

The Effect on Using Cells versus Spores of *Bacillus Sphaericus* on the Healing Performance of Self-Healing Mortar

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Abstract. Bacteria-based self-healing concrete has become an effective approach to mitigate microcracks in the concrete structure. However, there are still doubts about when to use vegetative cells or spores of *Bacillus sphaericus* as a healing agent. Thus, this research aims to give recommendations regarding this choice. Spores and cells were encapsulated into expanded clay aggregate to protect them from the harsh environment of fresh mortar. The viability of cells and spores after encapsulation was investigated. The 28- and 90-day mortar compressive strength was analysed. The healing performance of the resulting mortar samples that were cracked at the age of 28 and 90 days has also been observed. The results show that both cells and spores were still active after encapsulation. The yeast extract added to mortar containing spores decreased the compressive strength of the mortar compared to the reference sample. From the healing performance result, it seems that the spores are more suitable for mitigating microcracks in aged mortar, while cells are more suitable for mitigating early-age cracks in the mortar.

1 Introduction

A bacteria-based self-healing concrete has become an alternative approach to mitigate micro-cracks in concrete or mortar. Through the microbially induced calcium carbonate precipitation (MICP) mechanism, the bacterial activity could accelerate the production of carbonate that later reacts with the calcium in cement or nutrients, which can induce the formation of calcium carbonate that can close the cracks. Among bacterial metabolic pathways, urea hydrolysis gives rapid and satisfying healing performance [1–3]. *Bacillus sphaericus* has become one of favorite urea-positive strains due to its rapid healing performance in concrete [4–7].

In order to survive in the harsh environment of fresh concrete, the bacteria need to be encapsulated into a protector. An affordable protector that delivers excellent properties is expanded clay lightweight aggregate (EC LWA) [8–10]. The high amount of pores in expanded clay become a comfortable home for the bacteria.

Regarding the form of bacterial healing agent used in the system, most researchers used spores due to their high survival rates in the concrete [1,6,7,10]. However, when spores cannot germinate into cells due to unfavourable environmental conditions, the MICP mechanism will not succeed. A limited amount of reports could be found on directly employed cells as a healing agent. Initial research on utilizing cells of *B. sphaericus* as a healing agent found that mortar containing cells shows better healing performance than mortar containing immobilized spores [4]. However, in

that report, the cracks were fabricated at the age of 28 days, when autogenous healing mechanisms still largely occur.

Aiming to have an overview of suitable conditions to use spores or cells, in this research, spores and cells of *B. sphaericus* were encapsulated into EC LWA. The cracks were fabricated at 28 and 90 days to see whether the cells still survived after 90 days. By conducting this research, a recommendation can be formulated on when engineers need to use spores or cells depending on their needs.

2 Materials and Methods

2.1 Spores and Cells Cultivation

The vegetative cells of *Bacillus sphaericus* LMG 22257 were obtained by inoculating cells into sterilized yeast extract and urea medium, both with a concentration of 20 g/l. The culture was then incubated on a shaking table incubator (120 rpm, 28°C) for 24 hours. The pellets were obtained by centrifuging 24-hour-old culture (15050 g for 7 minutes), which were then re-suspended in yeast extract solution (5 g/l) and final concentration of the cells was 2×10^9 cells/ml.

The spores of *Bacillus sphaericus* LMG 22257 were produced by inoculating cells pellets into a sterilized sporulation medium that contained yeast extract (2 g/L), peptone (3 g/L), glucose (4 g/L), malt extract (3 g/L), K_2HPO_4 (1 g/L), $(NH_4)_2SO_4$ (4 g/L), $CaCl_2$ (0.1 g/L), $MgSO_4$ (0.8 g/L), $MnSO_4 \cdot H_2O$ (0.1 g/L), $FeSO_4 \cdot 7H_2O$ (0.001 g/L), $ZnSO_4$ (0.01 g/L) and

CuSO₄·5H₂O (0.01 g/L). The spore's culture was incubated in a shaking table incubator (120 rpm, 28°C) for 7-14 days until all cells transformed into spores. The method of harvesting spores was the same as harvesting cells. The spores with a concentration of 2×10⁹ spores/ml were re-suspended in saline solution. To kill the vegetative cells, the spore's suspension was pasteurized before being stored in the refrigerator for further use.

