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Development of Autonomous-Healing Mortar Using *Geobacillus stearothermophilus*

by M. A. Raden Maizatul Aimi, M. S. Hamidah, K. Kartini, H. Noor Hana, A. K. Khalilah, and E. Schlangen

Autonomous healing by the microbially induced calcite precipitation (MICP) mechanism has garnered significant interest in the sustainable approach to concrete repair and maintenance. Previous research works have reported that Bacillus pasteurii *and* Bacillus sphaericus *are the most commonly used in concrete associated with bacteria. However, there is limited information on other types of bacteria species. In this study, the vegetative cells of* Geobacillus stearothermophilus *were introduced and encapsulated into alginate-hydrogel before incorporation into the mortar. The urease activity, viability, swelling, and water retention properties of the bacterial* Geobacillus stearothermophilus *cell encapsulated in alginate-hydrogel were measured. The performance of alginate-encapsulated* Geobacillus stearothermophilus *(AE-GS) in the mortar mixture as a self-healing agent was measured by compressive strength, water absorption, and crack-healing efficiency. The precipitation of calcium carbonate of the AE-GS mortar was measured using thermogravimetric analysis (TGA). The highest level of crack healing was 63% (by the initial crack width) which was achieved by incorporating 15% AE-GS (replacement by total weight of the mortar). However, the lower result of compressive strength and the highest absorption rate were portrayed by the mortar specimens that contained 15% of AE-GS replacement compared with the control mortar (AE-R) and with those of AE-GS replacement level at 3 and 9%.*

Keywords: alginate-hydrogel beads; autonomous healing; bacteria; crack remediation; microbial $CaCO_3$.

INTRODUCTION

The biomineralization process is caused by the microbial activity that produces mineral precipitation, which has been proven to improve the behavior of concrete.[1-8] Carbonate-precipitating bacteria were added into concrete during the mixing process.[9-12] However, direct embedment of bacteria in concrete was discovered to result in the temporal activity of bacteria due to several factors, including the hardening process of concrete, which causes pore reduction to diminish the availability of bacteria.[8,13] The average size of *Bacillus*-type bacteria is 1.969×10^{-5} to 3.937×10^{-5} in. (0.5 to 1.0 μm) wide and by 3.937×10^{-5} to 1.575×10^{-4} in. (1.0 to 4.0 μm) long.[14] On another note, concrete and cement paste pore size is reported to be between 1.18×10^{-7} and 3.937×10^{-5} in. (0.003 and 1.000 μm) for young cement pastes, while mature cement pastes are between 1.18×10^{-7} and 3.937×10^{-6} in. (0.003 and 0.1 μm).[15] Moreover, the smaller pore size of concrete compared to the bacteria size make it impossible for bacteria to survive in concrete for a long period. Therefore, a suitable carrier is necessary to encapsulate the bacteria and protect it from destruction. The purpose of embedment of bacteria in the carrier is to ensure that the bacteria are dormant but viable in the concrete matrix, which will further enhance the self-healing potential.[16-18] Some of the carriers that have been used in concrete as self-healing agents include clay capsules,[8] silica gel or polyurethane,[16,17] and diatomaceous earth.[19]

The encapsulation method with sufficient water supply is necessary as most of the encapsulation methods were performed in full submersion or wet and dry cycles. Nevertheless, it should be noted that full submersion is not practical in many cases and difficult to be applied in construction work. Moreover, the encapsulation method using hydrogel was first introduced by Annamalai et al.[20] in 2012 using polyurethane-alginate. As proposed by Wang et al.,[21] encapsulation requires high water absorption capacity as well as the ability to retain a large amount of water without liquefaction. Following it, numerous kinds of research on self-healing concrete were performed using various types of hydrogel encapsulation, which include sodium alginate-polyurethane,[20] hydrogel,[21] poly-condensation reaction-based microencapsulation,[22] biological gel,[23] bio-based healing agent,[24] and methacrylate-modified alginate.[25]

In this study, *Geobacillus stearothermophilus* was encapsulated into sodium alginate gel beads as a carrier into the mortar matrix. *Geobacillus stearothermophilus* is high-resistant bacteria and also considered as thermophilic-type bacterium due to its capability of thriving in high temperatures (86 to 167°F [30 to 75°C]). Moreover, it can be found in a geothermally heated region such as a hot spring.[26,27] *Geobacillus stearothermophilus* is capable of producing endospore in the permitted harsh situation that enables them to lie quiescent but viable for a long period in an extreme condition as well as capable of being mobilized in the atmosphere and transported across a long distance.[28] More importantly, different cell concentrations of bacteria with different percentage replacement of alginate-encapsulated *Geobacillus stearothermophilus* (AE-GS) were introduced and investigated in this study. Finally, the outcome is hoped to be beneficial for various types of bacteria in crack repairs.

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RESEARCH SIGNIFICANCE

The purpose of this study is to demonstrate the survival ability of *Geobacillus stearothermophilus* when it is encapsulated into alginate-hydrogel as well as its capability to precipitate calcium carbonate ($CaCO_3$) when incorporated into the cementitious material. Autonomous-healing concrete is shown to have a significant impact as it provides both positive and negative results to concrete performance and has been proven by previous researchers. The success of using *Geobacillus stearothermophilus* is hoped to provide a basis of enhanced understanding in using microbes as self-healing agents in cementitious material, which is believed to increase the fundamental understanding of the substances in the pool of existing knowledge as a reference for future research.

