Bacteria as Self-Healing Agent in Mortar Cracks

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Abstract. This study was aimed to find the possibility of applying *Bacillus subtilis* integrated into mortar matrix as a self-healing agent to seal cracks. Bacterial spores at concentrations of 10^4, 10^5, and 10^6 cells/ml were directly added into pulverized fly ash as medium to protect bacteria in high alkaline conditions. The results show that the addition of *Bacillus subtilis* spores into the mortar mixture enhanced the compressive strength, especially at the cell concentration of 10^5 cells/ml. The bacterial mortar had a small ability to recover the stiffness of the mortar, amounting to 34.85% of its original stiffness. The effectiveness of crack sealant and resistance to water flow were limited to a maximum crack width size of 0.22 mm. Physical observation showed that the bacterial mortar is characterized by calcite precipitation as a product of ureolytic bacteria. The quantity and distribution of calcite precipitate depended on the precipitation weight, gravity direction and the oxygen availability. Meanwhile, chemical analysis using XRD and EDX showed that the bacterial mortar had a better crystallinity.

Keywords*: bacteria; crack; calcite; mortar; self-healing.*

1 Introduction

Concrete and mortar are important materials for building construction. These materials tend to crack under stress. The existing tensile stress in concrete may come from external loads, deformation, shrinkage, alkali-silica reactions or sulfate attacks. Without immediate and proper treatment, cracks tend to expand further and eventually require a high cost to repair. In the long term, the durability of concrete will be impaired by these cracks due to harmful liquids and gasses that penetrate through them.

The use of soil bacteria to precipitate $CaCO_3$ through the process of metabolism of urease enzymes has recently been investigated extensively. Some of the previous studies showed that bacteria have the potential to protect and heal concrete [1], to improve the strength of cement mortar [2], to restore historical building ornaments [3], and to remedy cracks in concrete surfaces [4,5]. Most

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researches used bacteria from the genus *Bacillus* as the agent to produce $CaCO_3$ precipitate. Calcium carbonate precipitation by bacteria is a complex mechanism depending on the bacteria concentration, ionic strength and pH level. Eqs (1) and (2) below summarize the chemical reaction processes that may occur in the precipitation of $CaCO_3$ by bacteria [5].

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (1)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \quad (2)$$

Self-healing mechanisms in cracked mortar (without direct improvement efforts) utilizing porous aggregates as bacterial immobilization medium were first introduced by Jonkers and are still in developed [6]. This study used impregnation of bacterial spores into pulverized fly ash medium to protect the bacteria in the mortar mixture with high alkaline conditions. *Bacillus subtilis* has been studied for use in bacterial concrete to improve the strength of mortar [7], but its use in self-healing concrete has not yet been studied extensively. Therefore, the purpose of this study was to investigate the possible application of *Bacillus subtilis* as a self-healing agent in mortar mixtures to seal cracks.

2 Materials and Methods

2.1 Culture of Bacteria

This research used *Bacillus subtilis* bacteria (IFO - 13719) obtained from the collection of the Nutrition and Food Research Centre, Gadjah Mada University, Indonesia. As the primary backup for the study, the bacteria was stored in nutrient agar and conditioned in a refrigerator at a temperature of ±4ºC until further use. The medium composition required for bacteria growth was 5 g/l peptone, 5 g/l NaCl and 3 g/l yeast extract. The bacteria were grown in liquid medium using a sealed sterile reaction tube. The bacteria were agitated at 125 rpm for 24 hours. Bacterial cells were counted using a microscope and the culture was diluted using 1% NaCl to obtain cell concentrations of 10^4; 10^5 and 10^6 cells/ml [7].

2.2 Mixture Proportion of Bacterial Mortar

The material used as mortar was a mixture of Portland cement, sand (passed through a 30-mesh sieve and retained on 100-mesh sieves), pulverized fly ash (PFA) and water. The mortar was prepared by blending cement and sand at a ratio of 1:3 and water to cement at a ratio of 0.485. PFA was used to replace 20% by weight of the cement content. This percentage was used based on a previous experiment [8], where the mechanical properties of bacterial mortar increased with 20% substitution of fly ash. The required materials per cubic meter of mortar are shown in Table 1.

Table 1 Required materials per cubic meter of mortar

Materials	Water	Cement	Sand	PFA
Required by weight (kg/m^3)	245.45	421.74	1511.78	84.35

The PFA was impregnated with bacteria cells in liquid media, 30% urea solution and 10% calcium acetate solution, separately. The percentage of PFA for each mixture were 20%, 20% and 60% for bacteria cells, urea solution, and calcium acetate solution, respectively. The impregnation was done using a desiccator vessel and suction machine with vacuum capability of up to ±40 cm.Hg. Before the impregnation process, the PFA was sterilized from possible microorganisms by heating it in an oven at a temperature of ±105ºC for 24 hours [9]. The material requirements for PFA impregnation are presented in Table 2.

Table 2 Required materials impregnated into PFA.