2.2 The Viability of Spores and Cells

A commercial expanded clay lightweight aggregate (EC LWA) from Argex Nv, which has 24h water absorption value of 20.85 % and an apparent density of 1.25 was used as a bacterial carrier. First, a vacuum pressure of -0.8 bar was applied to sterilized dried LWA in a sealed penicillin bottle, followed by a spores / cells injection. This vacuum pressure was kept for 30 minutes. A pressure of +1 bar was then introduced to the bottle. This pressure was kept for 24 hours at room temperature. Then, the bottle was uncapped, and the dried LWA containing cells was rinsed in demineralized water and transferred into sterile urea solution (20 g/l). For samples containing spores, the immersion solution contained 2 g/l yeast extract and 20 g/l urea. A sample was taken from the urea solution for analysing the urea decomposition at 0, 24 and 72 hours after immersion. The TAN value was measured using a spectrometer at wavelength of 425 nm. The decomposition of urea (DU) was calculated by equation (1).

$$DU = TAN \times dilution \times Mr \frac{Urea(\frac{60g}{mol})}{2 Nitrogen (2 \times \frac{14g}{mol})} \quad (1)$$

The viability of spores and cells were monitored by measuring the total ammonium nitrogen (TAN) formed [7]. During the MICP process the bacteria will accelerate the decomposition of urea into two ammonium molecules. Thus, measuring the ammonium by using Nessler test is an effective indirect measurement to monitor the activity of bacteria.

2.3 Mortar Production

The same EC LWA as described in section 2.2 was used in mortar production as river sand replacement (30% by volume). A certain amount of liquid (water/cells/spores) was entrapped into EC LWA through a vacuum (-0.8 bar) followed by application of pressure (+1 bar), as described earlier in section 2.2. The amount of liquid added was calculated based on the water absorption of EC LWA.

Cement type 1 52.5 from Holcim was used as a binder. Yeast extract (YE), urea, and calcium nitrate tetrahydrate (Cal) with 99% purity were added into the mortar mixture as a bacterial nutrient (Carl Roth, Belgium). To compensate for the water present in calcium nitrate tetrahydrate (4H₂O) the mixing water

was reduced equally. The mix proportion of one batch of mortar for three prisms of 4x4x16 cm³ is presented in table 1.

2.4 The Properties of Mortar

The mortar samples were produced by following EN 196 standards. The 28- and 90-day-old mortar samples were tested for their compressive strength in accordance with EN 196 standards. To investigate the healing performance of the resulting mortar at different age, multiple cracks were fabricated at the age of 28 and 90 days by introducing tensile load with a speed of 0.01 mm/s. The load was stopped when the crack width reached approximately 0.5 mm. The initial crack widths were observed under a microscope. The samples were then subjected to wet-dry curing (4 hours wet, 4 hours dry) for 30 days. The final crack width after being cured was observed under the microscope again. Finally, the healing ratio was the ratio between crack with reduction and initial crack width.

3 Result and Discussion

3.1 The Viability of Spores and Cells

The viability of spores and vegetative cells after being encapsulated into EC LWA is displayed in Fig. 1. The maximum theoretical amount of urea that can be decomposed in the solution is 20 g/l. When cells were encapsulated into EC LWA, all urea in the solution could be fully decomposed in 24 hours. In the meantime, when spores of *B. sphaericus* were encapsulated into EC LWA, it took 72 hours for spores to transform into cells and actively decompose urea in the solution. Based on the viability test result, it can be concluded that the encapsulation process did not damage the cells or spores. The spores and cells of *B. sphaericus* could still actively decompose urea in the solution after being encapsulated into EC LWA carrier. This result was in line with the previous findings stating that the pH and environment temperature are essential parameters that significantly affect the viability of spores rather than the encapsulation methods [7]. Other literature also reported that vacuum and pressure treatment did not damage the cells of *Diaphorobacter nitroreducens* and *Pseudomonas aeruginosa* [11]