MATERIALS AND METHODS

Bacterial strain

Geobacillus stearothermophilus (ATCC 12978) was used and cultured in the present study. Living cells were grown in a sterile nutrient broth (NB) which consists of beef extract (0.025 lb/gal. [3.0 g/L]) and peptone (0.042 lb/gal. [5.0 g/L]). Meanwhile, the pH of the medium was adjusted to 7.0. The culture was incubated in the broth at 149°F (65°C) and centrifuged at 110 rpm for 24 hours. Next, the bacteria cells were harvested by centrifuging the culture (8000 rpm, 39.2°F [4°C]) for 5 minutes. In this case, the best centrifuged cells remained, formed into pellets, and were washed twice with sterile distilled water before undergoing encapsulation process.

Encapsulation process

The encapsulation process was adopted in this study to protect the bacteria and act as an inert material. The encapsulation occurred through a cross-linking process in which the calcium ion in the solution cross-linked the polymers in the alginate that are attached at many points, resulting in gel beads. The cross-linker for each series of alginate beads includes 0.125 lb/gal. (15 g/L) sodium alginate ($C_6H_9NaO_7$) as gelling agent, 0.278 lb/gal. (33.3 g/L) of yeast extract, 0.278 lb/gal. (33.3 g/L) of urea (CH_4N_2O), 0.167 lb/gal. (20 g/L) calcium lactate ($C_6H_{10}CaO_6$) as a nutrient source of bacteria, and 0.093 lb/gal. (11.1 g/L) calcium chloride ($CaCl_2$).

Quantification of urease and viability of AE-GS in alkaline environment

In this study, cement slurry was produced by mixing the cement with distilled water to mimic the high pH environment inside the mortar to quantify the urease activity of AE-GS when incorporated into the mortar. The cement slurry was prepared by mixing 0.221 lb (100 g) cement with 0.026 gal. (100 mL) of water. Meanwhile, AE-GS with bacteria concentrations of 1×10^3 and 1×10^{11} lb^3/mL was prepared. The purpose of choosing the two bacteria concentrations was because they represent the lowest and highest concentration of bacteria used in this study. The amount of urea decomposed by AE-GS is a basis of evaluation for the bacterial viability. Beads of AE-GS, 0.353 oz (10 g) each, were placed into a tea bag, which was then submerged into the cement slurry for 24 hours at ambient temperature. Furthermore, the measurement of decomposed urea, as well as the cell viability of AE-GS, is described as a modification method that was adopted from the work conducted by Wang et al.[21,22]

After 24 hours of immersion, the urease activity by AE-GS was measured by taking out the tea bag from the cement slurry and rinsing it with autoclaved distilled water four times to remove the cement particles that were attached to the tea bag. Next, the tea bag was opened, and its contents were submerged into 0.026 gal. (100 mL) of NB (0.3% beef extract and 0.5% peptone). The amount of urea decomposed at 1, 4, and 7 days was measured by the total ammonia nitrogen (TAN)-Nessler method to determine whether the bacteria are still viable after the immersion in the mimicked mortar environment. Nessler refers to a type of ammonia test kit that consists of a mineral stabilizer, polyvinyl alcohol dispersing agent, and Nessler reagent. The experiments were carried out according to the manual produced by the Hach Company.[22] First, 2.642×10^{-4} gal. (1.0 mL) of broth with AE-GS beads was diluted in 6.340×10^{-3} gal. (24 mL) of distilled water in a 6.604×10^{-3} gal. (25 mL) graduated cylinder. Next, three drops of mineral stabilizer were added to the cylinder, followed by the polyvinyl alcohol dispersing agent. Then, the solution was hand-shaken after covering it with the graduated cylinder lid and thoroughly mixing it. After the solution was mixed, 2.642×10^{-4} gal. (1.0 mL) of the Nessler reagent was added into the cylinder. Once the final solution was prepared, its portion was transferred into a spectrophotometer cell and the ultraviolet (UV) light absorbance was measured. The wavelength of the light used in the measurement was set at 1.673×10^{-5} in. (425 nm). In the TAN test, the Nessler reagent (K_2HgI_4) reacted with the ammonia present in the sample (under strongly alkaline conditions) to produce a yellow-colored species. The intensity of the color was in direct proportion to the ammonia concentration. A blank solution that consisted of broth with encapsulation beads without bacteria was prepared for reference before the measurement of UV light absorbance of each water sample. Each test was performed in triplicate ($n = 3$). Moreover, the bacteria cell concentration in AE-GS beads is believed to reduce its number of cells during the encapsulation process. Hence, viability testing was conducted to measure the percentage of reduction in cell concentration to ensure that the required number of bacteria cells can be maintained. Meanwhile, the tea bag with 0.0176 oz (0.5 g) of AE-GS was submerged into the centrifuge tube with a phosphate buffer to measure AE-GS viability in the mimicked mortar environment. The phosphate buffer was used as a solution to break the alginate polymer chains to release the bacteria. The solution was vortexed to accelerate the capsule breakage to ensure that the immobilized bacteria would be released. The solution amount of 2.642×10^{-5} gal. (0.1 mL) was pipetted into the NB plate and incubated at 149°F (65°C) for 24 hours to determine its growth by cell counting. The result was used to adjust the final concentration of encapsulated bacteria before adding it to the mortar.

