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Production of self-healing mortar using bacterial spores encapsulated with jute fibre

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Abstract:
Cement-based composites have various advantages such as low cost, easy shaping, and high compressive strength. Therefore, they are widely used in the construction industry. However, their brittle structure makes them prone to cracking, which must be repaired promptly to avoid possible loss of strength and durability and ultimate structural failures. Microbially-induced calcite precipitation (MICP) offers an effective method for healing these cracks. Unlike other treatments, MICP method is a natural and environment-friendly option for self-healing of cracks. However, bacteria can be damaged by cement and mechanical impacts; therefore, encapsulation is necessary to protect them. Encapsulation in fibres is a simpler and more cost-effective method than the other techniques. This study tested the effectiveness of encapsulating Bacillus megaterium spores in jute fibres and added them to cement-based mortars. Different nutrient supplements were used, and physical analyses and compressive strength tests were conducted on 28-day cured cube specimens. Present findings revealed that Bacillus megaterium-type bacteria encapsulated in jute fibres improved the compressive strength recovery and healed the loading-induced cracks.

Keywords:
Bacteria; self-healing; fibre; MICP; crack healing

1 Introduction

Cracks can cause severe structural problems in cement-based materials. They can make a structure more vulnerable to external factors and reduce the overall durability and mechanical strength [1]. Although there are various methods for treating cracks, most use petroleum- and cement-based materials which release additional carbon emissions and chemicals into the environment [2, 3]. Recently, researchers have been seeking better ways to repair cracks in structures, because cracks tend to propagate into the inner structure. The supplementation of composite mixtures with bacteria may offer a cost-effective and environment-friendly method for repairing cracks. However, high pH, mechanical impact during production, and other external factors significantly reduce the efficiency of bacterial treatments [4]. In addition, the activity of bacterial spores decreases as the concrete age increases, and becomes negligible after the first two months [5]. Therefore, different encapsulation methods have been developed to protect against these harmful effects, while maintaining an active bacterial concentration. These capsules should be non-reactive to bacteria and media and resistant to alkaline damage and spoilage [6]. Various materials are used to encapsulate bacteria, such as polymer-based materials (hydrogels, alginates, melamine, and polyurethane), lightweight aggregates, nanomaterials, special minerals, and binder materials [7].

However, these encapsulation methods are expensive and require advanced technical processes. Therefore, bacterial encapsulation in fibres has emerged as an innovative and cost-effective method. Fibres are widely used in concrete mixtures to improve the mechanical properties and durability of cement-based materials. Owing to the bridging behaviour in concrete and mortar, the fibres significantly restrict crack widths [8]. This attribute of fibres is crucial, especially for producing self-healing bacterial concrete, because bacteria offer a more effective and faster treatment of crack widths [9, 10]. The working mechanism of the self-healing bacterial concrete with jute fibre is shown in Figure 1.

