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Effect of bacterial curing and bacterial additive on concrete properties

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Abstract:
In this study, calcium carbonate was formed on the surfaces and inner structure of concrete using the microbially induced carbonate precipitation method. Bacillus megaterium bacteria were supplemented into the curing water and concrete mixtures. Three types of concrete were tested: control concrete, bacteria-containing concrete, and concrete cured in bacterial liquid. Compressive strength, water absorption, capillary water absorption, scanning electron microscopy (SEM), and mapping analyses were conducted to investigate the effects of bacterial additive or bacterial curing to concrete specimens. Bacteria spore added to the concrete mixture and curing in bacterial media increased the compressive strengths of concrete by up to 9,52 % at the end of 28 days of curing. Bacterial curing and the addition of bacteria spores caused a reduction in water absorption rates owing to changes in the concrete structures. Calcite only formed on the surfaces of the samples treated with bacterial curing liquid, thereby limiting its effect on capillary water absorption. In contrast, capillary water absorption in the bacterial concrete decreased by 50 % compared to the control concrete. The crystalline structures of calcium carbonate and bacterial concrete were analysed through SEM imaging. Mapping analysis revealed that the primary elements of calcite were considerably more concentrated on the surface of bacterial concrete than in the control concrete.

Keywords:
bacterial concrete; bacterial curing; compressive strength; mapping; self-healing

1 Introduction

Concrete is the most commonly used construction material owing to its advanced properties, such as durability, low cost, high internal stability, and compressive strength. However, cementitious materials are exposed to various external factors throughout their service life, resulting in the formation of structural and non-structural cracks, spalling, and disintegration, which reduce the physical and mechanical strength of concrete. Therefore, renewable and recoverable composites are required to withstand the aforementioned effects and damage [1, 2].

Various materials, such as chemical admixtures, polymers, and cement-based products, are used to protect and repair concrete. However, the manufacturing processes and chemical compositions of these materials make them costly and hazardous to the environment [3-7]. Therefore, bacteria are used as biological additives in concrete to produce relatively environment-friendly, low-cost, and sustainable concrete. Bacteria can naturally generate calcite in various ways, a phenomenon called biomineralization [8, 9].

Calcite ($CaCO_3$) produced by bacteria fills the pores and cracks in concrete, resulting in a relatively compact structure. Thus, bacteria can improve the mechanical properties and durability of concrete. The microbially induced carbonate precipitation (MICP) method is expected to be beneficial in numerous civil and structural engineering applications. The chemical reactions of the MICP process are expressed in Equations 1 to 6 [10].

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \qquad (1)$$

$$NH_2COOH + H_2O \leftrightarrow NH_3 + H_2CO_3 \qquad (2)$$

$$H_2CO_3 \leftrightarrow HCO_3 + H^+ \qquad (3)$$

$$2NH_3 + 2\,H_2O \leftrightarrow 2NH_4^+ + 2\,OH^- \qquad (4)$$

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \qquad (5)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \qquad (6)$$

Through these reactions, highly pure calcium carbonate is formed around the bacterial cell, thereby improving the concrete properties [11]. The mechanism underlying calcite production by the Bacillus species is illustrated in Figure 1.

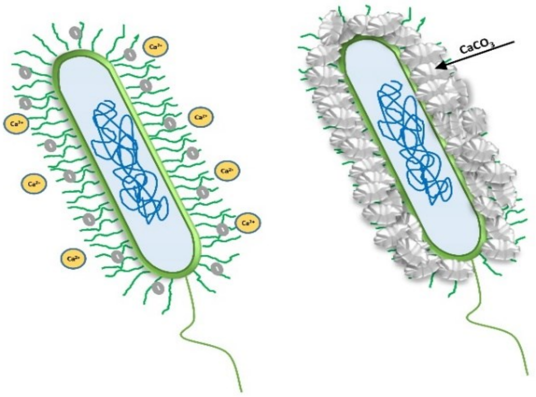


Figure 1. Schematic of calcium carbonate production by bacteria

Previous studies have indicated that bacterial supplementation of concrete mixtures positively and significantly influences their compressive strength. Andalib et al. [12] improved the compressive strength of concrete mixtures by supplementing them with different concentrations of bacteria. Krishnapriya et al. [13] and El-Enein et al. [14] found different bacterial species to have positive impact on concrete properties. Bai and Varghese [15] reported that MICP yielded positive outcomes for mineral additives. In addition to improved mechanical properties, bacterial concrete is relatively durable against the effects of high-temperature and acid attacks [16, 17].

