

Improvement of the Sand Quality by Applying Microorganism-induced Calcium Carbonate Precipitation to Reduce Cement Usage

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Abstract: - This research determines the Microbially Induced Calcium Carbonate Precipitation (MICP) process utilized by the bacteria found in Thailand. Many researchers typically use the high-efficiency MICP bacteria to precipitate calcium carbonate. However, it is only available in some countries, leading to a high import expense. Therefore, the methodology for using the bacteria capable of producing calcium carbonate in Thailand was investigated. The five pure bacteria strains are obtained from the Thailand Institute of Scientific and Technological Research (TISTR), i.e., Proteus mirabilis TISTR 100, Bacillus thuringiensis TISTR 126, Staphylococcus aureus TISTR 118, Bacillus sp. TISTR 658 and Bacillus megaterium TISTR 067. To screen urease production, the bacteria were spread on Christensen's Urea Agar (UA) slant surface via a colorimetric method. All bacteria strains can produce urease enzymes by observing the color changes in the UA. Berthelot's method was used to determine the urease activity. The result shows the bacteria's urease activity: 2389, 1989, 1589, 789, and 589 U/ml, respectively. These directly lead to calcium carbonate production: 3.430, 3.080, 2.590, 1.985, and 1.615 mg/ml, respectively. Despite the bacteria in this research having a low precipitation efficiency compared to the strain used in many research studies, they can improve sand stabilization in 7 days. Proteus mirabilis TISTR 100 was the most stable and effective strain for the MICP process in Thailand. Hence, this research reveals the ability of the local bacteria to bond with the sand particle. Briefly, the improvement of the MICP process in sand stabilization can be improved to reduce imported expenses. In addition, the MICP process can reduce the use of cement in sand stabilization work.

Key-Words: - MICP, Cement consumption, Biofilm, Improvement, Ureolytic bacteria, Cemented sand.

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1 Introduction

The current world is facing the issue of global warming, partly caused by environmental degradation resulting from the mining of limestone to produce cement. This process leads to pollution, such as dust and air pollution, and the destruction of natural habitats for wildlife and forests. Hence, the researchers have discovered the significance of the combination of biotechnology and industrial construction in developing innovative approaches to reduce cement usage for environmental sustainability.

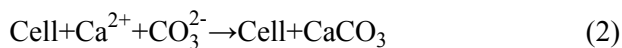
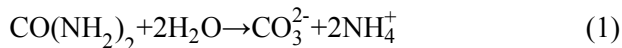
Microbially induced carbonate precipitation (MICP) may be defined as one environmentally

friendly approach that can improve the properties of soil and sand, [1], [2]. The MICP method uses the ureolytic bacteria which can produce the enzyme urease to accelerate the process of urea hydrolysis, leading to the formation of calcium carbonate, [3], [4].

This research selected the pure strain of each bacteria, mainly within Thailand from the Thailand Institute of Scientific and Technological Research (TISTR). The growth conditions of the bacteria strain were controlled by maintaining temperature and adjusting the pH value. The optimal conditions to ensure the production of urease enzymes, temperature, and pH are the major roles in inducing

calcium carbonate precipitation from various calcium sources, such as calcium lactate, calcium nitrate, [5], [6] and calcium chloride [7]. Calcium chloride is typically used in MICP processes due to the higher rate of calcium carbonate precipitation than the other sources, [8].

The MICP mechanism starts with the catalyzation of urea hydrolysis by urease activity. This reaction generates the carbonate and ammonium compounds forming, as shown in Equation 1. Subsequently, the presence of ammonium increases the pH value of the solution, promoting the precipitation of calcium carbonate, as shown in Equation 2, [9].



The MICP is mainly focused on calcium carbonate deposits in soil and sand to enhance their stability and reduce soil erosion, [10], [11], [12]. It can be used as a bonding between sand particles to improve their cohesiveness and formability, which is superior to untreated sand, [13], [14], [15], [16]. Furthermore, the bacterial biofilm formation effectively enhances the adhesiveness of the bacteria to sand surfaces. This biofilm formation also supports the efficacy of the MICP process, resulting in higher sand stability, [17], [18], [19], [20], [21].

This research aims to investigate the quality improvement of sand stabilization by applying the MICP process to precipitate calcium carbonate as a binding material between sand particles to enhance shape stabilization. This study potentially opts for the bacteria strains that found in Thailand. Experimental trials are conducted to determine the optimal concentration of calcium chloride solution that induces the highest calcium carbonate precipitation for various bacterial strains. These selected conditions will be further studied to improve the bonding characteristics and strength of the sand, which can be based on sustainable future developments in real-world construction.

