Functional mortars for conservation of cultural heritage structures

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Abstract. Mortar aging and deterioration are serious problem for architectural heritage conservation. The solution might be sought in advanced functional materials which could provide repair and lasting surface protection from atmospheric pollution and microbiological corrosion. In recent years, extensive studies have been conducted on the use of bacteria with biocalcification potential for self-healing effect in cements materials, but only a few publications deal with self-healing capacity of historical lime-based mortars. The main focus of our research was development of new bio-activated self-healing system and its application in laboratory conditions. The objects of the work were historical mortar samples from medieval Bač Fortress in Serbia and laboratory prepared and aged mortar models. Aiming to achieve high compatibility, laboratory models were prepared based on our previous results of historical mortars characterization. The bio-activated self-healing agent was made as two-component liquid system using bacterial cells of Sporosarcina pasteurii DSM 33 and nutrients. The components of the models were hydraulic lime, milled limestone, river sand, and crashed brick as aggregates, and water. Comparative characterization of historic mortars and aged models was performed by mechanical and colorimetric testing as well as examination of mutual interaction and cohesion between old and new material. The next step was efficiency evaluation of the external bacteria-based repair healing method in/on the laboratory samples. The detailed study of the cracks of the historical samples and the prepared models, and the bacterial suspension diffusion assessment were done by comparison of the results obtained by different complementary imaging techniques (optical and scanning electron microscopy). The experiments were performed on both samples of old and new materials treated with and without bio-activated self-healing agent. The obtained results are promising and support the development of the external bio-activated self-healing method. This solution represents functional system which could allow historical mortars and modern structures to heal themselves in the long-term, preserving their functional and aesthetic properties.

1. Introduction
In recent years, self-healing phenomenon of cement-based and limestone-based materials has been extensively studied, due to its increasing beneficial potentials in engineering and scientific practices. Mortars and concrete can under certain conditions develop capacity of natural or ‘autogenous’ healing,
while some approaches can also introduce ‘autonomic’ healing into/onto mortar materials [1]. The self-healing effect strongly depends on a number of factors including pH value of structure matrix, dissolved inorganic carbon, nucleation sites and presence of calcium ions available for chemical reaction with carbonate ions. Therefore, continued hydration of structure matrix cannot be solely responsible for a complete self-healing of cracks. To get more efficient self-healing ratio a simultaneous action of natural phenomena and engineered healing, such as microbiologically induced precipitation of calcium carbonate (MICP) may be required [1]. The bacterial-induced precipitation of calcium carbonate has large potential due to its fast and active crack repair, while also being environmentally friendly and providing efficient bonding capacity and compatibility [2]. When the calcifying bacteria are incorporated into the matrix, the viable bacterial cells can influence bioactivation of self-healing phenomena inducing the precipitation of inorganic mineral.

The self-healing of surface cracks can be promoted with addition of bacteria with biocalcification potential in crack zone inducing autonomic healing of materials. The efficiency of bacterial treatment depends on crack width, curing condition and age of samples. The mentioned bacteria may be used for healing of minor cracks in historical monuments, old buildings or for development of self-healing biocement [3]. Microorganisms can influence the precipitation of carbonaceous minerals, specifically calcium carbonate (CaCO\(_3\)) through metabolic processes such as organic acid utilization, urea hydrolysis and denitrification [4]. The result of these metabolic processes is a change in the pH and/or the concentration of dissolved inorganic deposit. It is believed that bacterial cell surfaces act as heterogeneous crystal nucleation sites in supersaturated matrix with Ca\(^{2+}\) and CO\(_3^{2-}\) ions. Moreover, microbes can alter the saturation state of an undersaturated solution by catalysing mineral precipitation [5]. The alkali-tolerant bacteria with high ureolytic activity have been extensively used for application on cementitious materials. These bacteria, such as *Sporosarcina pasteurii*, *S. urea*, *Bacillus sphaericus*, *B. megaterium*, have been used for improving self-healing of porous and cracked concrete. It is believed that ureolytic bacteria can metabolize carbonate ions trough generated urease enzyme as well as induce formation of 1 mol CaCO\(_3\) from 1 mol urea [6]. For example, *B. sphaericus* cells could induce formation of 60 g CaCO\(_3\) within 1 day under optimal conditions (10\(^8\) cfu/mL, 28 °C, 1M urea, 20 g/L yeast extract, 1M Ca\(^{2+}\)) [7], while *S. pasteurii* required less than 24 hours for conversion of calcium and urea into calcium carbonate [8]. Moreover, method of application of bacterial suspension and nutrients into cracks is very important. Until now, the external healing of surface cracks has been mainly restricted to porous limestone where surface deposit of calcium carbonate decreased water permeability by 65 to 90% depending on porosity [9].