Bacteria are integrated into cement-based materials through various methods, such as spraying, injection, direct addition to the mixture, use of glass tubes, and impregnation of the mixing materials. The direct addition of bacteria to the mixture is the most effective method of supplementation [18]. However, adding the same type of bacteria as external treatment or additive to the concrete mix and comparing the results would reveal the effectiveness of the treatment technique relatively clearly. Therefore, this study identified the most effective method of using bacteria by evaluating different ways of bacteria addition.

In this study, three different types of concrete were examined: bacteria-free control samples, concrete samples cured in a bacterial liquid, and concrete containing bacteria. Therefore, whether the bacterial spores are more effective in the concrete mix or as curing treatment was investigated. Compressive strength, water absorption, and capillary water absorption experiments were conducted to examine the effects of calcium carbonate formations on the concrete. As a result of the MICP mechanism on the concrete properties, the structures of calcium carbonate inside concrete were imaged using a scanning electron microscope (SEM). In addition, the intensity of calcite formation on the surface was monitored via EDS mapping analysis of the surfaces of both the bacteria-free and bacteria-supplemented concrete specimens.

2 Methodology

2.1 Bacteria characteristics

Bacillus megaterium bacteria were used as an additive in this study. The bacteria were obtained from a culture collection at the Refik Saydam Hygiene Institute (Ankara, Turkey). As they were not pathogenic, the bacterial species used in the present study had no negative impacts on human health.

2.2 Bacteria activation and broth medium

Bacillus megaterium was selected as the bacterial additive because of its high urease activity [19, 20]. The bacterial cells were maintained at -80 °C in 50 % (v/v) glycerol and cultured on nutrient agar plates at 30 °C for 24 h. Pure colonies were transferred to sterile nutrient broth and the culture was incubated at 30 °C with agitation at 150 rpm for 24 h. In addition, a curing liquid for bacterial concrete was prepared. Nutrient broth for this medium (5,0 g peptone, 1,5 g bovine extract, 1,5 g yeast extract, and 5,0 g sodium chloride per litre) was autoclaved and supplemented with filter-sterilized 2 % urea solution and 25 mM $CaCl_2$ (NBU medium). The medium also contained bacteria (Bacillus megaterium) at a concentration of $3×10^6$ cells/ml.

2.3 Cement

CEM I 42.5R Portland cement was used in the manufacture of all the mixtures. The chemical composition of the cement is presented in Table 1.

Table 1. Chemical composition of cement (% weight)

SiO_2	CaO	Al_2O_3	MgO	Fe_2O_3	K_2O	Na_2O	SO_3	Loss of Ignition
20,12	62,38	5,88	1,87	2,40	0,93	0,38	3,28	1,82

2.4 Aggregate

The aggregates used in the experimental processes were compliant with TS 706 EN 12620 standards [21]. Specific gravity was 2,70 for the coarse aggregate and 2,65 for the fine aggregate. The aggregate gradation based on a maximum aggregate size (Dmax) of 16 mm is shown in Figure 2.