2 Materials and Methods

2.1 Materials Preparation

The sample sand used in this research was obtained from the Chao Phraya River, Nonthaburi Province, Thailand. Sand passed through the sieve No. 30 and was retained on sieve No. 200 in a sieve shaker (RETSCH®, AS 200 Basic, Germany), which have a particle size within the range of 75-600 μm using a

sieve (RETSCH®, Woven Wire Mesh Sieves -Ø 200 mm., Germany) that complied with ASTM E11 standards, [21]. After that, the sand was washed thrice with deionized water (DI) and then dried in a hot air oven (Memmert, Universal oven UN30m, Germany) at 105°C for 48 hr.

The maximum dry density of the sand was conducted according to the ASTM D698 (Standard Efford Compaction), [22] and it was found that the sand in this research had an optimum moisture content of 17.5% and a maximum dry density of 1.60 g/cm³.

2.2 Medium Preparation and Cultural Method

2.2.1 Cultural Medium Preparation

The preparation of cultural medium using Nutrient Broth containing urea (NBU) began by weighing 13 g of the Nutrient Broth powder (HIMEDIA®, M002, India) and dissolving it in 1000 ml of distilled water. Then, 20 g of urea (KEMAUS, Australia) was added, and the medium was dissolved completely using a hot plate and magnetic stirrer (IKA®, C-MAG HS 7, Germany). Next, 100 ml of the prepared NBU was transferred into a 250-ml Erlenmeyer flask and then sterilized in an autoclave (HIRAYAMA, HICLAVE HVA-110, Japan) at a temperature of 121°C and a steam pressure of 15 psi for 15 min.

2.2.2 Urease Test

The urease screening by Christensen's Urea Agar (UA) (HIMEDIA®, M112, India) was prepared by dissolving 24 g of nutrient powder in 950 ml of distilled water. The medium was dissolved completely and sterilized in the autoclave at a temperature of 115°C and a steam pressure of 10 psi for 20 min. Afterward, the solution was left cool to 50°C.

The concentrated urea solution of 40 %w/v 50 ml was aseptically added to the UA. Finally, the UA 5 ml was transferred into the empty sterilized vials, and the vials were placed at an inclined angle of 30 degrees to allow for proper solidification (agar slant).

The urease screening by Stuarts' Urea Broth (UB) (HIMEDIA®, M111, India) by completely dissolving 18.71 g of the nutrient powder in 950 ml of distilled water. Subsequently, the solution is sterilized in the autoclave at a temperature of 121°C with a steam pressure of 15 psi for 15 min. Afterward, the solution was left cool to 50°C.

The 50 ml of concentrated urea solution was aseptically added to the UB solution. Finally, the 5

ml of UB solution is divided into empty sterilized vials.

2.2.3 Biofilm Detection

The preparation of Tryptone Soya Broth (TSB) (HIMEDIA[®], M011, India) for biofilm-forming of the bacteria. By weighing 30 g of the nutrient powder and dissolving it in 1000 ml of distilled water using the hot plate and magnetic stirrer. Then, 100 ml of the TSB solution is transferred into a 250-ml Erlenmeyer flask and sterilized in the autoclave at a temperature of 121°C and a steam pressure of 15 psi for 15 min.

2.2.4 Calcium Source Preparation

The calcium source for calcium carbonate precipitation in the research is a calcium chloride dihydrate solution (KEMAUS, Australia). It was dissolved by DI water to adjust the concentrations range from 100 to 300 mM. The various concentrations are modified to determine the optimal conditions for producing calcium carbonate in each bacteria strain.

2.2.5 Bacteria Cultural Method

The selection of pure bacterial strains used in the experiments was conducted at the Thailand Institute of Scientific and Technological Research (TISTR). Five pure strains were chosen, including *P. mirabilis* TISTR 100, *B. thuringiensis* TISTR 126, *S. aureus* TISTR 118, *Bacillus* sp. TISTR 658, and *B. megaterium* TISTR 067. Cell activation was performed by adding freeze-dried cells in an ampoule to vials containing Nutrient Broth (NB) as the culture medium, with a volume of 5 ml (comprising NB at a concentration of 13 g/L). The cells were incubated at 37°C for 24 hr. in an incubator. Subsequently, the bacterial strains were inoculated into the NBU medium to achieve a cell density of 1%. The cultures were further incubated at 37°C for 24 hr.