In the present study, the bacteria-based system for external self-healing of surface cracks in mortars was developed. It is a two-component healing agent (bacteria and nutrients) applied by injection in the visible cracks of the treated surface. The selected materials for these experiments were samples of historical mortar and laboratory prepared models of conservation mortar. The self-healing effect of the developed system was investigated. Moreover, the morphology of the deposits created in the surface cracks after the healing agent injection was monitored by optical microscopy and SEM.

2. Materials and methods
The materials used for this research include two groups of mortars: (1) samples of historical mortar from medieval Bač Fortress placed in northern Serbia (HM) and (2) laboratory prepared models of conservation mortar aimed for application on the Bač Fortress (MM). The aim of this work was to create artificially produced cracks which can be compared to the cracks on historical mortar samples and treat them with bio-activated self-healing agent.

2.1. Mortar models preparation and examination
Based on the results of chemical-mineralogical, textural, microstructural and mechanical examination of historical mortar samples (HM) the composition and production technology for mortar models was established. The components of the model mortars, with ratio of aggregates and binders 2:1, were the
following: hydraulic lime, crushed brick as pozzolanic material, river sand, and milled limestone as aggregates, and water.

The models of conservation mortar (MM), 18 in total, were prepared and artificially aged in laboratory. Artificial aging of the prepared model samples was done under room conditions and in the Binder Climate Chamber KBWF 240. After 5 days of exposure to room conditions (T = 20°C and RH = 60%), the moulds with the prepared mortars were placed in the chamber for one month (T = 30°C and RH = 80% for 5 days, and T = 0°C i RH = 60% for 24 h, total 5 cycles), after which they were again subjected to room conditions for 7 days prior to examination. Comparative characterization was performed on HM and MM to examine their compatibility. Mechanical properties were determined by a Drilling Resistance Measuring System (DRMS, SINT Technology), while colour aesthetic appearance was recorded on a CM-700D (Konica Minolta) spectrophotometer. The colour differences were calculated using CIE (Commission international de l’Eclairage) L*a*b* relative to a white background [10].

The surface crack properties on HM samples, such as location, orientation and dimensions, were studied in order to provide required information for creation of artificial cracks on laboratory MM. The surface cracks were created after the artificial ageing during the investigation of flexural characteristics. After evaluation of artificial crack properties [11], the samples were selected for the experiment.

2.2. Preparation of two-component self-healing agent

Due to the significant role of ureolytic bacteria in the MICP process, *Sporosarcina pasteurii* has been used in creating a model system of bacterial induced calcite precipitation in extreme environmental conditions. This sporogenic bacterium is well-known in different engineered process of self-healing, with the optimal pH value for growth and urease activity, between 7.5 and 9 [12]. Therefore, the ureolytic bacteria *Sporosarcina pasteurii* DSM 33 (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) has been used to induce precipitation of calcium carbonate in order to repair surface cracks in this experiment. In order to prepare inoculum, *S. pasteurii* DSM 33 was aerobically incubated on Trypton Soya Agar (TSA, HiMedia, India) with the addition of 20% urea (Difco, USA) at 30 °C for 6 days. The initial number of viable spores was estimated after thermal treatment (80 °C, 10 min) by counting colonies after the incubation at 30 °C for 48h on TSA with addition of 20% urea. As a nutrient medium for the MICP process, modified Urea-based broth (nutrient broth 30 g/L, NH₄Cl 100 g/L, NaHCO₃ 21.2 g/L, urea 200 g/L) was used.

2.3. Self-healing experiment

In order to examine the possibility of self-healing crack closure of the original historical mortar (HM) and models of conservation mortar (MM), two groups of materials were examined: (1) mortar samples (HM and MM) with bio-activating agent and nutrients (bio-samples), and (2) mortar samples (HM and MM) with nutrients only (blank samples).

In order to remove potential microbial contamination the samples were sterilised in autoclave prior to the application of bio-activated self-healing agent. The sterilisation process, naturally, will not be used in real-life application, but it was performed to validate that results obtained in laboratory can be evaluated as consequence of bio-activated self-healing process. All samples were first treated with nutrients. The nutrients (300 µL) were applied into the cracks of the upper samples’ surface. An aliquot of the bacterial suspension (100 µL) was applied on the bio-samples, while the blank samples were treated with 100 µL of sterile distilled water, instead of the bacterial suspension.