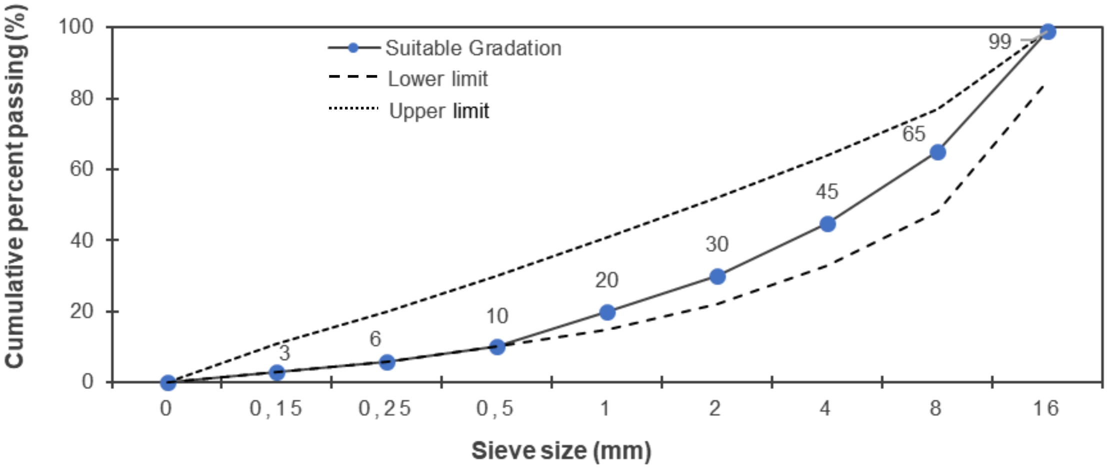


Figure 2. Gradations of aggregates

2.5 Water

Tap water was used as mixing water for CC and concrete specimens without bacteria (CC+cured). Bacteria spores were only added to the mixing water for BC.

2.6 Concrete mixture proportions and preparations

Concrete was produced in accordance with TS 802 standards [22]. Concrete cubic specimens (100×100×100 mm) were produced from the relevant mixtures. The maximum aggregate size (Dmax) was set to 16 mm and the water/cement ratio to 0,45. The mixing water for bacterial concrete (BC) was supplemented with 3×106 Bacillus megaterium bacteria. Mixing water containing bacteria was then added to the dry mixture. Fresh concrete was poured into cubic moulds and left to stand for 24 h. The BC samples and a portion of CC+cured specimens were subsequently placed in curing solutions prepared with bacterial spores and NBU medium. Furthermore, CC specimens were cured in standard tap water until the day of the experiment. The concrete mixture ratios are presented in Table 2.

Table 2. Material composition of concrete specimens for 1 m^3

Mixture name	**Coarse Aggregate** (kg/m^3)	**Fine Aggregate** (kg/m^3)	**Water** (kg/m^3)	**Cement** (kg/m^3)	**Bacteria addition in mixing** (cell/ml)	**Curing condition**
CC	942	766	195	433	none	Tap water
CC+cured	942	766	195	433	none	NBU media +bacteria (3×10^6)
BC	942	766	195	433	3×10^6	NBU media +bacteria (3×10^6)

2.7 Compressive strength test

Compressive strength tests were conducted on the cubic specimens in accordance with the TS EN 12390-3 standards [23]. CC specimens were cured in tap water and BC specimens in bacterial curing solutions for 7 and 28 days (Figure 3). Additionally, various CC samples (CC+cured) were cured and the effects of the curing solution on their compressive strength investigated.

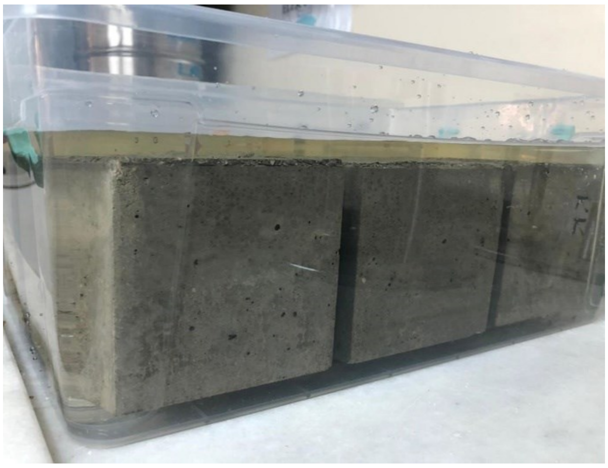


Figure 3. Bacterial concrete specimens in curing liquid

2.8 Water absorption test

Water absorption tests were conducted in accordance with TS EN 12390-7 standard [24]. At the end of the 28-day curing period, saturated weights of the concrete samples were determined. Afterwards, specimens were oven dried at 105 ℃ for 24 h, and dry weights were determined. Based on these weights, the water absorption of the samples was calculated using Equation 7.

$$\text{Water absorption ratio (\%)} = \frac{\text{Wsat} - \text{Wdry}}{\text{Wdry}} \cdot 100 \qquad (7)$$

Where Wsat is the surface dry mass of a concrete specimen soaked in water and Wdry is the dry weight of the concrete specimen.