Table 1—Mixture proportions of mortar with AE-GS

Batch	AE, %	BC, cfu/mL	Alginate encapsulation					Mortar		
			Σ dH_2O, mL	Sodium alginate, g	Calcium lactate, mL	Urea, mL	Yeast extract, mL	Cement, kg	Sand, kg	Water, L
AE-R 1	3	—	1039	20	80	80	133.2	10.8	32.3	5.4
AE-R 2	9	—	3116	60	240	240	399.6	10.1	30.3	5.1
AE-R 3	15	—	5194	100	400	400	666	9.4	28.3	4.7
AE-GS 1	3	1×10^3	1039	20	80	80	133.2	10.8	32.3	5.4
AE-GS 2	3	1×10^{11}	1039	20	80	80	133.2	10.8	32.3	5.4
AE-GS 3	9	1×10^3	3116	60	240	240	399.6	10.1	30.3	5.1
AE-GS 4	9	1×10^{11}	3116	60	240	240	399.6	10.1	30.3	5.1
AE-GS 5	15	1×10^3	5194	100	400	400	666	9.4	28.3	4.7
AE-GS 6	15	1×10^{11}	5194	100	400	400	666	9.4	28.3	4.7

Note: 1 mL = 0.0010566887 qt.; 1 g = 0.0352739907 oz; 1 kg = 2.2046244202 lb; 1 L = 0.2641721769 gal.

Swelling and water retention properties of AE-GS

The swelling and water retention properties of the AE-GS were investigated using two tests before introducing it into the mortar mixture. The swelling test of the AE-GS was conducted to examine the ability of alginate-hydrogel in absorbing water as it is serves as a water reservoir for bacterial activity when cracking occurs.[21] The modified method described by Wang et al.[21] was adopted in this study. In addition, the experiments were performed in triplicate (n = 3), while the swelling test was carried out in autoclaved distilled water (dH_2O) and the filtered cement slurry (FC) environment.

The cement slurry in this experiment had a concentration of 20 g cement/100 mL water, which was first mixed with water for 30 minutes and then filtered to remove undissolved particles. Meanwhile, both dH_2O and FC were measured using the pH indicator, whereby the pH for dH_2O and FC was found to be 7 and 12.5, respectively. In this test, two conditions of alginate encapsulation were employed, namely, alginate encapsulation without *Geobacillus stearothermophilus* (AE) and alginate encapsulated with *Geobacillus stearothermophilus* (AE-GS). Around 1 g of AE and AE-GS each was placed in the falcon tube and with 30 mL of dH_2O and labeled as AE-DW and AE-GSDW, respectively, for the immersion in dH_2O. Around 1 g of AE and AE-GS each was placed in the falcon tube with 30 mL of FC and labeled as AE-C and AE-GSC, respectively, for the immersion in FC. All the falcon tubes were placed in ambient temperature and closed tightly to avoid further evaporation. After 24 hours, the content in the falcon tubes was placed onto a filter paper that was saturated with dH_2O and FC beforehand. The unabsorbed water by the encapsulated alginate beads was weighed as W_1. The swelling ratio was calculated using Eq. (1). The wet AE-GS left on the filter paper from the swelling test was weighed as W_h

$$S_r = \frac{W_0 - W_1}{W_h} \quad (1)$$

where S_r is the swelling ratio (g/g); W_0 is the initial weight of water or FC (g); W_1 is the weight of water or FC (g) flowing into the falcon tube; and W_h is the weight of AE-GS.

The ability of AE-GS to retain water was measured through the water retention test. As explained by Wang et al.,[21] the water absorbed by hydrogels is slowly released into their surroundings. Hence, the objective of this test was to examine the capability of AE-GS to retain water and water releasing rates post encapsulation, because water is an essential element in bacterial activity. The test was performed using two types of hydrogels—AE and AE-GS—to compare their ability to retain water with or without the presence of bacteria. It is important to note that the water-saturated AE and AE-GS were exposed to ambient temperature. The weight was tested every hour for 96 hours to monitor the reduction in weight caused by the evaporation of the water, which then allows the amount of water released and retained to be measured. Furthermore, the measurement was repeated for three samples (n = 3).

DETERMINATION OF MORTAR MIXTURE PROPORTION AND CASTING PROCESS

In this study, the mortar mixture design was developed based on a ratio of 1:3 of the cement and the sand (S), with a water-cement ratio of 0.5. The materials used for encapsulation at 3%, 9%, and 15% replacement level were calculated by the total weight of the mortar. A total of nine batches were used, and the mixture proportions are shown in Table 1. AE-GS was prepared before the mixing process, while the cement and sand were mixed during the process, followed by the mixing of water and AE-GS. The mortar specimens were cast, demolded, and cured in distilled water. Distilled water was used to ensure there is no interference from other bacteria, unlike if drinking water was used. The detail of the testing is described in the following subsections.