Impregnation	Requirement	
	l/m^3	kg/m^3
PFA		16.87
Bacillus subtillis	10.68	
PFA		16.87
Urea	10.68	
PFA		50.61
Calcium acetate	32.03	
Total	**53.39**	**84.35**

2.3 Preparation of Test Specimens and Testing

Mechanical and physical properties tests and a chemical analysis were conducted. The mechanical properties tests were: compressive test (ASTM C-109/C109M, 50 x 50 x 50 mm, 6 specimens) [10], flexural test (ASTM C-348, 60 x 60 x 220 mm, 1 specimen) [11] and water permeability test of mortar (110 mm diameter and 20 mm thickness, 3 specimens). The compressive test was carried out to observe the effects of the addition of different bacterial cell concentrations (10^4, 10^5 and 10^6 cells/ml). The specimens were submersed in water or solution of 5% urea mixed with 1% calcium acetate, and the compressive test was conducted after 3, 7, and 28 days of submersion. The two treatments were conducted to compare the performance of the bacteria, because in the $CaCO_3$ precipitating process the bacteria still need an external source of nutrients. Other mechanical test specimens were made using the cell concentration that had the maximum value of compressive strength. The addition of a mixture of PFA-bacterial spores was followed by the addition of

mixtures of PFA-30% urea solution and PFA-10% calcium acetate solution as source of nutrients and calcium.

To find the bonding strength of a crack sealed by bacteria against flow and pressure of water, it is necessary to measure the coefficient of water permeability. Mortar permeability is measured by determining the rate of water flow through the specimen and is expressed as the coefficient of permeability k (m/s). The cracked mortar specimen for permeability testing was taken from a split tensile strength test. The mortar permeability is calculated based on Darcy's law as shown in Eq. (3).

$$k = \frac{a.T}{A.t} \ln \frac{h_0}{h_f} \tag{3}$$

where:

k = coefficient of water permeability (m/s)
a = cross-sectional area of pipette (m^2)
A = cross-sectional area of specimen (m^2)
T = specimen thickness (m)
t = time (s)
h_0 = initial water head (cm)
h_f = final water head (cm)

Microscopic observation of the bacteria mortar samples was performed using scanning electron microscopy (SEM). Moreover, an artificial crack of 0.2 mm was created in a specimen test of the mortar bacteria using zinc plates with depth variations of 10, 20, 30 and 40 mm (3 specimens each). The mortar specimens were then submerged for 60 days in nutrient solution for crack condition recovery. Then the specimens were cut in the crack direction to determine the distribution of calcite after the crack was sealed. A digital microscope with computer analysis using Image-J software version 1.47 was employed to observe the distribution of calcite precipitate in the mortar cracks. To analyze the chemical properties of the bacterial mortar, x-ray diffraction (XRD) assisted with MAUD software version 2.33 and equipped with energy dispersion x-ray (EDX) was used. The details of the specimen test and simulation are presented in Figure 1. The brittle mechanism in the flexural test specimens was controlled using 2 wires with a diameter of 1 mm (Figure 1(a)).