2.9 Capillary water absorption test

Capillary water absorption tests were conducted according to the TS 4045 standard [25]. At the end of the 28-day curing period, all concrete specimens were dried in an oven at 105 ℃ for 24 h and the dry weights of the specimens were measured. The sides of the dry specimens were covered with waterproof tape and placed in the test apparatus. The specimen weights were measured at 0, 15, 30, 60, 90, 135, 180, 225, 270, and 1500 min and the absorbed water quantities were determined. Based on these values, the capillarity coefficients were calculated using Equation 8:

$$N = \frac{\text{m1} - \text{m0}}{\text{A}\sqrt{\text{t}}} \qquad (8)$$

Where N is the capillarity coefficient ($\text{cm}^3/\text{cm}^2\ \sqrt{\text{min}}$), m0 the initial weight (g) of the specimen, m1 the weight of the specimen at the end of a certain period (g), A the water contact surface (cm × cm) of the specimen, and t the water contact duration (min).

2.10 Scanning electron microscopy

Scanning electron microphotographs were obtained using a Zeiss / Gemini 300 - EDS (Bruker /XFlash 6I100) instrument. Fragments obtained from the concrete samples were examined. The fragment widths ranged from 5-15 mm. The samples were dehumidified and vacuumed prior to SEM analysis. The microstructures of the samples were visualized using SEM. In addition, a mapping analysis was performed to observe the elemental distribution on the sample surface.

3 Results and discussion

3.1 Compressive strength

Compressive strength tests were performed on three types of concrete specimens (CC, CC+cured, and BC). The test results for the 7- and 28-day-cured samples are shown in Figure 4. The bacteria affected the compressive strength of the specimens even after seven days of curing. The compressive strengths of the CC and CC+cured samples after 7 days of curing were 40,49 and 40,61 MPa, respectively. Therefore, bacterial curing was observed to have no effect on compressive strength after 7 days. The compressive strength of the BC series, which contained bacteria in the concrete mixture, increased to 42,54 MPa. The compressive strength of the BC samples exceeded that of the CC samples by 5,07 %. Similar values have been reported in previous studies on Bacillus megaterium bacteria [12].

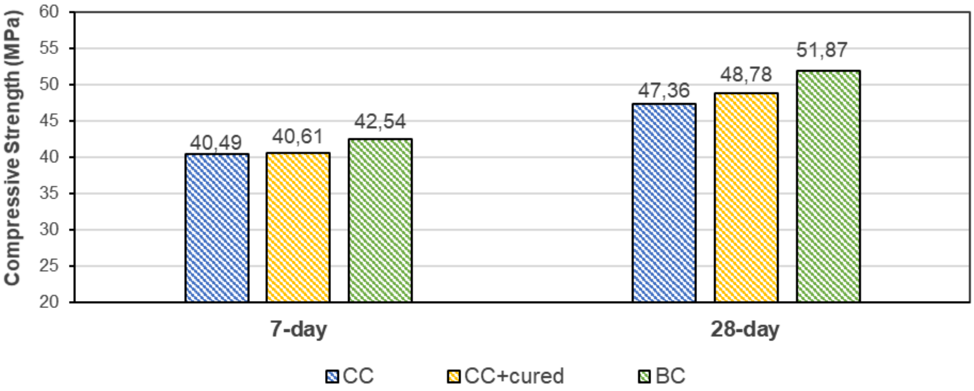


Figure 4. Compressive strength of 7- and 28-day-cured concrete specimens

The increase in compressive strength following bacterial supplementation is mainly attributed to $CaCO_3$ formation within the pores and surface voids of the concrete [26, 27]. At the end of 7 days, bacteria should be in the internal structure of the concrete if intended to increase the strength of the early age concrete. The bacterial cure did not contribute to the compressive strength at the end of the initial seven days.