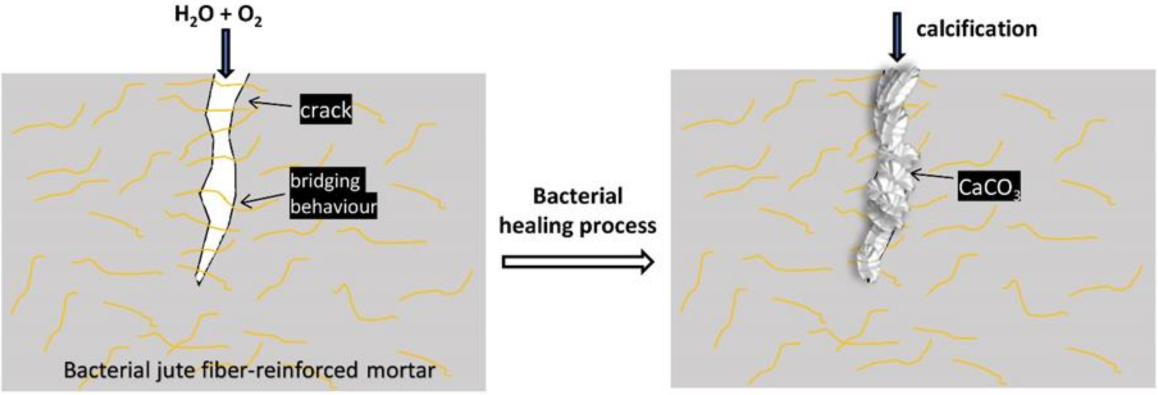


Figure 1. Working mechanism of self-healing bacterial concrete

There are a limited number of studies on this method and highly significant results have been published for each method. Singh and Gupta [11] used cellulose fibres to absorb a bacterial solution and encapsulate bacteria into the fibre structure, while protecting the bacteria from the high pH environment of cement, mechanical impacts during mixing, and negative external effects. It was also reported that fibre impregnation is the cheapest encapsulation technique. Rauf et al. [12] separately encapsulated three different bacterial species with different fibre types and demonstrated the positive effects of fibre impregnation on self-healing bacterial concrete. Guo et al. [13] encapsulated bacteria by absorbing them into the sisal fibre and indicated that the sisal fibre could successfully act as a protective barrier against bacteria. It was also reported that over 90,0 % of the cracks repaired by bacterial sisal fibres were between 0,8 and 1,0 mm. In addition, bacterial sisal fibre-reinforced concrete was found to have a 21,8 % higher compressive strength and a 28,7 % higher split tensile strength.

The utilisation of different bacterial species is crucial because they significantly affect calcite precipitation [14]. Bacillus megaterium-type bacteria, which have not been previously used in fibre encapsulation studies, were used in this study. Jute fibres are the preferred fibre type, which are increasingly being used and studied owing to their contribution to the mechanical properties and durability of composites [15, 16]. Furthermore, because jute is a natural fibre, it does not harm the environment, thus overlapping with the environmental philosophy of the MICP method. After the *Bacillus megaterium* spores were impregnated with jute fibre, cementitious mortar was produced. In addition, the contributions of calcium lactate and urea to MICP treatment were investigated using different ratios of mortar production. The produced mortar samples were damaged by compressive loads and cured in water for 28 days. The bacterial samples removed after curing were analysed for their physical appearance and tested for compressive strength.

2 Methodology

2.1 Materials

Bacillus megaterium was supplied by the Ankara Hygiene Institution and used as the bacterial additive in this study. Bacterial concentrations of 10^7 cells/mL were added to the mortar mixtures and jute fibres. CEN standard sand, CEM I -42.5R Portland cement, tap water, and jute fibre were used in the mortar production. Jute fibres are a type of natural fibre produced from jute plants whose chemical constituents include cellulose, hemicellulose, and microfibrils [17, 18]. These fibres were used in this study owing to their high water absorption capacity and strength. Jute fibres used in this study were supplied by KordSa Co. The filament structure of the jute fibre is shown in Figure 2.

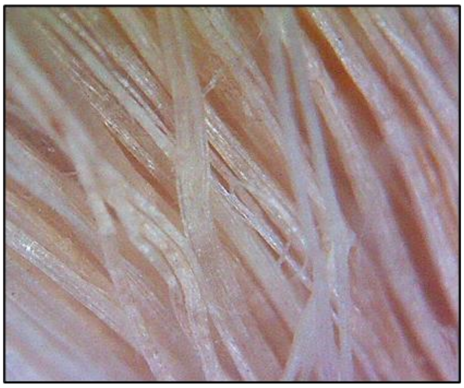


Figure 2. Filament structure of the jute fibre under microscope (200×)

The properties of the jute fibres and the chemical composition of cement are given in Table 1 and Table 2, respectively. A sieve analysis of standard sand is shown in Figure 3.

Table 1. Physical properties of jute fibre

Length (mm)	**Tensile strength** (MPa)	**Water absorption** (%)	**Filament diameter** (µm)	**Density** (g/cm^3)
6	400 - 700	160	10 - 30	1,3

Table 2. Chemical composition of cement (% by weight)