Following incubation, each bacterial strain was subjected to a growth curve analysis. The growth rate was measured at regular intervals using a visible spectrophotometer (JENWAY[®], 7200, United Kingdom) with a wavelength of 600 nm. This analysis aimed to determine the maximum growth rate for each bacterial strain, which would be utilized in subsequent experimental steps. From the reading O.D.₆₀₀, calculate the cell concentration using Equation 3, [23].

$$Y = 8.59 \times 10^7 \cdot Z^{1.3627} \quad (3)$$

Where, Y is the cells concentration (cells/ml)
Z is the reading O.D.₆₀₀

2.3 Microbiological Analysis

2.3.1 Gram Staining Method

The glass slides were cleaned using 95% ethanol and then passed through the flame of a Bunsen burner using a slide holder. Following this, a loop sterilized by flame was used to transfer a small amount of bacterial-suspended solution onto the glass slide. The slide was allowed to air dry and then held at one end while passing through the flame of the Bunsen burner several times with the smear-side facing up. Afterward, the smear on the slide was stained with crystal violet for 1 min. The slide was then gently rinsed with distilled water. Next, Gram's iodine was applied to the smear for 1 min., followed by elution with 95% ethanol until the eluted solution ran almost clear. The slide was rinsed again with distilled water and then stained with safranin O for 1 min. Subsequently, the slide was rinsed with distilled water and blot-dried before being observed under an optical microscope (Nikon, Eclipse E100, Japan) at 100x magnification.

2.3.2 Biofilm Detection and Screening

To investigate biofilm formation by the Microtiter plate assay of different bacterial strains' attachment to calcium carbonate granules, [17]. Each strain of bacteria was cultured in TSB at 37°C for 24 hr. Subsequently, the bacterial strains were diluted to a concentration of 1% in TSB and dispensed into a 96-well Microtiter plate. Incubate the plate at 37°C for 24 hr. After incubation, the bacteria were washed twice with phosphate-buffered saline (PBS) and allowed to air dry for 1 hour. The biofilm staining is performed by adding 1% crystal violet solution (200 µl) for 5 min., resulting in a purple coloration. Subsequently, the stain was rinsed off with distilled water thrice. Finally, the absorbance of the wells with 95% ethanol (200 µl) measures at 570 nm using a microplate reader (Metertech, M965+, Taiwan)—this measurement allowed for the quantification of light absorption, indicating the extent of biofilm formation.

2.3.3 Urease Screening

The bacteria were cultured on the UA slant using a sterile loop to screen bacteria capable of producing urease for urea hydrolysis reaction between urea and calcium chloride to form calcium carbonate precipitate. The slants were then incubated at 37°C for 24 hr. to test for any color changes in the culture medium. A pinkish-red color change in the culture medium indicated a positive result (+).

2.3.4 Urease Activity

Using Berthelot's Method, the enzyme samples were collected from the bacterial culture medium in Section 2.2. The bacterial culture was transferred into tubes and then centrifuged at 4000 rpm for 30 min. at 4°C (Eppendorf, 5810R - Benchtop Centrifuge, Germany). After centrifugation, the supernatant with 250 µl was collected. From the collected sample, a 20-fold dilution of the supernatant is performed with DI water. Potassium phosphate buffer with a pH of 8 and a concentration of 100 mM (1 ml) was added. Then, a starting solution of urea with a concentration of 100 mM (2.5 ml) was added. The solution was incubated at 37°C for 5 min. in an incubator (Memmert, Universal oven UN30m, Germany). Subsequently, detection of ammonia production by MICP process was measured by adding 1 ml of Phenol nitroprusside (Phenol 1% (w/v) and Sodium nitroprusside 0.05% (w/v)) and 1 ml of Alkaline hypochlorite (Sodium hydroxide 0.5% (w/v) and Sodium hypochlorite 0.84% (v/v)) to catalyze ammonia production. The solution was further incubated at 37°C for 30 min. Then, the absorbance of the solution using the visible spectrophotometer at a wavelength of 626 nm. The data were further analyzed to determine the urease activity produced by each bacteria strain.