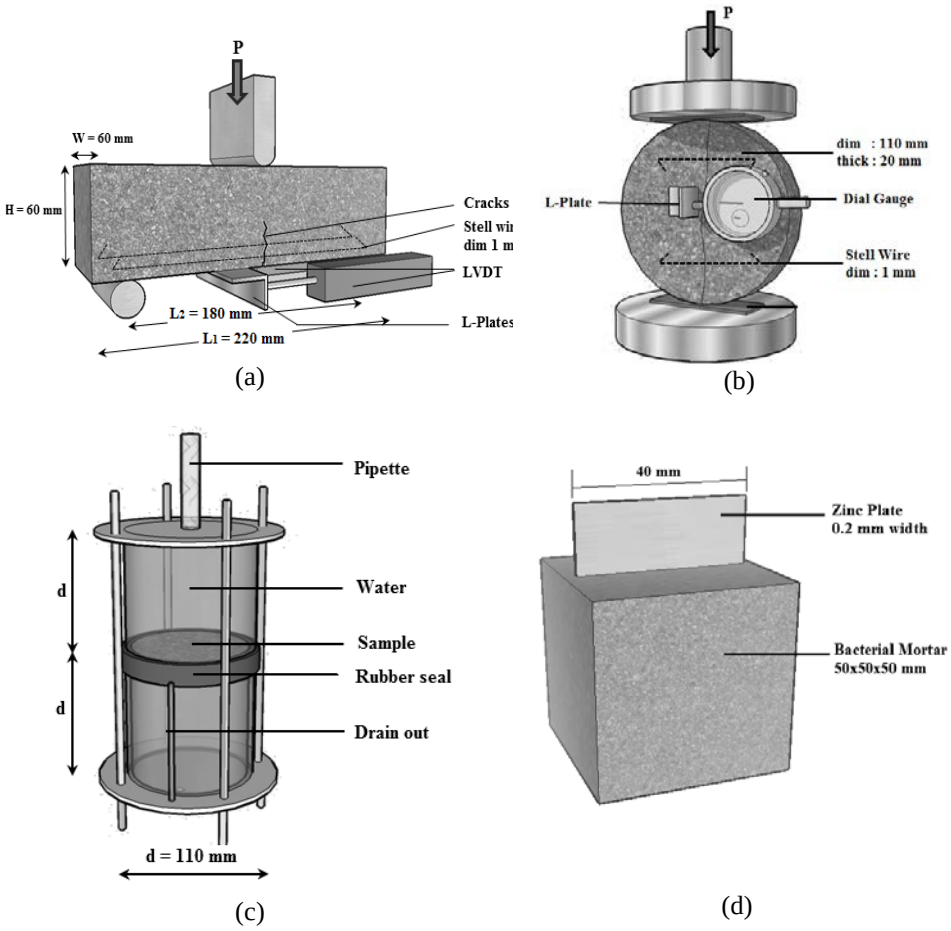


Figure 1 Simulation testing of (a) flexural strength, (b) crack specimen for permeability (c), water permeability, (d) artificial crack.

3 Results and Discussion

3.1 Mechanical Properties of Bacterial Mortar

3.1.1 Compressive Strength

Compressive strength values at 3, 7, and 28 days are presented in Table 3. Most of the compressive strengths of the bacterial mortar were higher compared to that of the control mortar. The bacteria *Bacillus subtilis* is able to hydrolyze urea into ammonium (NH_4^+) and carbonate (CO_3^{2-}), as well as the binding element of calcium (Ca^{2+}) in a solution of a compound of calcium acetate to

form calcite ($CaCO_3$). The calcite precipitate formation sealed the gap pores, making the mortar more dense [5].

Table 3 Percent increase in compressive strength of mortar.

Bacillus subtilis	**Average mortar compressive strength (MPa)**								
(cells/ml)	**3 days**	**SD**	**% increase**	**7 days**	**SD**	**% increase**	**28 days**	**SD**	**% increase**
Submersion in water									
Control	13.83	1.18	-	17.17	1.25	-	26.93	2.00	-
10^4	12.85	1.16	-7.06	17.08	2.15	-0.49	28.49	2.91	5.79
10^5	14.87	2.13	7.56	17.65	1.26	2.81	28.62	0.75	6.26
10^6	15.30	0.62	10.66	19.93	0.85	16.11	28.97	2.54	7.57
Submersion in solution of 5% urea and 1% calcium acetate									
0	11.95	1.62	-13.57	15.68	1.64	-8.66	25.10	2.30	-6.79
10^4	13.88	0.40	0.40	19.22	1.56	11.94	29.57	2.49	9.81
10^5	17.33	0.85	25.38	20.84	0.91	21.40	31.77	0.91	17.97
10^6	15.45	1.05	11.77	20.06	0.69	16.84	30.20	0.90	12.13

Note: SD = standard deviation

The optimum concentration of bacteria to obtain the highest compressive strength changes with different submersion solutions. In water submersion, the maximum value of compressive strength was obtained at a cell concentration of 10^6 cells/ml, while in the solution of calcium acetate and urea submersion, the maximum compressive strength was obtained at a cell concentration of 10^5 cells/ml. This suggests that the addition of nutrients from outside sources greatly affected the compressive strength of the bacterial mortar. Under conditions of excess nutrients, the bacteria concentration needed is smaller because for bacteria it is easier to live and breed in environmental conditions where water, oxygen, and nutrients are available. The percentage increases of compressive strength were 25.38%, 21.40%, and 17.97% for ages of 3, 7, and 28 days, respectively. The percent increase in compressive strength of the mortar was higher in the early treatment, because the pores of the mortar were filled with hydration products of the cement [12].

The decrease in compressive strength of the bacterial mortar at 10^6 cells/ml in the urea and calcium acetate solution submersion was due to an increase of ammonia and CO_2. The increase of ammonia was caused by the excess reaction of urease enzyme produced by the bacteria. The excess of ammonia alkaline compounds was indicated by the presence of ammonia and the increase of pH levels in the bath solution [5]. In this experiment, the addition of bacteria at

10^6 cells/ml yielded a pH of more than 10.5 as measured with a pH meter. Some bacteria may die due to the high pH. However, some bacteria cells may survive in the porous material.

Table 4 shows water absorption of bacterial mortar. The smallest decrease in water absorption was obtained at cell concentrations of 10^5 cells/ml. The deposition of calcite produced by the bacteria was able to seal part of the pore cavities in the mortar. Calcites are compounds that hardly dissolve in water, therefore the presence of calcite reduces water absorption, seals the gap pores and potentially increases the compressive strength.

Table 4 Water absorption of bacterial mortar at 28 days.

***Bacillus subtilis* cells/ml**	**absorption (%)**	**decrease in water absorption (%)**
control	11.28	-
10^4	8.23	27.04
10^5	6.89	38.92
10^6	8.52	24.47

PFA grains have a finer diameter than cement grains. The finer grains and the porous nature of this material increase the contact surface, thus increasing water absorption [13]. In this case the PFA contributed to higher water absorption by the mortar mixture, however it reduced the formation of pores after the mortar hardened.