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

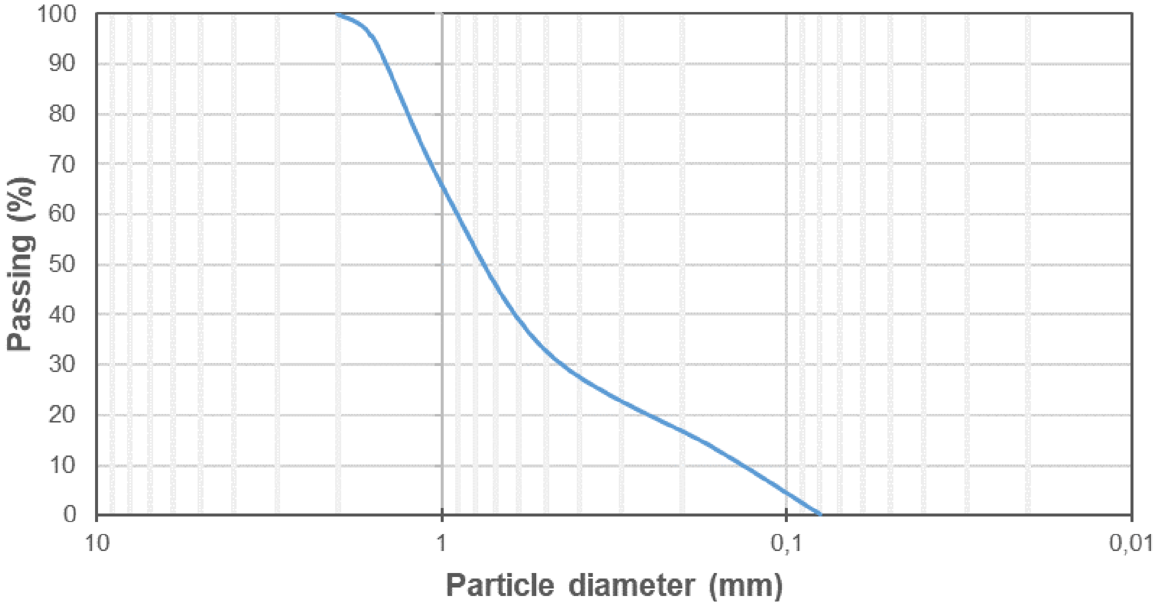


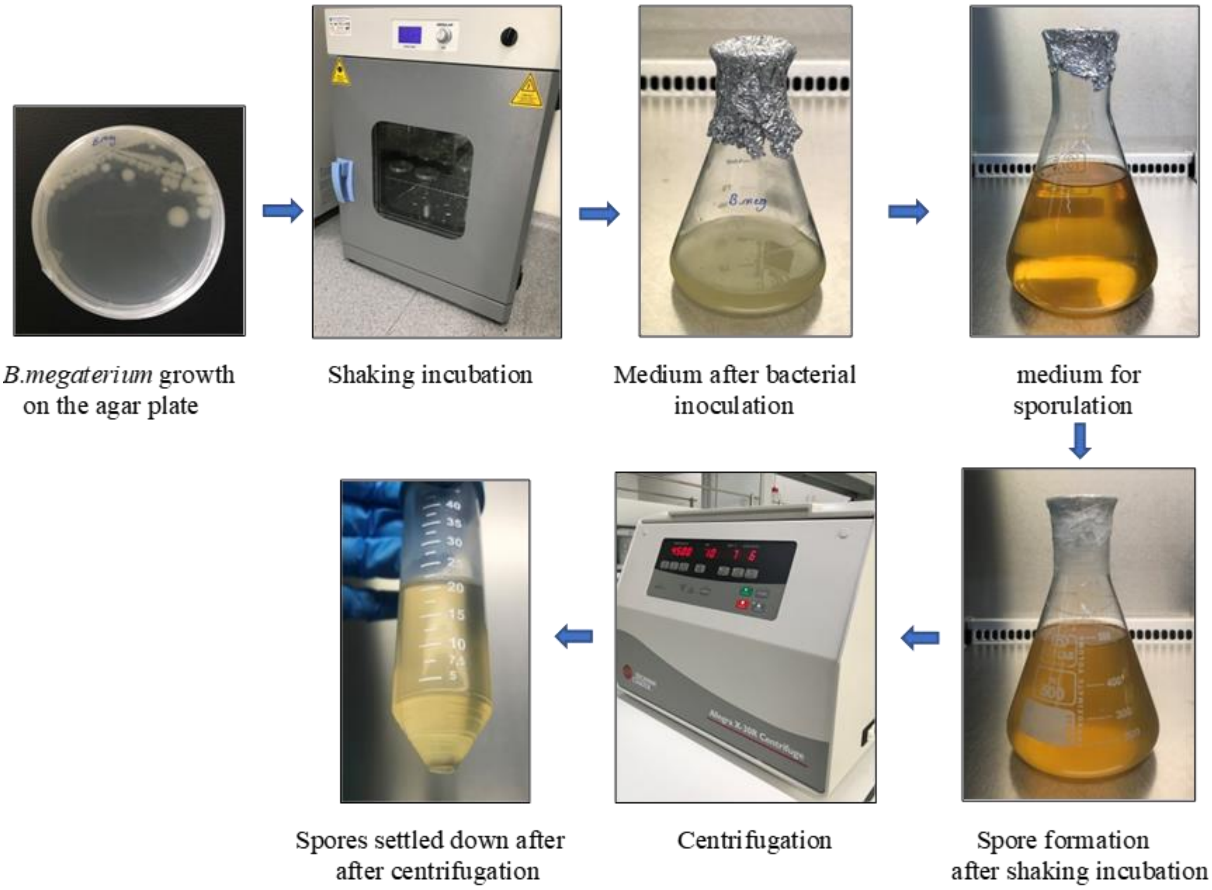
Figure 3. Sieve analysis of standard sand