Compressive strength

The compressive strength of the mortar specimens was tested according to BS EN 12390-3:2009. Compressive strength testing of the specimens was performed when the specimens were at 7 and 28 days of age. The specimens' size

was 1.969 x 1.969 x 1.969 in. (50 x 50 x 50 mm), and they were operated at a pacing rate of 674 lbf/s (3.0 kN/s).

Water absorption

In the present study, water absorption testing was carried out according to BS EN 1881-122:1983. The test was conducted at 7, 28, and 60 days of age. Meanwhile, the weight of the specimens was measured before the immersion process as M_1. The specimens were removed from the water tank after being immersed for 30 minutes. Their surfaces were wiped to remove any excess water and weighed as M_2. The absorption was calculated as the increase in mass resulting from the immersion, which is expressed as a percentage of the mass of the dry specimens as per Eq. (2)

$$\text{Water absorption (\%)} = \frac{M_2 - M_1}{M_1} \quad (2)$$

where M_1 is mass dried of specimens; and M_2 is mass of wet specimens.

Healing efficiency

The evaluation of healing was conducted by creating realistic cracks in the specimens. Furthermore, a cylinder with 40 mm diameter size of specimens and 100 mm height was used to evaluate the self-healing efficiency. The realistic cracks were made by applying controlled load using a universal testing machine (UTM) by placing the specimen in the machine between the grips and a suitable jig placed above the table load. The pacing rate, fixed at 18.66 lbf/s (0.083 kN/s) compression load, was applied for all specimens to create multiple realistic crack widths. Moreover, the visualization of crack filling was performed using a portable stereomicroscope camera. Next, the images of the cracks in the specimens were taken after the crack creation, in which six positions were marked on each crack of the specimens that were homogeneously distributed along the crack length. Initial images and final images (after 7, 28, and 60 days of incubation under wetting-and-drying cycles) were taken with the markers located in the center of the images. In each image, crack widths at two locations nearby the marker were analyzed using the camera. On top of that, the cracked specimens incorporated with alginate hydrogel without *Geobacillus stearothermophilus* was prepared as control purpose. The healing efficiency was measured in terms of the reduction in width and calculated using Eq. (3).[20] The self-healing that was performed with the aid of bacteria is due to microbial precipitation of calcium carbonate, whereby the deposition will act as an indicator for healing efficiency[21]

$$\text{Healing, (\%)} = H\,\frac{Cw_i - Cw_f}{Cw_t} \quad (3)$$

where Cw_i is initial crack width; Cw_f is final crack width; and Cw_t is total initial crack width.

The healing efficiency measurement is limited to the observation of the closure of the cracks. More importantly, the filling materials of the cracks were scratched and examined using a scanning electron microscope (SEM) to substantiate that the filling materials were $CaCO_3$ precipitation induced by the encapsulated *Geobacillus stearothermophilus* bacteria.

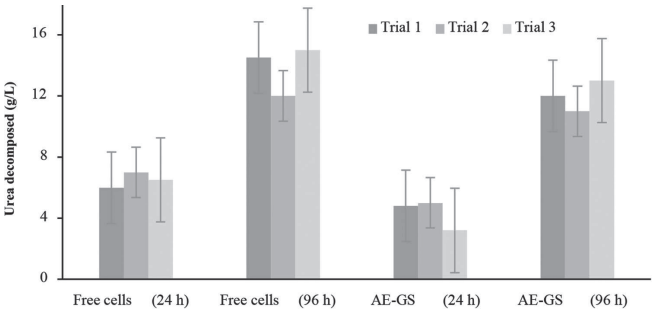

Fig. 1—Urea decomposed in NB for first 24 and 96 hours respectively, in comparison to free live cells of Geobacillus stearothermophilus *toward the alginate-encapsulated* Geobacillus stearothermophilus *(AE-GS).*

Thermogravimetric analysis (TGA)

To perform TGA, a total of three samples—labeled as AE-R3, AE-GS 5, and AE-GS 6, respectively—were taken out, dried at ambient temperature, and pounded into fine powder after curing at 28 days to fit the sample pan of the TGA instrument. Moreover, 10 g of powder was added to the sample pan of the TGA instrument using dry powder from the specimens. The temperature was increased from room temperature to 1832°F (1000°C) at a heating rate of 50°F/min (28°C/min) in the nitrogen atmosphere. The weight loss of the sample during heating was recorded to confirm the $CaCO_3$ formation that was derived from the bacteria.

RESULTS AND DISCUSSIONS

Quantification of urease activity by AE-GS in free cells state and after being encapsulated

The urease activity was measured using the TAN method developed by Nessler. Figure 1 shows the urea decomposition of free cells and alginate-encapsulated cells after 24 and 96 hours of being immersed in the modified NB. Moreover, a small amount of urea was decomposed both in the live cells state and after being encapsulated in the first 24 hours. Meanwhile, the urea decomposition at 96 hours' immersion was found to increase due to the increase in the growth of the bacterial cell as a result of the multiplication. In addition, the free cells managed to obtain a higher concentration of urea at 0.054 to 0.058 lb/gal. (6.5 to 7 g/L) compared with the encapsulated *Geobacillus stearothermophilus* at 0.027 to 0.042 lb/gal. (3.2 to 5 g/L). However, the higher concentration of urea was measured with the free live cells obtained at 0.100 to 0.125 lb/gal. (12 to 15 g/L) compared with the encapsulated *Geobacillus stearothermophilus* at 0.092 to 0.109 lb/gal. (11 to 13 g/L) after 96 hours of immersion. The slower decomposition rate of AE-GS was caused by the decrease of bacterial activity after encapsulation, compared with the free cells. Therefore, a longer time is needed for the encapsulated bacteria to achieve the same amount of decomposed urea compared with the non-encapsulated bacteria, as supported by findings by Wang et al.[16]