3.1.2 Flexural Strength

In this study a bending load test was conducted twice on the same specimen. This test is to determine the effect of the addition of *Bacillus subtilis* on the flexural strength of the mortar and the possibility of stiffness recovery due to calcite precipitation inside cracks. First the specimen was tested after 28 days, then it was immersed again in water for 60 days before the second load was conducted. Visual observation showed that the cracks of the mortar had been sealed.

The flexural strength loading cycle of the specimen without bacteria (control) is presented in Figure 2. It can be seen that after the first load, the bending strength is characterized by a linear elastic line and reached a maximum load of 2.30kN. It was followed further by cracking and the reduction of strength. The loading was stopped when the crack reached a width of 0.3242 mm. When the

first loading was released, the crack width decreased to 0.2552 mm. The second loading was conducted after the specimen had been submersed in nutrient solutions for 60 days. It can be seen that three was no stiffness recovery. This may be due to the inability of the mortar to seal the gap resulted by the first loading process.

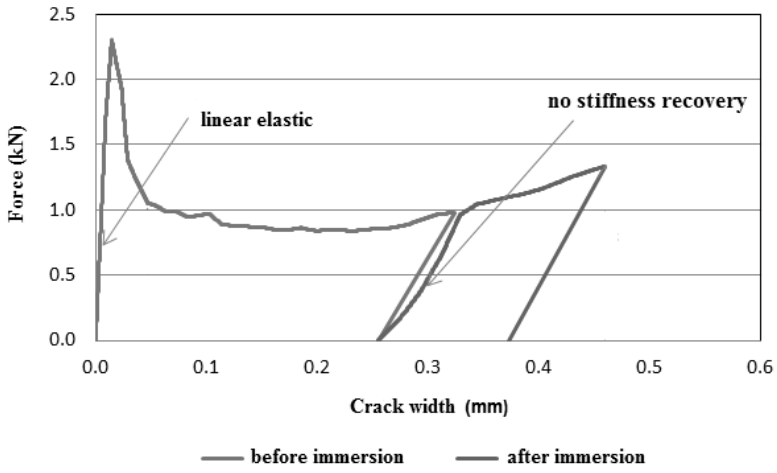


Figure 2 Loading cycle of control mortar before and after nutrient immersion.

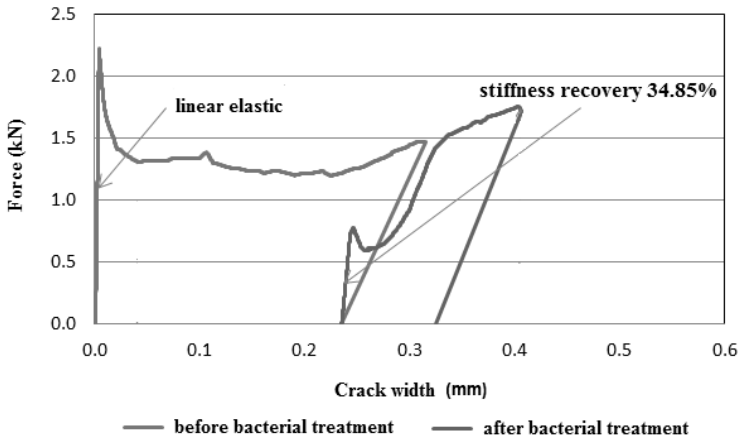


Figure 3 Loading cycle of mortar before and after bacterial treatment.

The flexural strength loading cycle of the bacterial mortar is presented in Figure 3. At the first load, the maximum value of flexural strength was 2.23 kN. The loading was continued until the crack width reached 0.3152 mm where the value of flexural strength decreased. After the first load was released, the crack

width decreased to 0.2355 mm. The specimen was submersed for 60 days in solution of 5% urea mixed with 10% calcium acetate, then the second load was conducted. At the second loading, the maximum force reached 0.78 kN. This means that a stiffness recovery of 34.85% of the original strength was achieved by the bacterial mortar.

Calcite precipitation by the addition of bacteria was not strong enough to bind the filler material. The calcite precipitation tended to occur at the outer surface of the mortar and the slit pore. Calcite precipitation inside the mortar depends on the gap and the supply of oxygen because *Bacillus subtilis* is aerobic bacteria type. The bond strength of this mineral formation is a biological product affected by the environment, the quantity and the type of bacteria used. There is also the possibility of a disruption of the cement bond due to urea compound, which is classified as organic material.

3.1.3 Permeability Test

The permeability coefficient of the bacterial mortar was smaller compared to that of the control mortar (without addition of bacteria), as shown in Figure 4. The bacterial mortar was more resistant against the pressure and flow of water, both in normal condition and in cracked condition after submersion for 60 days in nutrient solution. However, the effectiveness of crack healing and resistance to water flow was confined to a maximum crack width of 0.22 mm. At a higher width, the crack calcite precipitation was no longer able to withstand the pressure and flow of water.