In the preparation of the standard ammonium chloride solution, ammonium chloride (Carlo Erba™, Germany) was dissolved in distilled water to achieve concentrations of 200, 400, 600, 800, and 1000 µmol. Then, each concentration of ammonium chloride solution was prepared the same as the enzyme solution. The absorbance of these solutions was measured at a wavelength of 626 nm against a blank solution. The obtained absorbance values were plotted on a graph representing the calibration curve of ammonium chloride solution, as shown in Figure 1.

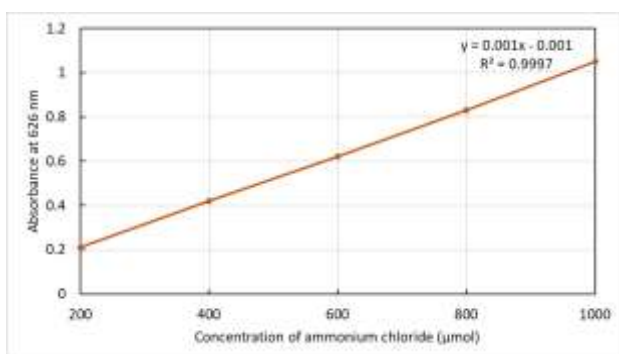


Fig. 1: Calibration curve of ammonium chloride

The absorbance to analyze urease activity for each bacteria strain was plotted on the calibration curve. Ammonium chloride concentration (X) in each bacteria strain was calculated using Equations 4 and 5 derived from the calibration curve of ammonium chloride. Subsequently, the urease activity was determined using Equation 6, utilizing the calculated concentration (X).

$$(y \times \text{dilution}) = ax + b \quad (4)$$

$$x = \frac{(y \times \text{dilution}) - b}{a} \quad (5)$$

Where, y is the reading O.D.₆₂₆
 X is an NH₄Cl (µmol)

$$\text{Urease activity} = \frac{X \times V_t}{V_{\text{sub.}} \times t_i \times V_{\text{smp.}}} \quad (6)$$

Where, V_t is the total volume (ml)
 V_{sub.} Is the volume of the substrate (ml)
 t_i is the incubation time (min.)
 V_{smp.} is the volume of enzyme (ml)

2.3.5 Calcium Carbonate Precipitation

Add the calcium chloride solution with a concentration of 100-300 mM, [15], to the bacterial-suspended solution to induce the precipitation of calcium carbonate. After adding the calcium chloride solution, incubate the resulting solution in an incubator at 37°C for 1-7 days. [8], filter the precipitate obtained using a vacuum filtration system through a Büchner funnel with Whatman No.4 filter paper. Then, dry the precipitate by subjecting it to hot air and drying it at 60°C for 24 hr.

2.3.6 Criteria for Microorganism Selection

Select the concentration of calcium chloride solution that produces the highest level of calcium carbonate precipitation among the five bacterial strains by weighing the dried precipitates obtained from each strain and comparing them.

2.3.7 Chemical Composition by SEM

This experiment was conducted to observe the microstructure and evaluate the chemical composition of the calcium carbonate precipitated using a Scanning Electron Microscope (SEM) with energy-dispersive X-ray Spectroscopy (EDS) (FEI QUANTA 450). The experiment captures the microstructure of the calcium carbonate precipitate at 1000x magnification.

2.4 Sand Sample Preparation

The sand samples obtained from Section 2.1 were molded into cube shapes with 5 cm in width, length, and height dimensions, as shown in Figure 2. The sample was achieved by taking 200 g of sand per sample, and three samples were prepared for each bacterial strain. The sand was then mixed with the bacterial-suspended solution obtained from Section 2.2.3, with a bacterial volume of 35 ml. The sand was compacted into molds, providing a height of 5 cm. Subsequently, an optimum concentration of calcium chloride solution, selected based on the criteria outlined in Section 2.3.6, was added to the sand. The volume of the calcium chloride solution was also 35 ml, marking the completion of one cycle. Following this, a mixture containing NBU and a calcium chloride solution, each with a volume

of 35 ml, was added to the molds every 24 hr. for 7 days.



Fig. 2: Casting mold

3 Results

3.1 Growth Curve

Based on the study, growth rates were determined by measuring the light absorbance at a wavelength of 600 nm, and cell concentration was calculated as shown in Table 1.