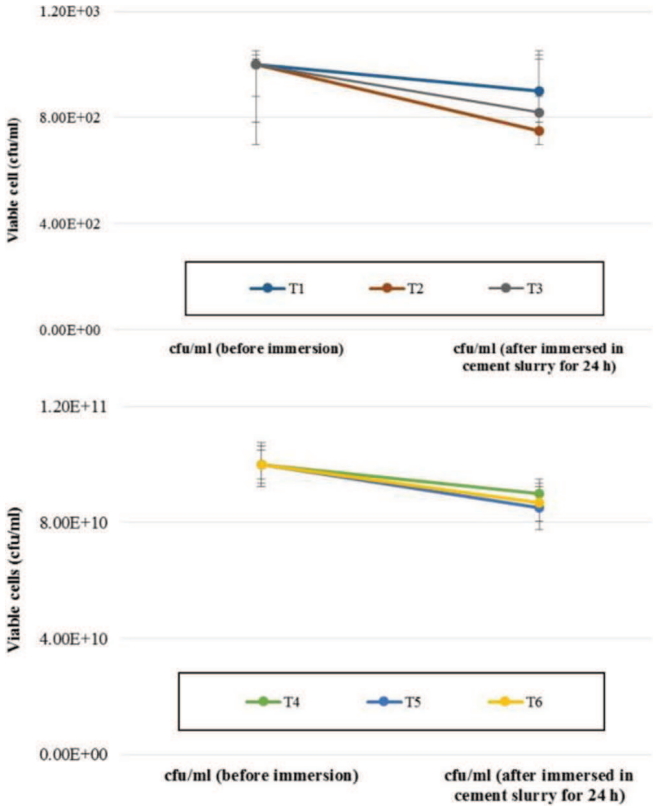

Fig. 2—Viability of Geobacillus stearothermophilus *in mimicked mortar environment after 24 hours.*

Viability of AE-GS in mimicked mortar environment

In the present study, the serial dilution method was employed to count the embedded *Geobacillus stearothermophilus* in the alginate beads. Trials 1 to 3 are the result of the reduction number for bacterial concentration at 1×10^3 cfu/mL, while Trials 4 to 6 represent the result for bacterial concentration at 1×10^{11} cfu/mL. As shown in Fig. 2, the reduction of cell numbers occurs at 10% to 15%, whereby the reduction number occurs between 8.5×10^{10} and 9.0×10^{10} cfu/mL at the concentration of 1.0×10^{11} cfu/mL. On the other hand, the concentration of 1.0×10^3 cfu/mL was found to reduce up to 8.5×10^2 to 9.0×10^2 cfu/mL.

Swelling and water retention properties of AE-GS

Figure 3 depicts the swelling capacity for AE and AE-GS in cement slurry (C) as well as distilled water (DW). As can be observed, the swelling capacity for alginate-encapsulated without *Geobacillus stearothermophilus* in cement slurry (AE-C) is approximately 0.254 oz/oz (7.2 g/g), while the alginate encapsulated with *Geobacillus stearothermophilus* in cement slurry (AE-GSC) is approximately 0.314 oz/oz (8.9 g/g). On the other hand, the swelling capacity is found to be around 0.187 oz/oz (5.3 g/g) for alginate encapsulated with *Geobacillus stearothermophilus* immersed in distilled water (AE-GSDW), and 0.236 oz/oz (6.7 g/g) for alginate encapsulated without the bacteria immersed in distilled water (AE-DW). The alginate encapsulation loaded with bacteria has a higher water uptake compared with the non-loaded bacteria-alginate encapsulation for both immersion conditions. This further indicates that the inclusion of *Geobacillus stearothermophilus* has a direct relation with

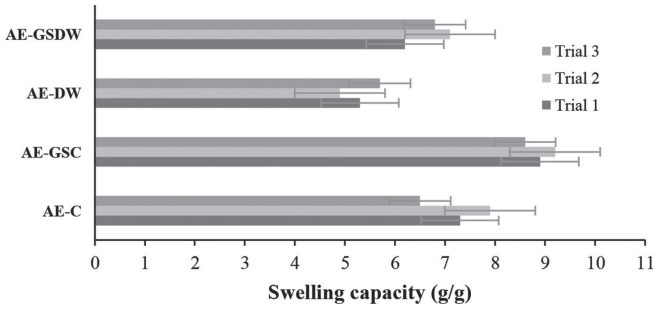

Fig. 3—Swelling test results of AE-GS immersed in water and cement slurry.