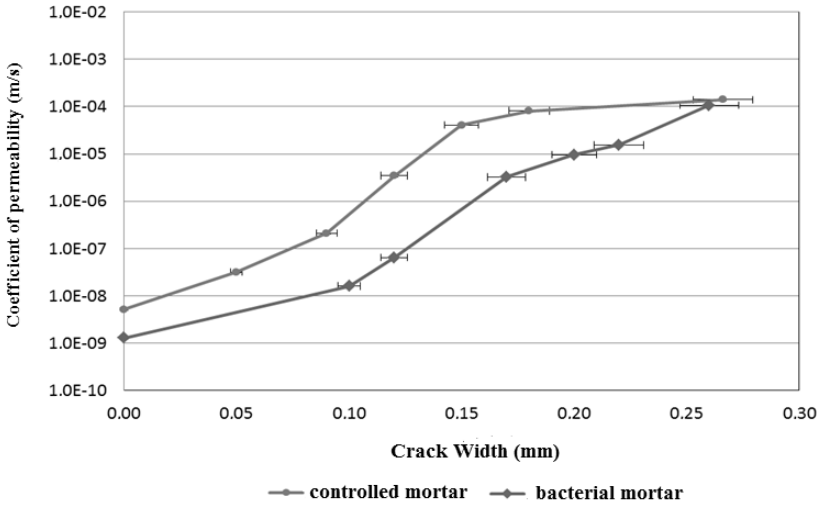


Figure 4 K values of mortar with and without bacterial treatment.

Figure 5 shows crack repair and the condition of the calcite precipitation after the permeability test with water pressure. It seems obvious that the crack deposition began to be eroded by water at a crack width above 0.22 mm, however it did not happen at crack widths below that value because the calcite precipitation could resist the water pressure in this test.

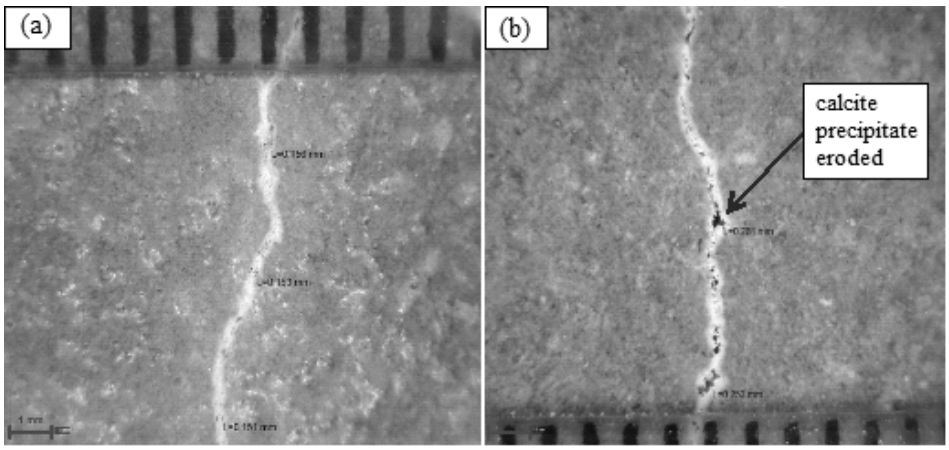


Figure 5 Crack healing after water permeability test: (a) crack width of 0.15 mm, (b) crack width of 0.25 mm.

3.2 Physical Properties of Bacterial Mortar

3.2.1 Microscopic and SEM Observations

Figure 6 shows some physical characteristics of the bacterial mortar after it was tested and conditioned in a water bath. Figure 6(a) shows the presence of calcite precipitate that began to form in the cracks after submersion for 7 days. Calcite crystals were formed from the calcium precipitate (Ca^{2+}), starting from the bottom surface. This is due to the influence of gravity and the weight of the material that triggers the formation of precipitation. Figure 6(b) shows the precipitation of calcite in the deepest part of the mortar cracks. The calcite precipitation was formed not only from the inside of the cracks but also from the inside of the pores that allowed water to enter and triggered a reaction of the bacterial urease enzyme.

A similar case was also observed in the flexural strength test specimen, where calcite precipitate was formed in the crack (Figure 6(c)). Details observation in Figure 6(d) showed that the calcite precipitate began to form at the bottom and partly also in the middle of the crack. Calcite precipitation starts at the bottom

due to the influence of gravity and the aerobic nature of *Bacillus subtilis* that needs the supply of oxygen.