Superplasticiser additive meeting the TS EN 934-2 standard with a density of 1,09 g/cm^3, a 36,35 % solids content ratio, and a pH value of 3,82 was used to maintain constant flowability. Urea (SIGMA) and calcium lactate (SIGMA) were added for bacterial media.

2.2 Bacterial spore production

Lyophilised *Bacillus megaterium* strain was activated using a tryptic soy broth (TSB, Merck) and stored at -80 °C using 30 % glycerol for further use. *Bacillus megaterium* was incubated in a sterilised nutrient broth at 30 °C and 150 rpm shaking rate. For bacterial spores, basal salt medium ($MgSO_4.7H_2O$ 0,3 g/L, $MnSO_4$ 0,02 g/L, $Fe_2(SO_4)_3$ 0,02 g/L, $ZnSO_4.7H_2O$ 0,02 g/L, $CaCl_2$ 0,2 g/L, tryptose 10 g/L, yeast extracts 2 g/L) used in the study by Kalfon et al. [19] was prepared and sterilised. Approximately 100 mL of the basal salt medium was inoculated with 1 % (v/v) of *Bacillus megaterium* culture and incubated for one day and left for 7 days at 30 °C and 150 rpm. At the end of incubation, it was kept at 85 °C for 15 minutes for spore formation. Bacterial counts were performed before and after pasteurisation to determine sporulation. For this purpose, the culture was diluted and inoculated on nutrient agar and left to incubate at 30 °C for 24 hours.

The spores were harvested by centrifugation at 4 °C and 5000 rpm for 15 minutes. The supernatant was removed, and the remaining part was washed three times with distilled water. The spores were suspended in sterile distilled water, and the cell density was adjusted to the McFarland Standard 2. This value is equivalent to turbidity, which is approximately 6×108 colony-forming units (CFU)/mL. The suspension was stored at 4 °C for further use [12]. The spore production process is illustrated in Figure 4.



B.megaterium growth on the agar plate

Shaking incubation

Medium after bacterial inoculation

medium for sporulation

Spore formation after shaking incubation

Centrifugation

Spores settled down after after centrifugation

Figure 4. Process of spore formation

2.3 Encapsulation of bacteria with jute fibre

Jute fibres were added to the bacterial spore solution and allowed to absorb, as shown in Figure 5. The fibres that thoroughly absorbed the bacterial spores were then directly added to the mortar mixture without further processing.