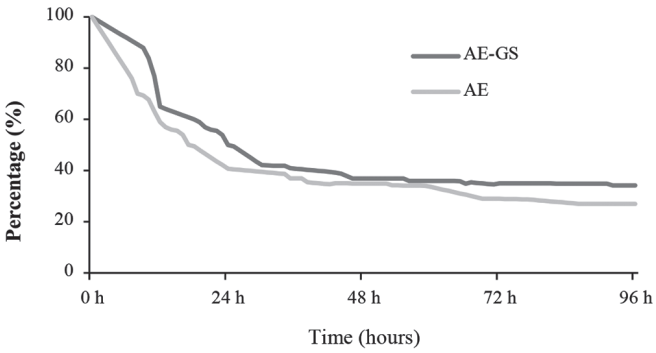

Fig. 4—Weight changes in alginate-hydrogel after being immersed in water for 24 hours.

the water uptake through the alginate membrane. Moreover, it is depicted that the swelling of the beads in cement slurry is higher, similar to that in water due to the different viscosities of water and cement slurry, which plays a significant role in determining the absorption rate.

The swelling capacity will be able to determine the extent to which the alginate hydrogel will swell on the basis of the presence of water upon the formation of the crack. Furthermore, it is important to note that the excessive swelling capacity will cause premature breakage and release the bacteria and the nutrient embedded inside the hydrogel before the appearance of cracks. Apart from that, the alginate-hydrogels have direct contact with the high pH mixture and start to take up water during the mortar-mixing process. However, this will be offset by the cement hydration, which gradually causes alginate-hydrogel to lose its water content and shrink, thus leaving additional voids in the mortar matrix. The swelling properties of alginate-encapsulated without bacteria (AE-C and AE-DW) and with bacteria (AE-GSC and AE-GSDW) are found to be consistent with the range reported by other researchers. Therefore, both AE and AE-GS are in an acceptable state and will not cause serious breakage that will affect their efficiency.

The water retention properties are shown in Fig. 4. In this case, AE-GS can retain 65% of the absorbed water for the first 12 hours, while 59% of water is found to be retained for AE. The weight of the alginate beads with and without *Geobacillus stearothermophilus* managed to reach the equilibrium state at 48 hours (37%) and 84 hours (27.4%) after the beads were taken out from 24 hours immersion in water (Fig. 4). Hence, this indicates the capability of alginate beads to retain their water for bacterial activity.[21] On the

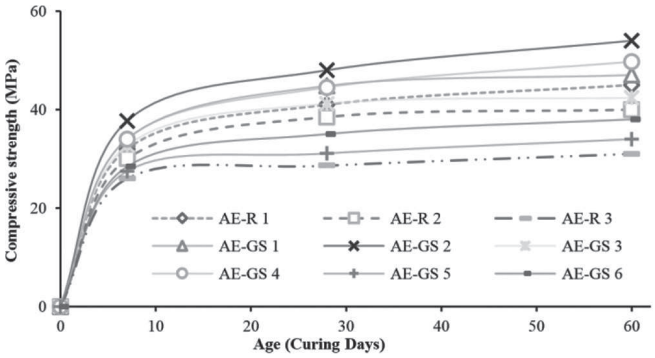

Fig. 5—Compressive strength of mortar cubes.

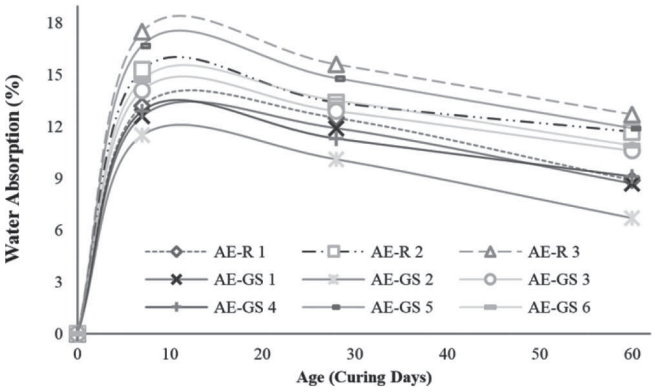

Fig. 6—Water absorption of mortar cubes.

other hand, the hydrogels need to be able to release water at a slow rate to the mortar surroundings to prevent the reduction of mortar strength through an increase in the water-cement ratio.

Compressive strength

Figure 5 depicts that higher incorporation of AE-GS can cause a reduction in strength, particularly at 15%. Nevertheless, both lower levels of replacement at 3% and 9% of AE-GS do not substantially affect the strength. The compressive strength of the AE-GS mortar was compared with that of the control mortar (AE-R 1, AE-R 2, and AE-R 3) to determine the effect of incorporating encapsulated beads containing bacteria. The results obtained show that mortar incorporated with AE-R mortar specimens tends to record lower strength compared with the AE-GS series. In addition, the AE-GS2 mortar containing 3%, 1×10^{11} cfu/mL of *Geobacillus stearothermophilus* bacteria cells are observed to have a higher compressive strength. On the other hand, the lowest compressive strength is detected for those mortar specimens containing 15%, 1×10^{3} cfu/mL bacteria cells (AE-GS5). More importantly, it is noted that incorporating encapsulated bacteria up to 3%, 1×10^{11} cfu/mL cell concentration managed to improve the compressive strength of the mortar. Hence, it can be concluded that the effect of bacteria are lower at higher AE-GS content up to 15%.