The compressive strength results of 28-day-cured samples revealed that calcification continued. The average compressive strengths of 28-day-cured CC, CC+cured, and BC samples were 47,36; 48,78 and 51,87 MPa, respectively. The compressive strengths of CC+cured and BC samples were 3,00 % and 9,52 % greater compressive strengths, respectively. As the sample age increased, the rate of increase in the compressive strength increased because the amount of $CaCO_3$ produced by MICP increased.

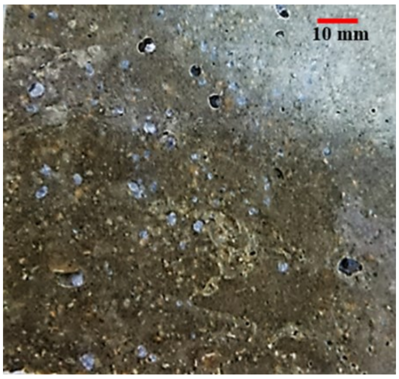


Figure 5. Seven-day $CaCO_3$ formations on BC concrete surface

Calcite formation on the surface of seven-day-cured concrete samples was visible to the naked eye. These formations tended to occur in the pores, as shown in Figure 5. The compressive strength test results indicated that direct bacterial supplementation into the concrete mixture and curing solution was more effective than supplementation only in the curing solution. However, the use of bacteria for MICP contributed positively to compressive strength.

3.2 Water absorption

The results of the water absorption tests for the 28-day-cured specimens are presented in Table 3. The BC samples were determined to have lower water absorption ratios than the CC and CC+cured samples. The average water absorption value of 4,87 % for CC samples decreased to 4,67 % and 4,23 % for CC+cured and BC samples, respectively. The BC samples absorbed 13,35 % less water than CC samples. This reduction in the water absorption of BC samples was mainly attributed to $CaCO_3$ formations within the pores and on the surface of concrete specimens. Calcite ($CaCO_3$) formations on the specimen surfaces are presented in Figures 5 and 7. CC+cured samples absorbed 4,10 % less water than CC. Because calcite, which can form on the surface owing to curing, is not formed in the internal structure of concrete, its effect on the water absorption rate is less than that on the compressive strength of the BC specimens. However, its formation on the surface led to a reduction in water absorption.

Table 3. Water absorption of samples according to weight (%)

Sample name	CC	CC+cured	BC
Water Absorption (%)	4,87	4,67	4,23

3.3 Capillary water absorption

Three concrete samples were used for capillary water absorption tests. The capillary water absorption curves are presented in Figure 6.