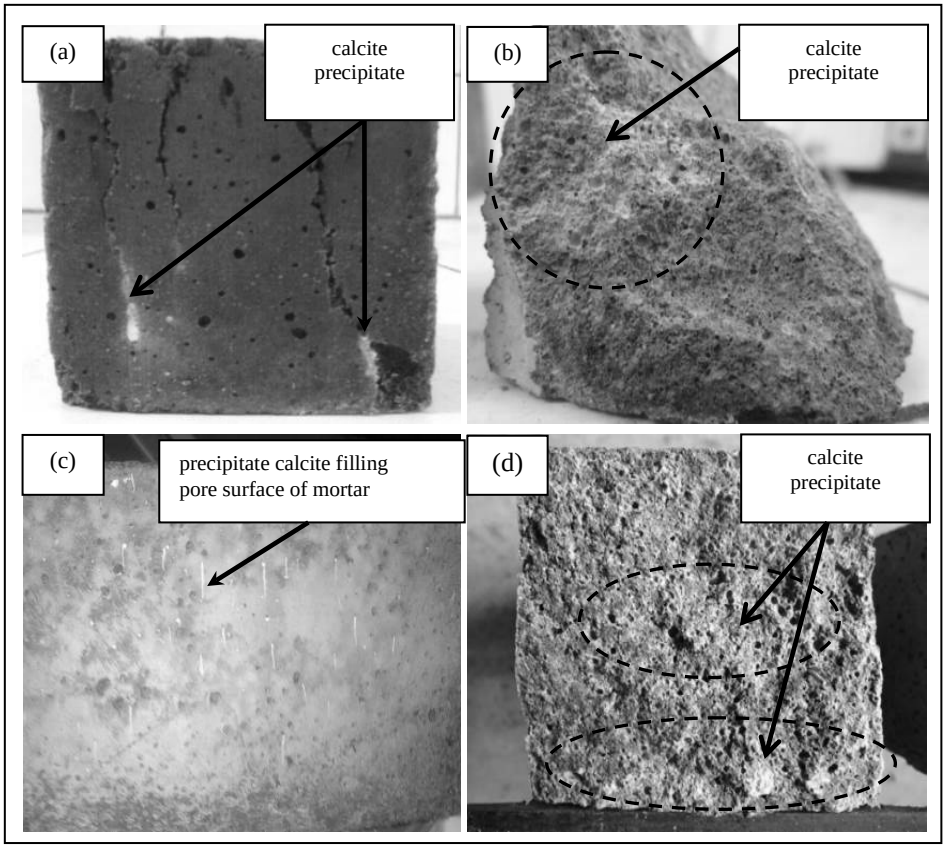


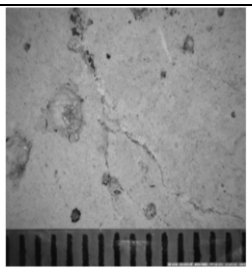
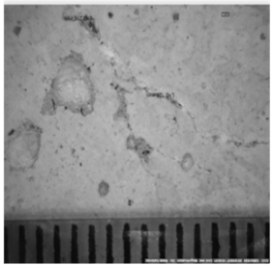
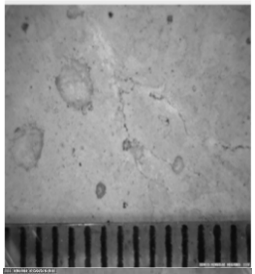
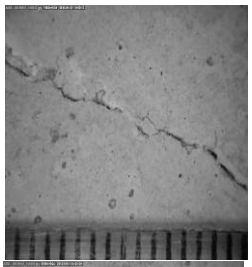
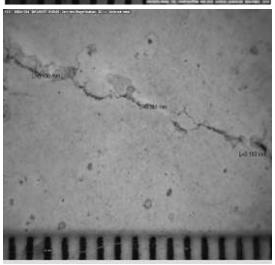
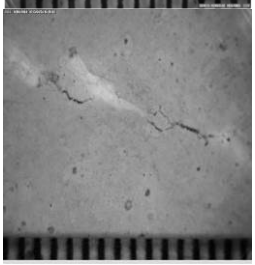
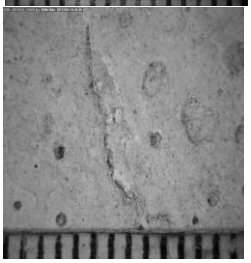
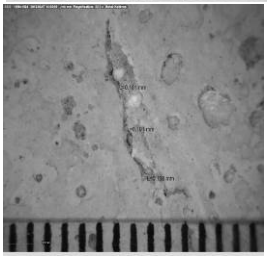
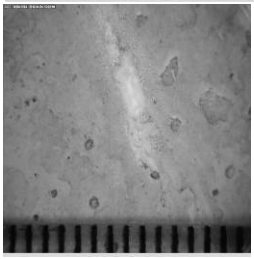
Figure 6 Calcite precipitation in the cracked bacterial mortar: (a) calcite deposition in the crack of the compressive strength test specimen, (b) calcite in the pores of the compressive strength test specimen, (c) calcite filling the pore surface of the mortar, (d) surface crack in the flexural strength test specimen.

Table 5 shows the sealing process of some cracks, from the initial state to the condition after 7 days and 60 days of submersion in a solution of urea and calcium acetate.

The SEM results are shown in Figure 7. It can be seen that in the control mortar (Figure 7(a)), the bond between silica sand and cement formed calcium silicate hydrate. In the mortar with 10^4 cells/ml bacteria, as shown in Figure 7(b), in addition to the structure of calcium silicate hydrates formed, it can be seen that the porous PFA granules did not bond with the cement. Apart from functioning

as cement filler, the PFA grains become a repository of bacterial spores medium and visible calcite precipitate began to form around the grains of PFA.

Table 5 Bacterial mortar crack sealing process by calcite precipitate.