Water absorption

Figure 6 shows that the lowest absorption belongs to the mortar with the lower replacement of AE-GS mortar, which is 3%. Meanwhile, AE-GS1 and AE-GS2 show lower water

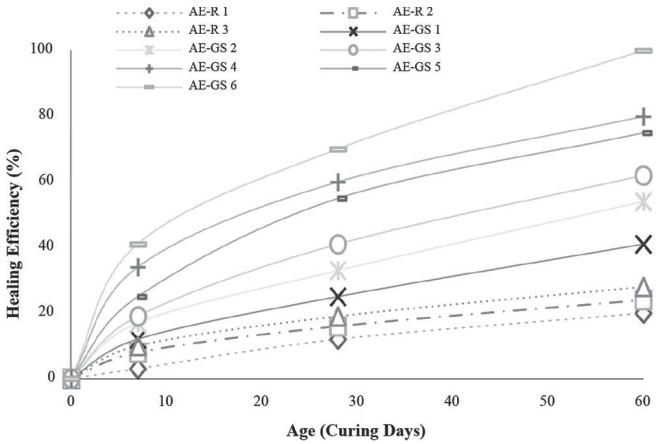

Fig. 7—Healing efficiency of AE-GS mortar.

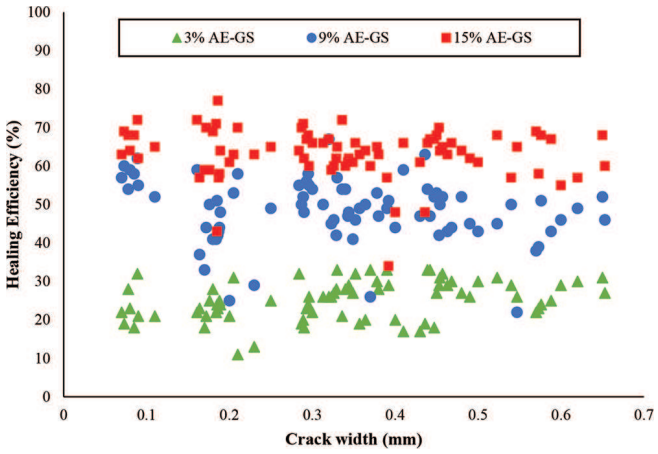

Fig. 8—Healing efficiency of AE-GS mortar with different crack width.

absorption with the replacement of 3% containing 1×10^{3} and 1×10^{11} cfu/mL compared with the mortar without bacteria (AE-R1). Moreover, the same trend applies to those with the replacement of 9% and 15% mortar, which depict a lower absorption rate. More importantly, it is shown that the bacteria $CaCO_3$ precipitation aids the closure of voids, which contributes to the reduction of the absorption rate.

Healing efficiency

In Fig. 7, the mortar that contains 9% and 15% AE-GS is shown to charter higher healing efficiency compared with that of 3%. Complete healing efficiency (100%) managed to be achieved for alginate encapsulation with the inclusion of 1×10^{11} cfu/mL cells at age 60 days. The minimum healing efficiency was found for AE-GS 1 (1×10^{3} cfu/mL, 3%) on the basis of results at 60 days. The crack width ranging from 5.906×10^{-3} to 0.026 in. (0.15 to 0.65 mm) and the tabulation view of healing efficiency are shown in Fig. 8. The correlation between healing efficiency and total replacement of AE-GS managed to be achieved is based on the variation of tabulated healing efficiency, in which a higher healing percentage is observed with the increase in the percentage of total replacement of AE-GS in the mortar. This is believed to be caused by the higher dependency of healing efficiency on the distribution of AE-GS in the mortar, which further

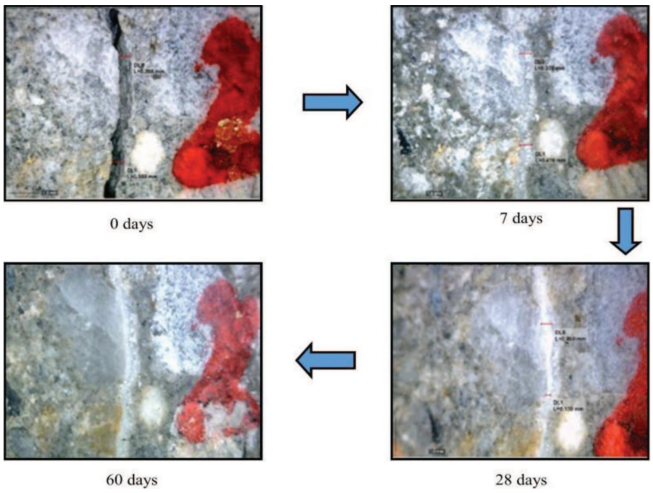

Fig. 9—Crack filling with whitish precipitation by Geobacillus stearothermophilus *(specimen in series AE-GS 5).*