Figure 5. The appearance of jute fibres impregnated with bacterial spores

2.4 Mix proportions and mortar production

The mixing was performed in accordance with the ASTM C109 standard. The contents and proportions of the mortar produced are listed in Table 3. The water to cement ratio was kept constant at 0,485. Jute fibre was added to the mixtures at a rate of 1 % by weight of cement. Seven different mortar mixtures were prepared. Initially, control mixtures that did not contain jute fibres or bacteria were prepared. Then, fibre-reinforced mortar samples containing bacteria-free jute fibres were produced. Subsequently, mortar samples containing different urea-to-calcium lactate ratios were produced. Samples were named "CM" for control, "JF" for jute fibre, "JFB" for jute fibre with bacterial spore, and "cl" for calcium lactate. A total of 84 cube specimens (5×5×5 cm) were produced (six samples of each type). Where JF denotes jute fibre, JFB jute fibre with bacterial spore.

Table 3. Mortar mix proportions (g)

Specimen	Cement	Sand	Water	JF (%)	JFB (%)	Bacteria (cell/mL)	Urea	Calcium lactate
CM	500	1375	242,5	-	-	-	-	-
JF	500	1375	242,5	1	-	-	-	-
JFB	500	1375	242,5	-	1	-	-	-
JFB+cl	500	1375	242,5	-	1	-	-	25,0
JFB+cl+bs	500	1375	242,5	-	1	10^7	-	-
JFB+cl+urea	500	1375	242,5	-	1	-	12,5	12,5
JFB+cl+urea+bs	500	1375	242,5	-	1	10^7	12,5	12,5

The mortars produced were removed from the moulds at the end of 24 hours and subjected to water curing. At the end of the 7-day curing period, the compressive strengths were determined. Subsequently, 90 % of these compressive strengths were loaded, and cracks of various sizes were generated. The damaged samples were then cured in water for 28 days.

2.5 Physical analyses

The crack widths and calcite formation were examined in the samples removed from curing. Healing images were captured using a digital camera and an optical microscope at 200× magnification.

2.6 Compressive strength

The 28-day compressive strength values of 5×5×5 cm cube specimens were determined in accordance with the ASTM C109 standard. The compressive strength test process is shown in Figure 6.

Figure 6. Compressive strength test process

3 Results and discussions

3.1 Physical healing

The mortar samples were damaged and exhibited numerous cracks on their surfaces after being loaded. Consistent with the findings of previous studies [20-22], jute fibres exhibited bridging behaviour, restricted crack widths, and prevented sudden breakage of mortars. The bacterial treatment process was continued for 28 days on the pre-crack mortar samples. After 28 days, healing was observed in the cracks on the mortar surfaces. Images of the sample surfaces before and after bacterial treatment are shown in Figure 7. It can be seen that the bacteria-induced calcite formation heals loading-induced cracks.

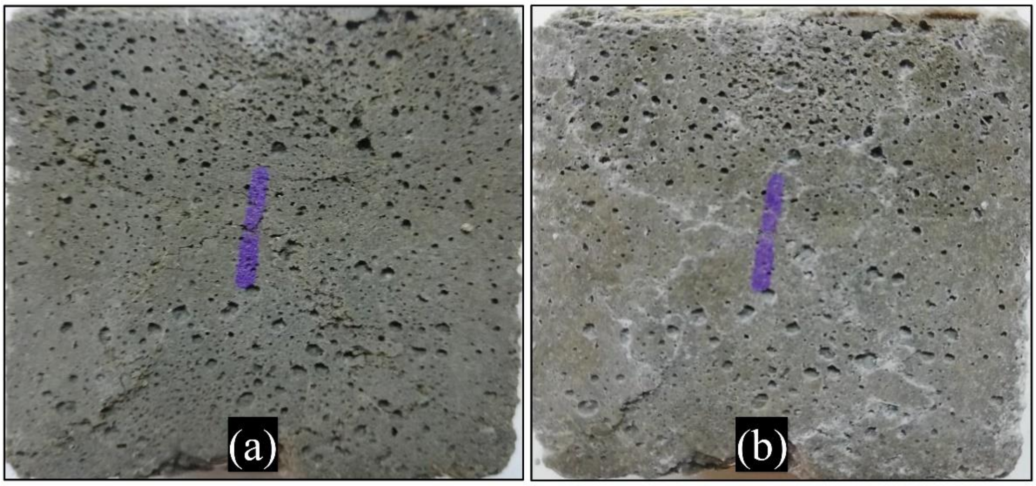


Figure 7. The appearance of mortar surface before (a) and after (b) bacterial treatment