In order to maintain constant humidity required for incubation of the self-healing agent, the samples were kept in sterile distilled water (approximately 1/3 sample height was in water) in Petri dishes during 150 days at 30 °C. The number of vegetative cells of *S. pasteurii* DSM 33 was periodically estimated by streaking onto on TSA with addition of 20% urea. The sampling was done every 7 days within the first month, and until the end of incubation period the samples were taken
every 30 days. The pH value onto crack zone was monitored in the same sampling time as bacterial viability by HANNA HI 99161 pH/temperature meter.

2.4. Characterization of mortar models and evaluation of crack closure ratio

The change of surface cracks was monitored every 7 days during the first 28 day-incubation, and every 30 days until 150-day period. It was performed with the Portable Digital Microscope ViTiny Pro10-3 (ViTiny, USA) on samples kept in sterile distilled water. During the first month of the incubation period, the viability of the bacterial cells/spores and pH values of the crack surfaces were monitored weekly and further monitoring was performed on monthly basis. The surface morphology of the formed calcium-carbonate precipitate into the surface cracks was examined by SEM after 28 days of incubation period.

3. Results and discussion

3.1. Mortar compatibility assessment

Aiming to evaluate the compatibility of MM with HM samples, mechanical and colorimetric measurements were performed, as well as the study of mutual interaction and cohesion between original and new mortars examined using stereo-optical microscope (OMANO OMXTL/V7 Articulated Boom Microscope).

Based on the results given in Table 1 it is confirmed that the designed model mortars (MM) are suitable for conservation purposes since they show lower values of average drilling force compared to the original historical mortar samples (HM) [11]. The total colour difference ($\Delta E = 0.94$) is lower than 3 which mean MM are visually highly compatible with the HM (the difference could not be detected with naked eye) [10]. Moreover, colour coordinates for MM samples are approximately the same as in the case of HM.

| Method/Properties | Drilling resistance measurements | Spectrophotometry colour coordinates and $\Delta E$ |
|-------------------|--------------------------------|-----------------------------------------------|
|                   | Average value of drilling force [N] | Max. value of drilling force [N] | L* | a* | b* | $\Delta E$ |
| Historical mortar (HM) | 0.5 | 0.6 | 79.08 | 1.45 | 10.77 | 0.94 |
| Model mortar after artificial aging (MM) | 0.3 | 0.5 | 79.32 | 2.36 | 10.77 | |

The mutual interaction and cohesion of MM samples with the original historical mortar (HM) was investigated in order to further test their compatibility. In the fresh prepared MM samples a small fragments of HM were put inside and analysed after the artificial ageing. During the carbonization/hydration process in the designed MM samples a contact zone without cracks was formed between these two materials, as shown in Figure 1.
The morphology of the contact zone, Figure 1, shows that the formed area possesses satisfied appearance (without cracks). After compatibility between original and designed mortars has been proven, the newly designed self-healing system was tested on both HM and MM samples.

3.2. Self-healing crack repair / bio-activated crack repair
The change of surface cracks after the application of bio-activated self-healing system was continuously monitored by digital optical microscope (Table 2). The blank samples treated with nutrient only were also monitored.

Table 2. Microscopic images of HM and MM bio- and blank samples

| Incubation period (days) | 0 days | 7 days | 28 days | 150 days | 150 days |
|--------------------------|--------|--------|---------|----------|----------|
| HM BIO-SAMPLES           |        |        |         |          |          |
| x10                      | ![Image](image1) | ![Image](image2) | ![Image](image3) |          |          |
| x40                      | ![Image](image4) | ![Image](image5) | ![Image](image6) |          |          |
| MM BIO-SAMPLES           |        |        |         |          |          |
| x10                      | ![Image](image7) | ![Image](image8) | ![Image](image9) |          |          |
| x40                      | ![Image](image10) | ![Image](image11) | ![Image](image12) |          |          |
| HM BLANK                 |        |        |         |          |          |
| x10                      | ![Image](image13) | ![Image](image14) | ![Image](image15) |          |          |
| x40                      | ![Image](image16) | ![Image](image17) | ![Image](image18) |          |          |
| MM BLANK                 |        |        |         |          |          |
| x10                      | ![Image](image19) | ![Image](image20) | ![Image](image21) |          |          |
| x40                      | ![Image](image22) | ![Image](image23) | ![Image](image24) |          |          |
Based on the microscopy, the process of surface cracks sealing was clearly visible on both HM and MM samples treated with the bio-activated self-healing agent (bio-samples), in comparison to the blank samples. The self-healing efficiency was more pronounced in the case of MM bio-samples, therefore the SEM investigation was performed on MM bio-samples 28 days after the bio-activated self-healing agent application aiming to reveal the nature and structure of the precipitate, Table 3. On the SEM photos the difference is easily observed between blank and bio-samples of MM due to the presence of CaCO$_3$ crystals in the precipitate of the bio-samples.