Crack width (mm)	Cracks before submersion	After 7 days	After 60 days
0.11			
0.13			
0.19			

In Figure 7(c) it is clearly shown that the precipitate of calcite crystals formed and covered most of the mortar surface. Precipitate calcite crystals were shown to be optimum at a bacteria cell concentrations of 10^5 cells/ml, whereas at a cell concentration of 10^6 cells/ml, only a slight amount of calcite precipitate was formed because of the excessive bacteria concentration conditions (Figure 7(d)).

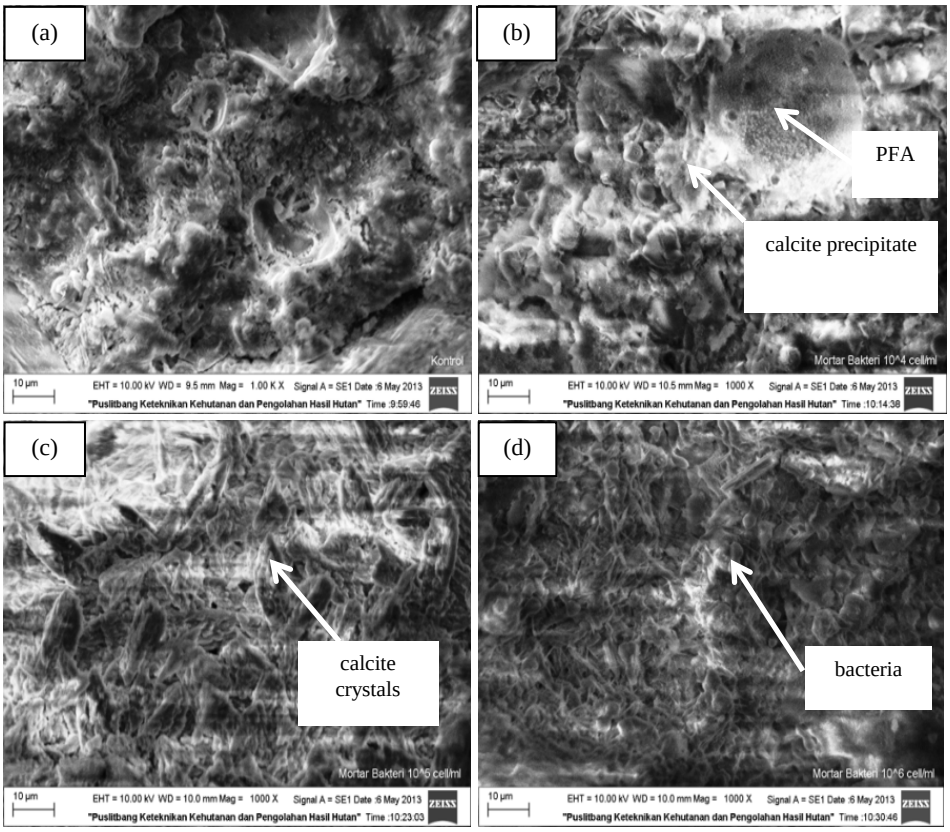


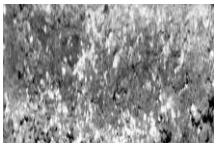
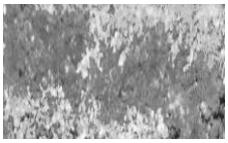
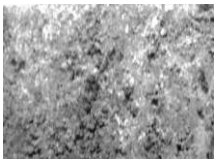
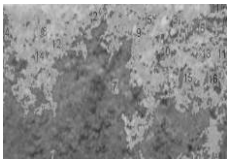
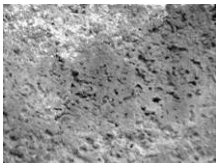
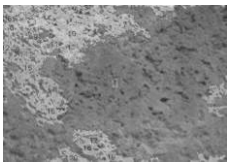
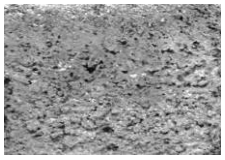
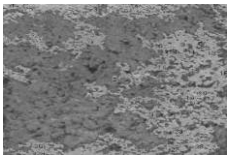
Figure 7 SEM observations: (a) control mortar, (b) bacterial mortar – 10^4 cells/ml, (c) bacterial mortar – 10^5 cells/ml, (d) bacterial mortar – 10^6 cells/ml.

3.2.2 Observation of Cracks

In this experiment, artificial cracks were deliberately made to observe the effectiveness of calcite distribution that was produced by the bacteria to seal the cracks. Observations were made in the inside surface of the mortar using photographic results and further analyzed using Image-J software version 1.47.

Calcite deposits were mostly formed in the lower and upper areas of the cracks (Table 6). The calcite cover on the cracks did not follow a particular pattern, except a tendency of calcite formation at the bottom and top of the cracks. In general, the deeper cracks is the smaller the percentage of precipitate. This may be due to factors such as the influence of gravity and the basic nature of the aerobic bacteria. The aerobic bacteria mostly deposited on the mortar surface because there it is easier to get a supply of oxygen.