The MICP treatment creates a suitable environment for bacteria to form calcite. In Figure 8, $CaCO_3$ formations are observed in the deep cracks on the edges and spills, owing to the fracture mechanism of the damaged mortar. The bridging behaviour of the jute fibres prevented surface shedding, and damaged areas were reattached to the structure owing to the bacteria forming calcite. Previous studies have reported that Bacillus megaterium can successfully produce calcite [23-25]. The findings of the present study also revealed that Bacillus megaterium species, which were not used previously in studies using the fibre encapsulation method, could also be used to successfully produce calcite.

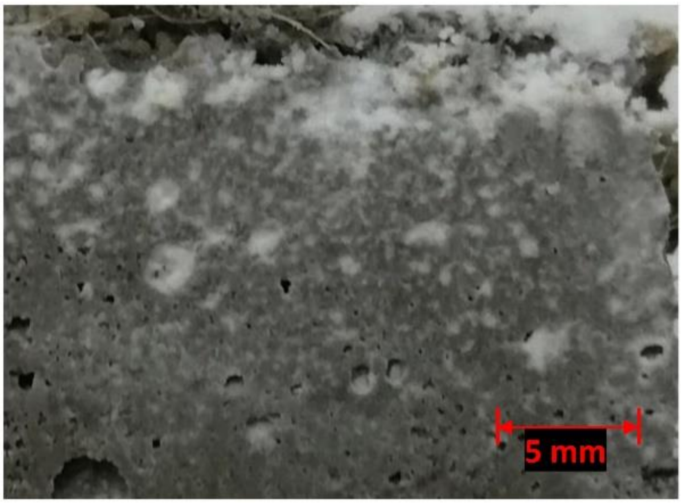


Figure 8. The calcite precipitation on the surface of the damaged mortar

In terms of closed crack widths, the widest crack healing was observed in the mortar samples containing bacterial jute fibres using calcium lactate and urea as the medium. This demonstrates that the use of calcium lactate alone is insufficient for *Bacillus megaterium* species and that the presence of urea in the mortar mixture is necessary. The widest healed crack in this series was 0,62 mm, and is shown in Figure 9. A study by Rauf et al. [12] reported that a 0,40 mm concrete crack was healed by bacterial species impregnated with jute fibre and showed that bacterial species were effective in repairing crack widths. Similarly, cracks of different sizes were closed by *Bacillus megaterium* in this study. Dhami et al. [26] and Dick et al. [27] also showed that the amount of calcite produced by biological means can be highly variable, depending on the bacterial species.

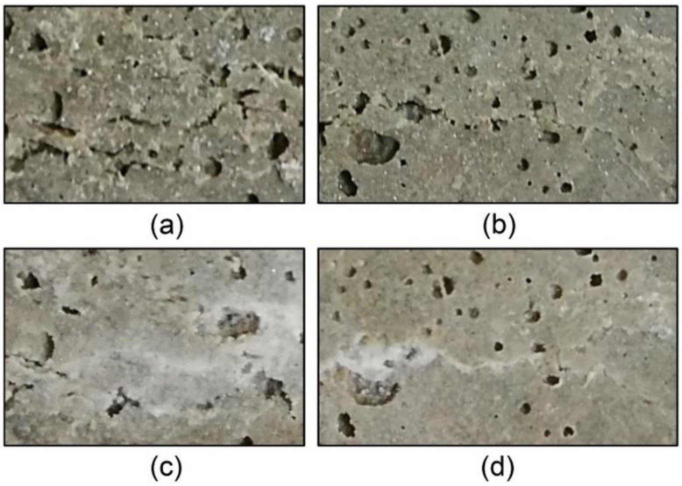


Figure 9. Width 0,62 mm of cracks appearance before (a, b) and after (c, d) bacterial treatment

3.2 Compressive strength

The compressive strengths of the mortar samples after 28 days of water curing are listed in Table 4. It was seen that the jute fibres preserved their compressive strength at a higher rate. Jute fibres prevented the expansion of cracks by acting as a bridge between cracks. Thus, the jute fibre-reinforced mortars absorbed more energy and demonstrated higher compressive strengths than that of the control mortars. The bridging behaviour of the jute fibres is shown in Figure 10.