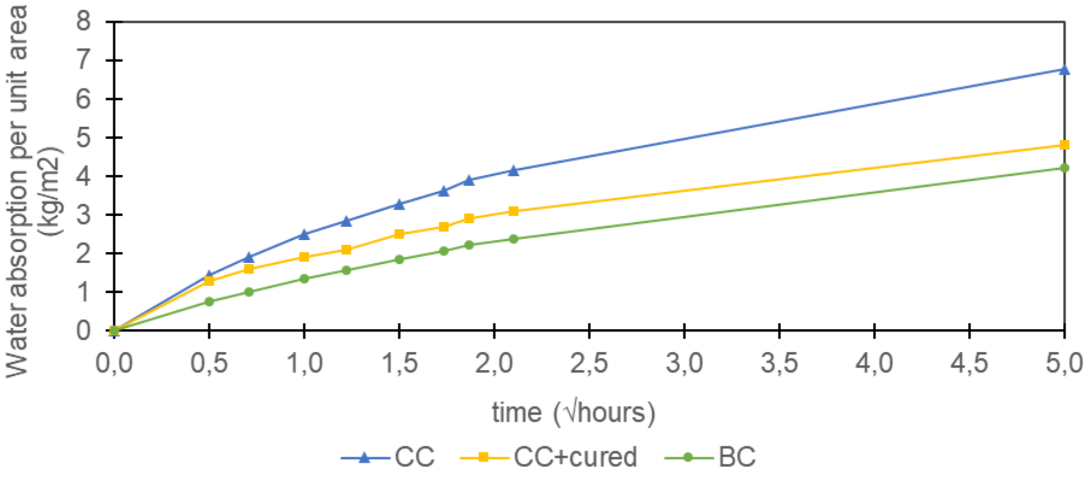


Figure 6. Capillary water absorption of concrete samples

Bacterial supplementation was effective in the capillary water absorption of BC and CC+-cured samples, even after the initial 15 min, at which point the samples absorbed 50 % less water than the CC samples. In addition, during the remainder of the testing procedure, the BC samples constantly absorbed less water (approximately 50 %) than the CC samples. After 1500 min (25 h), BC samples had 38,23 % less capillary than CC samples. When the sample surfaces on which the capillary water absorption tests were conducted were examined, the calcite formations filled most of the pores and gaps on the surface of the concrete sample, as shown in Figure 7. Chahal et al. [28] and Krishnapriya et al. [13] reported that bacteria led to a reduction in water absorption values.

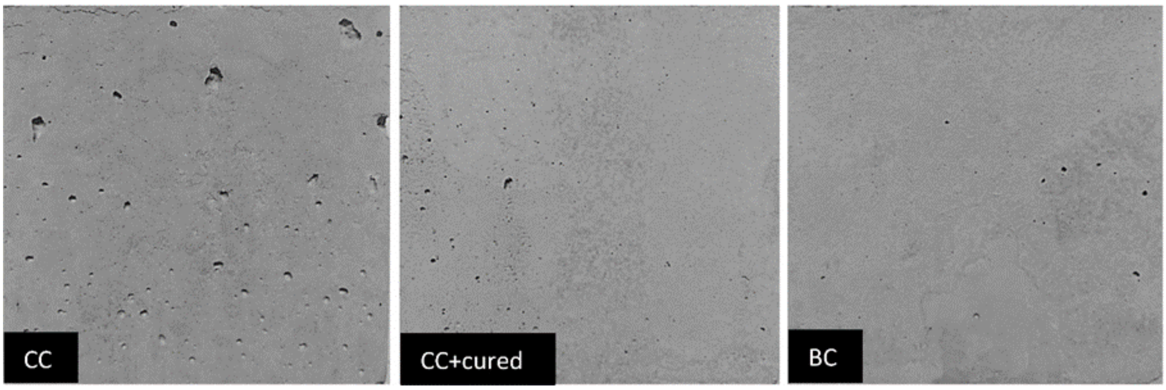


Figure 7. Surfaces of concrete samples used in the capillary test

Low water absorption was observed in the CC+cured samples in the initial measurements of the experiment; however, as the weighing time progressed, the water absorption rates approached that of the CC samples. This is because the calcite formed only on the surface of CC+-cured samples and not in the inner structure. In the initial measurement, calcite formation occurred on the concrete surfaces, blocking the capillary paths and preventing water sorption to the inner concrete structure. However, because of the insufficient formation of bacterial products in the internal concrete structure, the capillary pathway remained open and could not sufficiently prevent capillary water absorption.

The capillarity coefficients derived from the amount of water absorbed are shown in Figure 8. In the measurement performed after 15 min, the CC+cured series exhibited a value close to that of the BC series. However, as the measurement time progressed, the capillarity coefficient converged to that of the CC samples in parallel with the amount of water absorbed. Moreover, the BC series yielded considerably higher capillary coefficient values from the beginning to the end of the experiment owing to the calcium carbonate formed by the bacteria. If the bacterium was present in the concrete mix, it filled the capillary pathways in the inner structure with calcite and created more impermeable concrete. Previous studies reported that concrete-containing bacteria provide superior results in test methods related to capillarity, such as water permeability and rapid chloride permeability tests [29, 30].

