, and M. C. van Loosdrecht, "Potential soil reinforcement by biological denitrification," *Ecological Engineering*, vol. 36, no. 2, pp. 168–175, 2010.

[52] F. Su, Y. Yang, Y. Qi, and H. Zhang, "Combining microbially induced calcite precipitation (MICP) with zeolite: a new technique to reduce ammonia emission and enhance soil treatment ability of MICP technology," *Journal of Environmental Chemical Engineering*, vol. 10, no. 3, Article ID 107770, 2022.