Table 1. Maximum growth rate

Microorganisms	Maximum growth rate (hr.)	Cell concentration (cells/ml)
<i>P. mirabilis</i> TISTR 100	12	1.60×10^8
<i>B. thuringiensis</i> TISTR 126	32	1.98×10^8
<i>S. aureus</i> TISTR 118	22	1.61×10^8
<i>Bacillus</i> sp. TISTR 658	17	1.75×10^8
<i>B. megaterium</i> TISTR 067	30	1.91×10^8

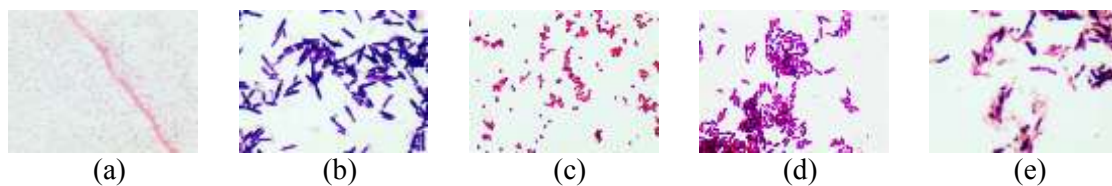


Fig. 3: Gram strained bacteria: (a) *P. mirabilis* TISTR 100, (b) *B. thuringiensis* TISTR 126, (c) *S. aureus* TISTR 118, (d) *Bacillus* sp. TISTR 658, and (e) *B. megaterium* TISTR 067

Table 2. Gram stain

Microorganisms	Gram
<i>P. mirabilis</i> TISTR 100	-
<i>B. thuringiensis</i> TISTR 126	+
<i>S. aureus</i> TISTR 118	+
<i>Bacillus</i> sp. TISTR 658	+
<i>B. megaterium</i> TISTR 067	+

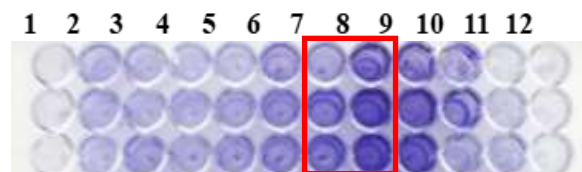


Fig. 4: 96-well plate of biofilm detection. Where 1 and 12 are control (blank) 2-3: biofilm formation from *P. mirabilis* TISTR 100, 4-5: *B. thuringiensis* TISTR 126, 6-7: *B. megaterium* TISTR 067, 8-9: *Bacillus* sp. TISTR 658, and 10-11: *S. aureus* TISTR 118

3.2 Gram Strain

Based on the study, Gram staining following the method of Hans Christian Gram was performed, [24] and observations were made using a microscope with a magnification of 100x. The results are shown in Figure 3, indicating that among the five bacterial strains examined, *P. mirabilis* TISTR 100 was the only strain that exhibited Gram-negative (-) characteristics, as shown in Table 2.

3.3 Biofilm Detection and Screening

From the results of the experiment on biofilm formation, as shown in Figure 4, it was observed that each bacterial strain could produce biofilms. This phenomenon can be attributed to the binding of crystal violet, which affects the adhesion of bacteria and the resulting production of calcium carbonate.

Intense staining indicates higher biological adhesion compared to faded staining, [25].

The measurement of O.D.₅₇₀, as shown in Table 3, confirms these findings.

3.4 Urease Screening and Activity

It was observed that all bacterial strains could produce the enzyme urease, which catalyzes the hydrolysis of urea. This reaction occurs with the presence of urea as the substrate in the medium, increasing the pH of UA and UB. Consequently, the color of the medium changes from the control (-) to a positive (+) result, exhibiting a pinkish-red color, [26], as depicted in Figure 5.

Table 4 shows the urease activity using Berthelot's method. The result was found that *P. mirabilis* TISTR 100 had the maximum urease activity, approximately 2389 U/ml.

Table 3. The reading O.D.₅₇₀ (nm) of biofilm

1	2	3	4	5	6	7	8	9	10	11	12
0.073	0.273	0.239	0.218	0.188	0.245	0.302	0.638	0.582	0.316	0.170	0.102
0.103	0.220	0.172	0.201	0.213	0.238	0.332	0.468	0.543	0.387	0.193	0.107
0.101	0.175	0.237	0.199	0.215	0.218	0.267	0.357	0.512	0.177	0.225	0.085