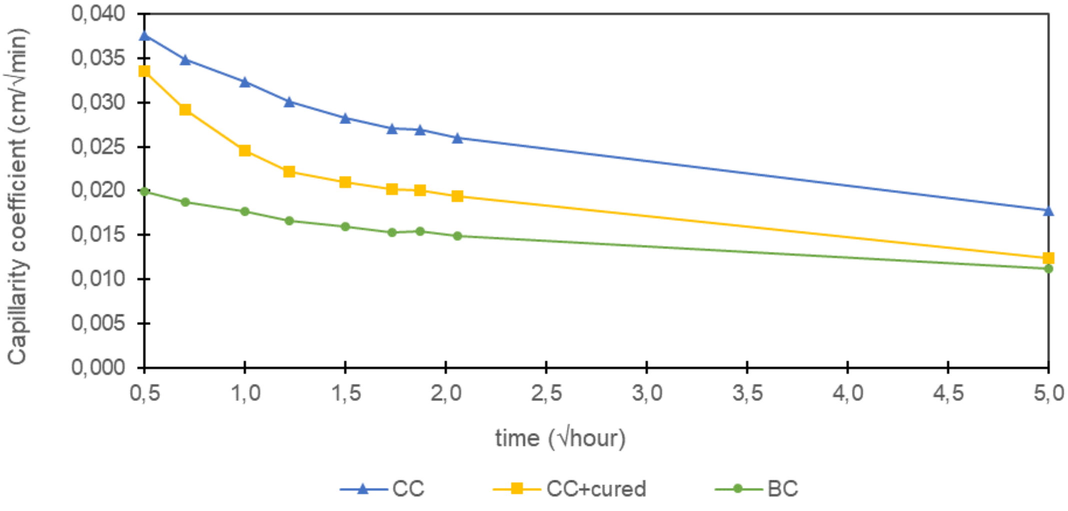
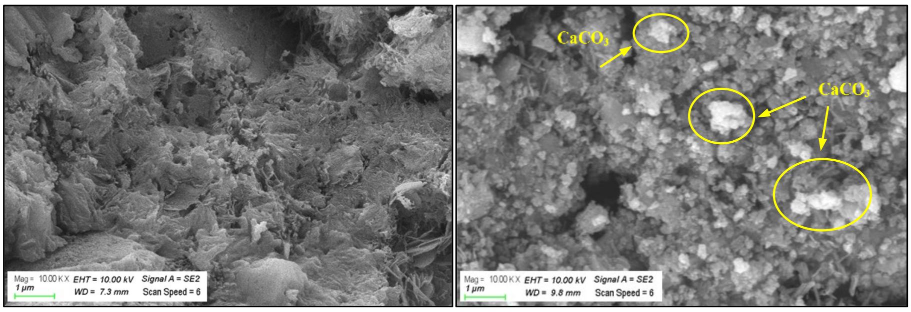


Figure 8. Capillarity coefficient of concrete specimens

3.4 Scanning electron microscopy

Calcite products were formed within and on the surface of 28-day-cured bacteria-supplemented specimens. The products formed in the specimens are shown in Figure 9. The structure of the resulting $CaCO_3$ product is shown in Figure 10.



(a) (b)

Figure 9. Internal structure of control concrete (a) and bacterial concrete (b) specimens

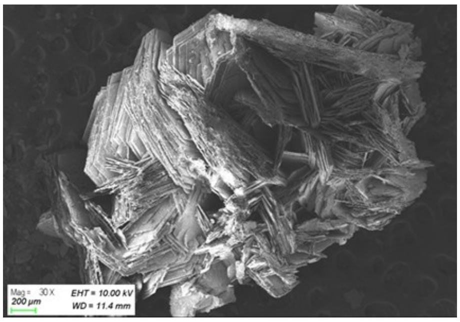
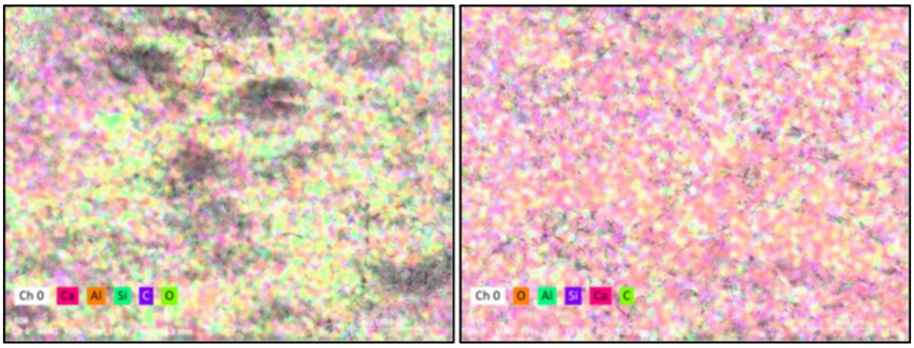