Table 6 Analysis of calcite precipitate in artificial cracks.

Artificial crack 0,2 mm	Image of cracks on bacterial mortar	Calculation *Image-J Software*	Precipitate (%)
depth : 10 mm length : 40 mm			42.16
depth : 20 mm length : 40 mm			41.57
depth : 30 mm length : 40 mm			32.54
depth : 40 mm length 40 mm			37.35

3.3 Chemical Properties of Bacterial Mortar

The XRD analyses of the control mortar and the bacterial mortar are presented in Table 7 and XRD spectra are presented in Figure 8. The $CaCO_3$ compound consists of vaterite, aragonite and calcite. It can be seen that the crystallinity and calcite minerals of the mortar bacteria were higher than those of the control mortar. The bacterial mortar contained a relatively smaller aragonite and vaterite than the control mortar. This may be due to the effects of temperature, the pH of the solution and the addition of additives.

Table 7 Crystallinity and relative content of $CaCO_3$.

Sample	Vaterite %	Aragonite %	Calcite %	Crystallinity %
Control mortar	46.00	28.90	25.20	42.30
Bacterial mortar	41.50	18.80	39.70	62.36

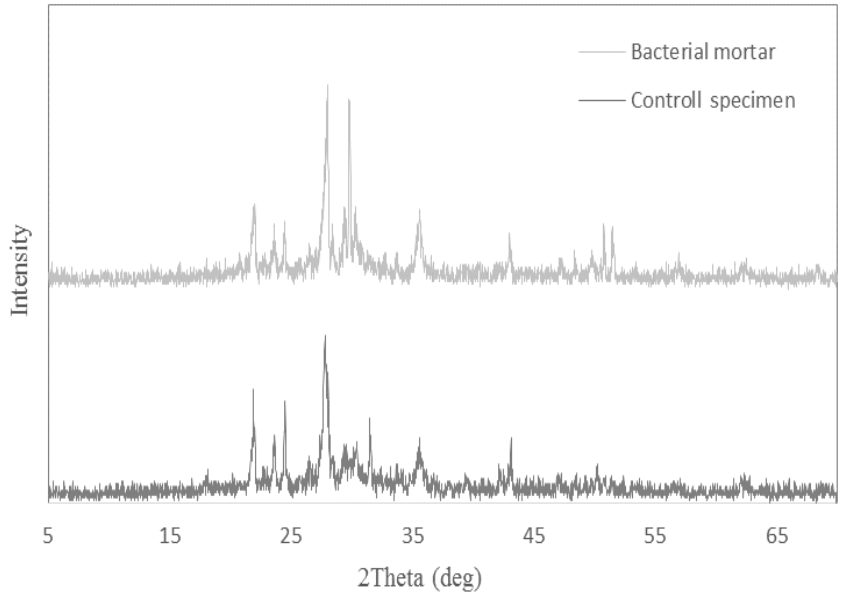


Figure 8 Diffraction spectra of XRD test.

Table 8 shows the analysis results of the mortars using EDX. It can be seen that the more bacteria were added in the mortar, the more Ca (calcium) was contained in the mortar. Calcium is not a single element but part of the bonding compound of lime or calcite. The higher calcium content was found in the mortar added with a concentration of 10^5 cells/ml bacteria. At a cell concentration of 10^6 cells/ml the calcium content slightly decreased because the excessive bacteria increased the ammonia content and pH in the solution.

Table 8 Analysis results of mortars using EDX spectrum acquisition

Element content	Concentration of *Bacillus subtilis*			
	0 cells/ml	**10^4 cells/ml**	**10^5 cells/ml**	**10^6 cells/ml**
	%	**%**	**%**	**%**
Carbon	6.89	2.21	7.96	6.70
Oxygen	57.87	54.54	52.69	51.31
Sodium	1.19	1.72	0.29	1.28
Magnesium	0.61	1.54	0.19	1.42
Aluminium	3.85	4.87	0.96	4.83
Silicon	6.23	11.31	2.87	8.42
Calcium	18.28	16.58	29.44	20.37
Iron	1.11	2.49	0.64	1.44
Antimony	3.98	4.73	4.97	4.23

4 Conclusion

The addition of *Bacillus subtilis* spores, calcium acetate and urea in pulverized fly ash medium in a mortar mixture enhanced the compressive strength, especially at a concentration of 10^5 cells/ml. At that concentration the increase of compressive strength compared to the control mortar were 25.38%, 21.40%, and 17.97% after 3, 7 and 28 days, respectively.

The analysis of flexural loading showed that the bacterial mortar had a small ability to regain strength, amounting to 34.85% of its original strength. The effectiveness of crack healing and resistance to water flow were limited to a maximum crack width of 0.22 mm.

Physical observation showed that the bacterial mortar was characterized by calcite precipitation as a product of ureolytic bacteria. The quantity and distribution of the calcite precipitate depended on the precipitation weight, the gravity direction and the oxygen availability. Meanwhile, the chemical analysis using XRD and EDX showed that the bacterial mortar had a better crystallinity.

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