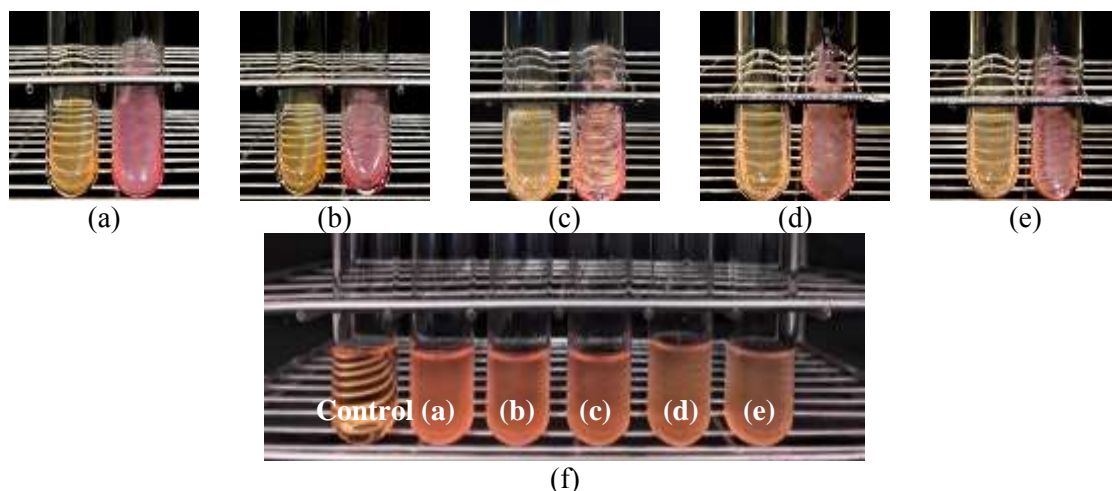


Fig. 5: Urease screening on UA: (a) *P. mirabilis* TISTR 100, (b) *B. thuringiensis* TISTR 126, (c) *S. aureus* TISTR 118, (d) *Bacillus* sp. TISTR 658, (e) *B. megaterium* TISTR 067, and (f) urease screening on UB of all bacteria strains

Table 4. Urease activity of each microorganism

Microorganisms	Urease activity (U/ml)
<i>P. mirabilis</i> TISTR 100	2389
<i>B. thuringiensis</i> TISTR 126	1989
<i>S. aureus</i> TISTR 118	1589
<i>Bacillus</i> sp. TISTR 658	789
<i>B. megaterium</i> TISTR 067	589

3.5 Calcium Carbonate Precipitation

The induction of calcium carbonate precipitation through microbial calcification was investigated in the experiment outlined in Section 2.3.5. Interestingly, distinct variations in the characteristics of the calcium carbonate precipitates were observed among the different bacterial strains, as illustrated in Figure 6.

Furthermore, it was found that the maximum yielding of calcium carbonate precipitation, represented by the formation of precipitates, differed among the various strains of bacteria in the presence of calcium chloride as the inducing agent. The calcium carbonate precipitation rate is directly increased with urease activity. Therefore, the maximum calcium carbonate precipitation rate was found in *P. mirabilis* TISTR 100, followed by *B. thuringiensis* TISTR 126, *S. aureus* TISTR 118, *Bacillus sp.* TISTR 658, and *B. megaterium* TISTR 067. This phenomenon occurred at a pH range of 7.3 to 8.8, as depicted in Figure 7.

3.6 Chemical Composition by SEM

From the result, the calcium carbonate precipitate from each bacteria has a different shape. The physical shape was classified into three groups: regular, round, and irregular compared to the

analytical grade calcium carbonate (KEMAUS). The regular group comprises analytical grade calcium carbonate (a small rhombohedral), *B. thuringiensis* TISTR 126, and *B. megaterium* TISTR 067 were found in a regular shape with a bigger particle than the control, as shown in Figure 8a), Figure 8c) and Figure 8f).

The round group comprises *P. mirabilis* TISTR 100 and *Bacillus sp.* TISTR 658. However, the calcium carbonate precipitated showed a different physical shape in the group, for *P. mirabilis* TISTR 100 precipitated with a small round particle, and *Bacillus sp.* TISTR 658 precipitated with a round, flat appearance, as shown in Figure 8b) and Figure 8e). The irregular group comprises *S. aureus* TISTR 118, which has several shapes, as shown in Figure 8d).

The chemical composition of the calcium carbonate precipitated was determined using the EDS method, as shown in Table 5. The elements found in the sample were Calcium (Ca), Carbon (C), Oxygen (O), Phosphorus (P), and Chlorine (Cl). The analytical grade calcium carbonate contains only Ca, C, and O. For the calcium carbonate precipitated calcium carbonate from each bacteria found an additional P and Cl.