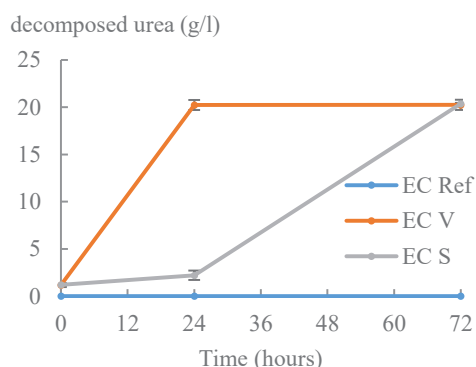


Fig 1. Urea decomposition activity of spores and cells after encapsulation into EC LWA. The error bar represents the standard deviation (n=3).

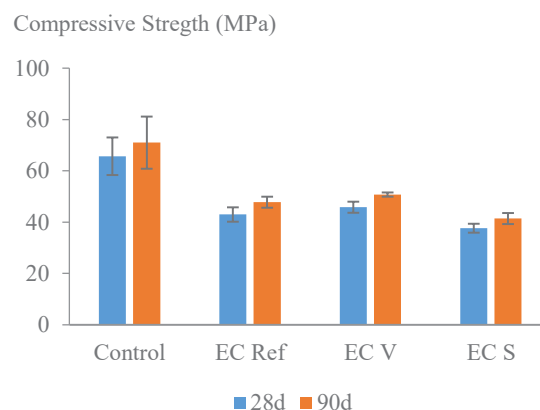


Fig 2. The 28- and 90-days compressive strength of mortar. The error bar represents the standard deviation (n=3).

Table 1. Mix Design of Mortar

Type	LWA (g)	Cells/spores/water (ml)	Sand (g)	Cement (g)	Water (g)	Y.E. (g)	Urea (g)	Cal* (g)
Control	0	0	1350	450	225	0	0	0
EC Ref	186	17	1164	450	214	0	18	36
EC V	186	17	1164	450	214	0	18	36
EC S	186	17	1164	450	214	1.53	18	36

*Cal : Calcium nitrate tetrahydrate

3.2 The Mechanical Properties of Mortar

Based on data presented in Fig. 2, the presence of cells and spores slightly affected the compressive strength of the resulting mortar. At all mortar ages, the effect of replacing 30% by volume of sand with EC LWA decreased the compressive strength of resulting mortar up to 38%. A further slight decrease in compressive strength in sample EC S versus EC V was likely due to the presence of yeast extract in the mixture. As reported previously, yeast extract containing sugar could delay the hydration reactions, leading to a strength decrease of the resulting mortar [9,12]. Similar results were also found in our previous report on encapsulated spores into EC LWA. The presence of spores alone did not significantly affect the strength of the resulting mortar [11].

The role of EC LWA as a water reservoir could be seen in the increasing mortar's strength at the age of 90 days. The water inside the LWA was slowly released and reacted with unhydrated cement in the mortar. This phenomenon was also found in a previous article that investigated the durability performance of mortar containing EC LWA [11,13,14]. The denser ITZ between LWA and cement paste caused an improvement in the compressive strength of the resulting mortar [13,14].

3.3 The Healing Performance of Mortar

When cracks were created at 28 days, it is quite challenging to differentiate whether the cracks closed due to continued hydration or the existence of a healing agent (Fig. 3). Still, in the same age group, cracks with a width range of 0.2-0.3 mm in EC Ref, EC V, and EC S were completely closed after being cured for 30 days. At a larger crack width range (0.3-0.4 mm), EC Ref and EC V had a healing ratio of more than 80%, while the EC S sample had a lower healing ratio. An excellent healing performance in the EC Ref sample group is a proof that EC LWA could act as a water reservoir in the mortar. Thus, when cracking occurs, water trapped inside the pores of EC LWA could react with unreacted cement and closed the cracks. The role of LWA as internal curing agent that could promote continued hydration was also reported in the literature [15].