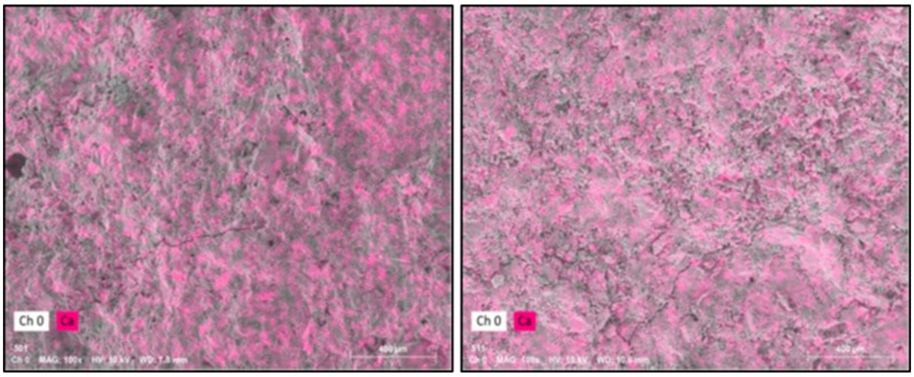
Figure 10. Structure of CaCO3 formed on the concrete surface

Mapping was performed to determine the distribution of the main calcite components (Ca, C, and O) on the surfaces of concrete specimens. In Figure 11, the elements are observed on the 28-day-cured control (a) and bacterial (b) concrete surfaces. The abundance of calcium was more remarkable in bacterial concrete than in control concrete. Only the calcium distribution is shown in Figure 12. The control sample has less and also more dispersed calcium, while the bacterial sample has more intensive calcium on its surface (b). When analysed as a percentage, while the calcium distribution of the control sample surface is 48,1 %, the bacterial sample surface has a calcium distribution of 57,0 %. Because of the MICP mechanism, $CaCO_3$ components will be observed more intensely in samples containing bacteria [31, 32].



(a) (b)

Figure 11. Mapping analysis of control (a) and bacterial (b) specimens



(a) (b)

Figure 12. Calcium mapping on the surface of control concrete (a) and bacterial concrete (b) specimens

4 Conclusions

The main findings and conclusions of the present study are as follows:

- After the initial 7 days, although the effect of bacterial supplementation on compressive strength was minimal, the compressive strength increased by 5,07 % when the bacteria were supplemented into both the curing solution and concrete mixture. After 28 days of curing, the compressive strength of CC+cured and BC samples increased by 3,00 % and 9,52 %, respectively. For increased mechanical strength, bacteria must be present in both the curing liquid and concrete mixture.
- Owing to the MICP mechanism, the water absorption ratio decreased by 13,35 %. The capillary water absorption tests yielded lower capillary water absorption values for the BC samples than for the CC samples. In the CC+cured series, because of the calcite formations that occurred on the surface, less water was absorbed by the capillary in the first measurement of the experiment, whereas it approached the values of the CC series over time. At the end of the test, the bacteria-treated concretes absorbed 37,53 % less water. Similarly, the capillarity coefficient decreased by 44,20 % with bacterial supplementation.
- Calcite formation was visualised using SEM. Microbial calcification yielded a relatively compact (void-filled) structure.
- Mapping analysis revealed greater quantities of calcite components on the surfaces of bacterial concrete samples.
- Based on the present study findings, bacterial supplementation of the curing solution and concrete mixture generated a relatively compact concrete structure with improved mechanical properties and reduced water absorption. Although the sole use of bacterial cure had positive effects, the addition of bacterial spores to the concrete mix relatively improves the concrete properties.

Acknowledgments

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