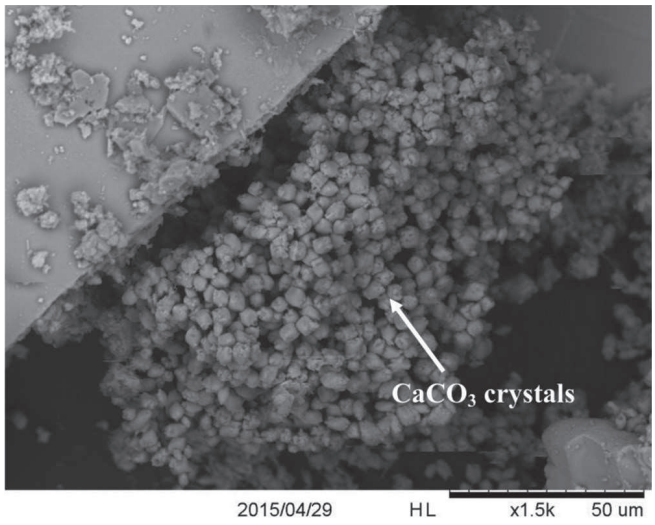

Fig. 10—SEM micrographs showing calcium carbonate ($CaCO_3$) precipitation from crack-filling material.

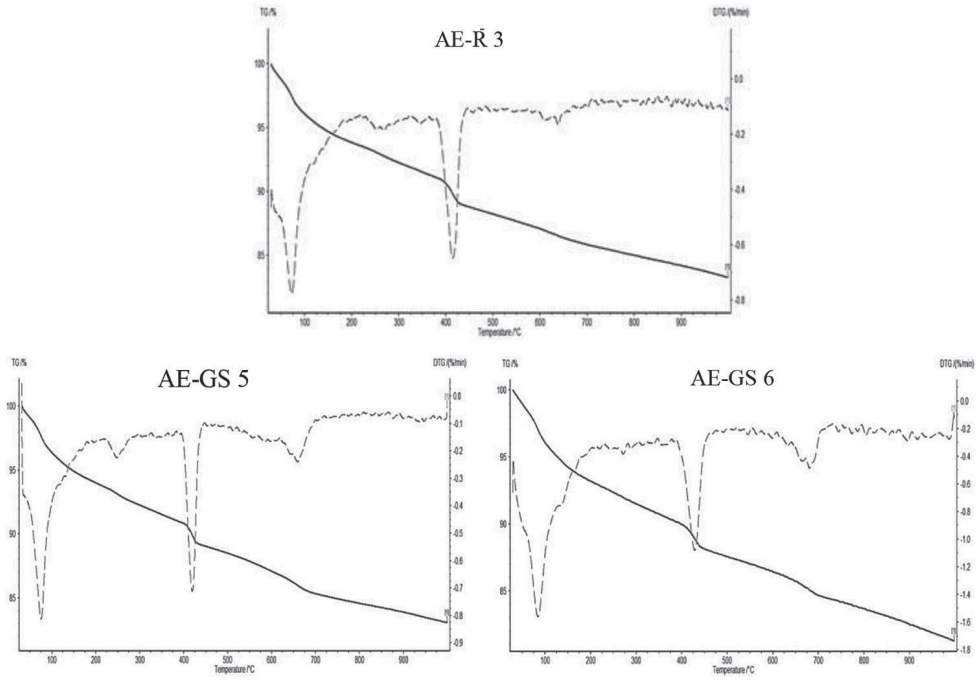

Fig. 11—TG/DTG curves for AE-R3, AE-GS 5, and AE-GS 6 at 28 days.

indicates that the increase in the potential of healing efficiency will incorporate higher AE-GS content.

The healing effect was assessed in terms of crack width and healing efficiency. The healing image is presented in Fig. 9, which shows that the crack widths at 0.015 and 0.019 in. (0.38 and 0.47 mm) are completely healed. The crack path is filled with whitish precipitation, derived by the incorporation of AE-GS into the mortar. Denser precipitation was observed with the longer incubation period. The SEM micrographs illustrated in Fig. 10 portray that the formation of $CaCO_3$is in the rhombohedral shape, which is considered as the most stable form of calcite.[29,30]

TGA

Figure 11 shows the TGA results of the decomposition mass of AE-R, AE-GS 5, and AE-GS 6 mortars. As can be observed, the decomposition weight (in mg) occurs at 392 to 752°F (200 to 400°C),[25] while the decomposition of $CaCO_3$ occurs at a temperature of 1202 to 1292°F (650 to 700°C).[31] In addition, the TGA result presents that the decomposition mass for 392°F (200°C) to 842°F (450°C) occurs at a similar temperature for both AE-R and AE-GS. However, distinct weight loss was noticed in the mortar incorporated with AE-GS compared with AE-R, which was evidenced by the presence of $CaCO_3$ that contributes to the healing.

CONCLUSIONS

In the present study, *Geobacillus stearothermophilus* vegetative cells were successfully encapsulated into alginate-hydrogel. The reduction of *Geobacillus stearothermophilus* was expected, whereby approximately 10 to 15% excess of bacterial cells were added to ensure that the final

cell numbers could be achieved. Meanwhile, the swelling and water retention properties of alginate-hydrogel were also found to increase with the addition of bacteria. The potential of AE-GS in self-healing concrete is evidenced by the presence of whitish precipitation of bacteria through the crack filling along the crack line. The precipitation of $CaCO_3$ by bacteria managed to fill the mortar pores, which was further proved by the lower rate of water absorption compared with that of the nonbacterial mortar. Finally, the maximum crack width healed was found to be about 0.025 in. (0.63 mm) mortar incorporated with AE-GS.

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