Fig. 6: Precipitated calcium carbonate: (a) *P. mirabilis* TISTR 100, (b) *B. thuringiensis* TISTR 126, (c) *S. aureus* TISTR 118, (d) *Bacillus sp.* TISTR 658, and (e) *B. megaterium* TISTR 067

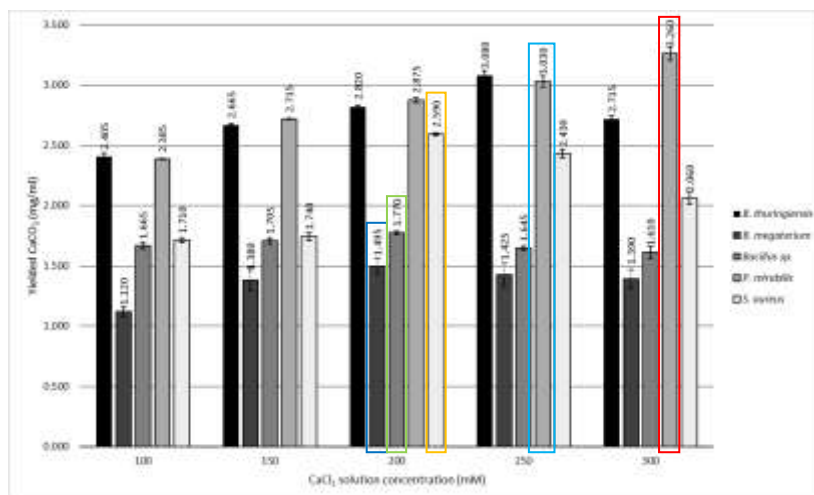


Fig. 7: Calcium carbonate yield of each bacteria strain

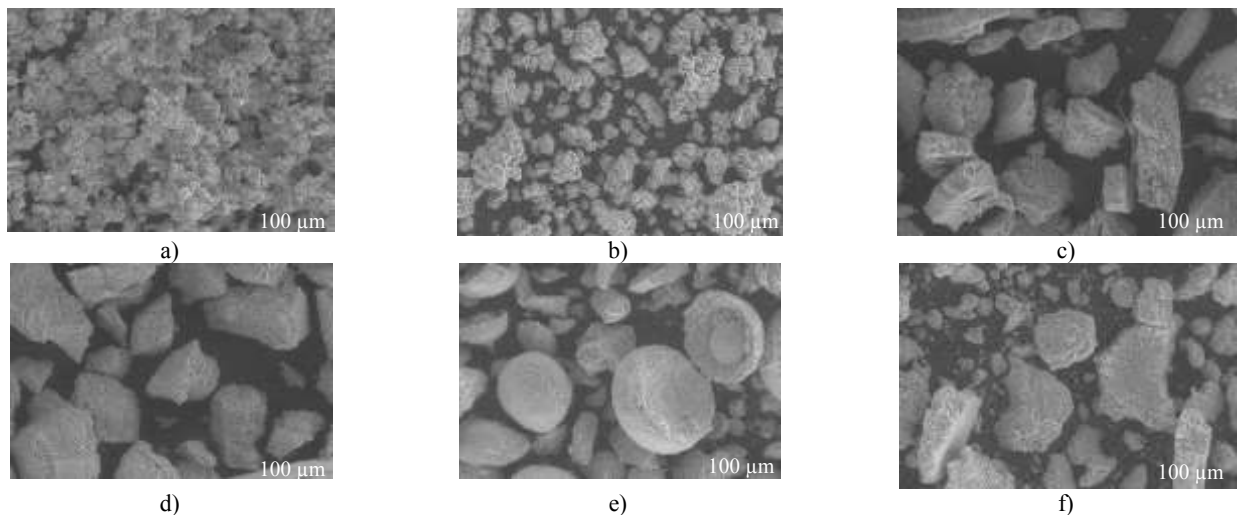


Fig. 8: Microstructure of the precipitated calcium carbonate: (a) the analytical grade calcium carbonate, (b) *P. mirabilis* TISTR 100, (c) *B. thuringiensis* TISTR 126, (d) *S. aureus* TISTR 118, (e) *Bacillus* sp. TISTR 658, and (f) *B. megaterium* TISTR 067, [27], [28]