Compared to Intarasootron et al.'s previous work, in which *B. sphaericus* was encapsulated into calcium chloride cells, the crack width closed in 28- days old EC V samples is two times higher [4]. Intarasootron et al. also suggested using vegetative cells for young mortar, as it gives better performance than spores[4].

On the other hand, when cracks were fabricated at the age of 90 days, the effect of introducing a healing agent into the mixture could be clearly seen. In sample EC S where spores were incorporated into EC LWA, cracks with the width range of 0.2-0.3 mm had an average healing ratio of 91%. In sample EC V (cells), with the same crack width range, the average healing ratio reached 45 %, slightly higher compared to the reference sample (EC Ref), which had a healing ratio of 35 %. It seems that the cells in the mixture could not survive as well as spores in the mortar until 90 days. Bacterial cells are in an active phase, and they need enough oxygen and nutrients to survive in mortar, while during the hydration process the pores reduce in size and the available oxygen decreases as the mortar ages.

A decrease in healing performance was also found in a previous report on mortar containing granulated cells. The initial crack width that could be closed in mortars containing granulated cells cracked at the age of 6 months was only 0.15 mm, while larger crack width was reported to be completely closed when cracks were fabricated at a younger age (28 days) [16]. In previous work by Yang et al., it was also recommended to keep the crack width below 0.15 mm for young-age mortar specimens to maintain the healing performance[17]. However, at larger crack width range (0.3-0.5 mm), mortar with granulated cells has better healing performance compared to the result in this research [16].

In conclusion, vegetative cells could be an alternative healing agent when cracks need to be mitigated at an early age. However, when cracks need to be mitigated at later age, spores would be more suitable as healing agent. It is also important to mention that the effective crack width that could be closed when cracks were fabricated at later age is in the range of 0.2-0.3 mm, while larger crack width could be closed when cracks were fabricated at the age of 28 days.

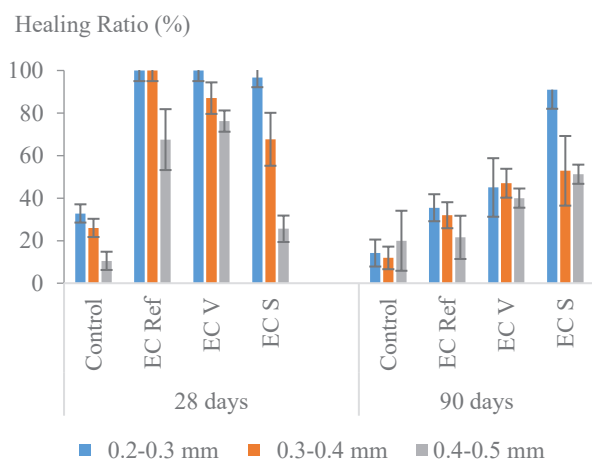


Fig 3. The healing ratio of the tested mortar. Cracks were created at the age of 28 and 90 days. The error bar represents standard deviation.

4 Conclusions

Based on results presented above, several conclusions could be drawn:

1. The spores and cells of *B. spharicus* are still viable after being encapsulated into EC LWA.
2. The addition of spores and cells has minor effects on the compressive strength of the resulting mortar. A slight decrease in the strength of mortar-containing spores compared to the reference sample was mostly caused by the presence of yeast extract in the matrix.
3. The autogenous healing mechanism due to continued hydration was observed mainly when samples were cracked at 28 days.
4. In the crack width range of 0.2-0.3 mm, cells are more suitable for mitigating early-age cracks, while spores could be used for healing cracks in mature mortar.

The first author would like to thank the Ministry of Education, Culture, Research and Technology of Indonesia for their financial support. The assistance and help from all technicians in the Magnel-Vandepitte Laboratory and CMET at Ghent University is also acknowledged.

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