Figure 10. Bridging behaviour of jute fibre

A minimal increase was observed in the compressive strength of the JFB series with bacterial impregnation of jute fibres. Considering the absence of visible calcite formation, it was observed that the bacteria were not beneficial without any nutrient sources in the mortar mix. However, the bacterial spores absorbed into the fibres did not cause any damage to the structure of the jute fibres and did not cause a decrease in the mechanical properties.

A serious loss of compressive strength was observed in "JFB+cl" series with calcium lactate in the mortar mixture. Calcium lactate decreased the compressive strength by affecting cement hydration. A similar result was obtained by Cunniffe [28] who used a similar ratio of calcium lactate. In the JFB+cl+bs series containing bacterial spores and calcium lactate, the rate of strength loss decreased with the addition of bacteria. The bacteria reduced the compressive strength loss by 8,97 %. It was observed that calcium lactate initiated the formation of calcite by activating bacterial enzymes in the mixing water. Amaj et al. [29] reported positive outcomes of bacteria absorbed into sisal fibre using only calcium lactate as a medium. However, it was determined in this study that Bacillus megaterium bacteria could not produce an effective amount of $CaCO_3$ using only calcium lactate because Bacillus megaterium-type bacteria have a high urease capacity and are gram-positive bacteria; therefore, CO_3^{2}- should be separated from urea [30, 31]. Although it provides the necessary calcium from calcium lactate, it cannot form large amounts of calcite, because it cannot collect sufficient amounts of carbonate from the environment. Consistent with a study by Schreiberová et al. [32], it was discovered that urea is the primary source of self-healing composites that contain ureolytic bacterial species.

Table 4. 28-day compressive strength values (MPa) of damaged mortar samples

Sample	Compressive strength	Increase/decrease rates (%)
CM	35,92	-
JF	37,14	+3,40
JFB	37,51	+4,43
JFB+cl	18,92	-47,33
JFB+cl+bs	22,14	-38,36
JFB+cl+urea	39,97	+11,28
JFB+cl+urea+bs	42,11	+17,23

In the JFB+cl+urea series with both urea and calcium lactate, a 11,28 % increase was observed in the compressive strength compared to that of the control mixtures. Thus, it was shown that bacterial spores embedded in jute fibres were activated using the urea medium. The resultant $CaCO_3$ formation had a positive effect not only on crack healing, but also on the mechanical properties. The highest compressive strength values were observed in the "JFB+cl+urea+bs" series, in which bacteria were absorbed into the jute fibre and added to the mortar mixing water. In addition to providing physical capsule protection, the high water absorption rate of jute fibres creates a more humid environment in the mortar structure, making it more suitable for bacteria. Thus, a 17,23 % higher compressive strength was obtained with the MICP treatment.

4 Conclusions

In this study, fibre-reinforced mortars were produced using jute fibres containing bacterial spores. The effects of the Bacillus megaterium species and growth media on these mortars were investigated. The following conclusions were drawn from the findings:

- Jute fibres reduced cracks through their bridging behaviour, facilitated autogenous healing, and preserved high compressive strength.
- Jute fibre absorbed the bacterial solution owing to its high water absorption capacity. Thus, they were determined suitable for use in self-healing composites.

- *Bacillus megaterium* species impregnated into jute fibres were able to repair cracks of up to 0,62 mm width and provided a mutualistic improvement in fibre behaviour.
- Jute fibres successfully encapsulated Bacillus megaterium species, but no significant contribution of *Bacillus megaterium* was observed without any nutrient supplementation.
- Calcium lactate reduced the compressive strength of mortar when used at a rate of over 2,5 % by cement weight.
- The use of calcium lactate with urea considerably increased the compressive strength. This showed that urea should be included in the mixture, especially for *Bacillus megaterium* species.
- The highest compressive strength values were achieved in mixtures with bacterial spores added to both the fibres and mixing water.

In future studies, it is recommended to use fibres with varying water absorption capacities and different bacterial species to produce self-healing composites. This will allow for a comparative examination of healing abilities based on the fibre and bacteria types.

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