**Table 3. SEM analysis of MM blank and bio-samples, 28 days of incubation**

| BLANK | BIO-SAMPLES |
|-------|-------------|
| ![SEM photo x200](image1) | ![SEM photo x200](image2) |
| ![SEM photo x1000](image3) | ![SEM photo x1000](image4) |

The growth profiles of *S. pasteurii* DSM 33 on HM and MM bio-samples is shown in Figure 2.

**Figure 2.** Enumeration of *S. pasteurii* DSM 33 on MM and HM bio-samples
The initial number of bacterial spores of *S. pasteurii* DSM 33 in suspension was 8.3 log/mL. After application of 100 µL of suspension into the crack, the number of bacterial cells was above 5.5 log/mL dependent of sample type (HM or MM). Within the first two months of incubation, concentration of vegetative cells was increased for minimum 1 log unit. The decreasing trends for both bio-samples were observed to the end of incubation period. At the end of experiment, concentration of vegetative cells of *S. pasteurii* DSM 33 was above 6 log unit. According to the obtained results shown in Figure 2, it can be suggested that the reached concentration of vegetative cells can provide the MICP process and induce precipitation of crystal form in a crack zone.

Considering that the pH value can affect bacterial growth, urease activity as well as precipitation of CaCO$_3$ [12] the change in pH value during incubation time can be hindering factor for efficiency of the MICP process by *S. pasteurii* therefore the pH monitoring was required during the whole experiment. In Figure 3 are shown results of pH monitoring for blank and bio-samples.

At the start of the experiment, HM samples had lower pH value (approx.7), while MM samples were in alkaline zone (approx.8.5). The mentioned pH values are in the optimal pH zone for bacterial growth as well as ureolytic activity of *S. pasteurii* [12]. In the case of HM samples, the pH value of the bio-sample did not change within the first 7 days which is in correlation with the lag (initial) growth phase of *S. pasteurii*. After initial stagnation, the pH value of HM bio-sample increased suggesting the start of ureolytic activity. During the next 21 days, the pH value and the number of vegetative cells shared increasing trends (Figures 2 and 3a). Besides further increase of vegetative cells number, the pH value of the HM bio-sample did not follow this trend and dropped to the initial pH value. These results suggest that ureolytic activity started, but bacterial-induced precipitation slowed down due to the lack of free calcium ions in historical samples. Moreover, it might be concluded that ureolytic bacteria did not have optimal conditions for the MICP process in HM samples, but high number of vegetative cells, indicated the possibility of bioactivation.

In the case of MM samples, the pH change of bio-samples followed similar trend as for bacterial viability. After short decreasing period at the start, pH value reached the initial level and kept this value until 60 days of incubation. Due to decrease in number of vegetative cells and ureolytic activity, pH value of MM bio-sample was decreasing too, until the end of incubation with the final value of 7.

The HM blank sample remained stable during incubation period, while MM blank sample was affected by continues hydration process and had steep changes and lower final pH value than initial value.

4. Conclusion

The present study of the bio-activated self-healing agent based on *Sporosarcina pasteurii* DSM 33 bacterial culture showed a promising path to further investigation of bacteria-based systems for self-
healing effect on historical and conservation mortars. The monitored viability of S. pasteurii DSM 33 cells during the 150-day experiment confirmed potentials of the chosen bacterial culture for self-healing of the selected substrates. Higher efficiency of the bio-activated self-healing system was obtained in the case of conservation mortar (MM) samples compared to historical ones (HM). It was expected due to lack of free calcium ions in the substrate as well as in nutrient matrix for the MICP process in historical samples. However, high number of vegetative cells in historical samples indicate the possibility of their bioactivation, while the obtained results in model samples confirmed the tested system efficiency with the presence of CaCO$_3$ crystals in the precipitate. These were supported with optical microscopy of the cracks sealing and SEM analysis of the precipitate taken from the cracks undertaking the sealing process. The lack of any self-healing effect in blank samples suggests bioactivated precipitation of CaCO$_3$ in bio-samples.

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