Table 5. The chemical composition of each bacteria

Sample	Chemical composition (% by weight)					Ca C O content (%)
	Ca	C	O	P	Cl	
Analytical grade CaCO ₃	41.2	7.8	51.0	0.0	0.0	100.0
<i>P. mirabilis</i> TISTR 100	32.2	13.4	44.5	7.7	2.2	90.1
<i>B. thuringiensis</i> TISTR 126	35.0	13.8	41.6	8.9	0.7	90.4
<i>S. aureus</i> TISTR 118	37.0	11.0	44.1	5.5	2.4	92.1
<i>Bacillus</i> sp. TISTR 658	41.7	11.3	42.0	3.3	1.7	95.0
<i>B. megaterium</i> TISTR 067	30.0	27.3	34.7	5.2	2.8	92.0

3.7 Improvement of Sand Bonding

The improvement of sand quality by selecting calcium chloride concentrations that induce optimal calcium carbonate precipitation was investigated. This process resulted in the formation of the highest level of calcium carbonate precipitates for each bacterial strain, as observed in the experimental results depicted in Figure 7, as shown in Figure 9.

It was observed that the sand samples that experienced quality improvement, which allowed them to maintain their structure and stability most effectively, had undergone quality improvement through applying *P. mirabilis* TISTR 100, as represented in Figure 9a. This finding aligns with the results obtained from the investigation on the

highest capability to produce calcium carbonate precipitates.

Using the natural microorganisms present within the sandy soil can precipitate calcium carbonate to form bonds in the sandy soil particles, [29]. It was found that naturally existing bacteria still depend on nutrients and calcium sources used to precipitate calcium carbonate appropriately. Using *Sporosarcina pasteurii* to precipitate with the optimum conditions were able to cause the highest calcium carbonate precipitation in sand, [30]. When compared with the bacteria strains in this research (according to the optimum conditions of each strain), they can form calcium carbonate bonding, leading to the sand maintaining its shape.

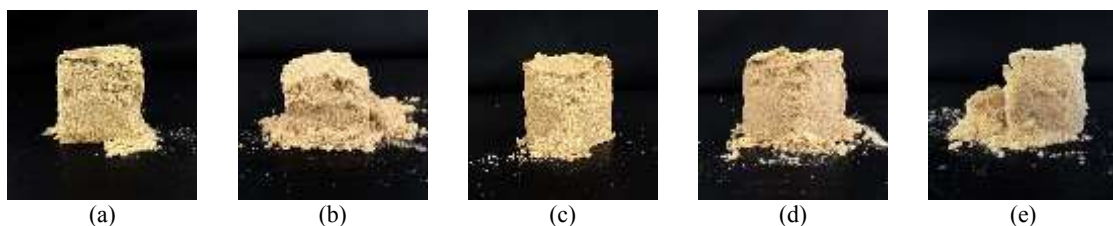


Fig. 9: Improvement of sand: (a) *P. mirabilis* TISTR 100, (b) *B. thuringiensis* TISTR 126, (c) *S. aureus* TISTR 118, (d) *Bacillus* sp. TISTR 658, and (e) *B. megaterium* TISTR 067

4 Conclusion

In this study, we proposed and demonstrated the MICP for sand improvement as a new eco-friendly bio-mineralization process. The procedure involved the selection of bacteria capable of producing urease enzymes to facilitate the precipitation of calcium carbonate through urea hydrolysis, which occurred when the pH was shifted from neutral to mildly alkaline conditions. The selected bacteria were found to form biofilms with varying intensities of coloration, which directly correlated with the amount of biofilm formation and inversely correlated with the sand's structural stability.

This investigation used the various strains of bacteria to improve bonds in sand using the MICP process. All bacteria strains showed the forming of calcium carbonate bonds between the sand particles, resulting in the sand maintaining its shape even when de-molded. From the experimental result, *P. mirabilis* TISTR 100 produces the highest urease activity and calcium carbonate precipitation. Therefore, the MICP process may be a method that can reduce the usage of cement in sand improvement.

This study has shown that the bacteria isolated in Thailand have a similar ability to form calcium carbonate bonding as bacteria typically used abroad. Future research and development are warranted to optimize the MICP process parameters, including the selection of specific microorganisms and their cultivation conditions, and to evaluate the long-term performance and durability of the MICP-treated sand in a stable application. Nevertheless, the results of this study highlight the potential of MICP as a viable and environmentally friendly technique for sand improvement, contributing to the advancement of real-world sustainable construction practices.

Acknowledgement:

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly in order to check the grammar of this context. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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