Biotreatment of ceramic bricks: the impact of the application method of an innovative bioproduct on biomineralization

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Abstract

The effect of biological treatments on protection of exposed construction materials in the built heritage may have great potential. This work reports the efficacy of several techniques (dropping, spraying, brushing, poulticing and absorption by capillarity) to apply a new bioproduct, produced by *Escherichia coli* cultures supplemented with iron, aiming to treat the surface of ceramic bricks. The results showed that most biotreatments improved the ceramic bricks resistance to water absorption, depending on the method of application of the bioproduct. Nevertheless, within the error range, the most efficient biotreatments were observed when the bioproduct was applied by dropping, brushing and spraying. The bioproduct analyzed in the present study can be stored and easily transported to construction sites, where it can be readily prepared by resuspension of the dried bioproduct in tap water. The water resistance effect of the bioproduct was attributed to the presence of a gelatinous biofilm and to the formation of amorphous biosilica aggregates (biomineralization by biosilicification) that filled the pores and voids of bricks samples. Acting as a mild water repellent agent, it may be a promising solution for protection of ceramic bricks, reducing the degradation rate, namely for compatible and ecological architectural heritage conservation practices.

Keywords: Bacterial based bioproduct, biotreatment, biomineralization, biosilicification, reversibility, water absorption.

1. Introduction

Exposed construction surfaces are made from different materials and, with ageing, may present different states of degradation. Therefore, a significant part of exposed construction materials in the built heritage requires protective interventions. These types of interventions are indubitably preferable to the replacement of weathered materials, for economic, ecological and technical issues, while ensuring the preservation of cultural identity. Conservation and restoration of ancient ceramic bricks and tiles represent a worldwide problem for the preservation of architectural heritage, where brick masonry and terracotta decorations were commonly used in historical constructions (Franzoni et al. 2014).

Biotreatment is an innovative technique that have awakened interest for consolidation, protection and improvement of the properties of building materials (Parracha et al., 2019). This biotechnology is based on the activity of enzymes or bacterial cells that frequently involves precipitation of inorganic compounds. In this technique a bioproduct is applied at the surface of porous materials or in localized areas, as cracks. Although applied at the surface of construction materials and depending on the type of construction material, they can create an in-depth treated layer (Parracha et al., 2019). These effects result in consolidation of the material, reducing erosion, acting as a coating, reducing the absorption of water and increasing durability. Biotreatments can be more ecological and compatible with old construction materials in comparison with available synthetic surface treatments. Surface treatments can be applied by different methods, such as brushing, spraying, pipetting, dropping, capillarity, among others.

According to Alvarez de Buergo et al. (2004) consolidation is one of the most important conservation and restoration techniques, using inorganic or organic agents, natural or synthetic. The authors also referred that, beside the consolidation capacity, some of these agents also provide slight waterproofing, improving the materials durability.

The analysis of compatibility, effectiveness and durability of new consolidants is important to prevent the use of improper treatments that alter the physical, chemical and aesthetic properties of treated materials, creating further damage. The physical-chemical compatibility of a consolidation product with the material to be treated is a fundamental condition for its use in heritage preservation (Sierra-Fernandez et al., 2017). Moreover, van Hees et al. (2016) claim that a consolidation treatment can be considered compatible when it does not cause technical (material) or aesthetic damage to the treated materials.

The reversibility principle has been, for a long time, considered relevant by conservatives and states that no treatment should be used, unless it can be later removed (Doehne and Price, 2010). Nowadays, consolidation treatments are considered irreversible actions (Pinto and Delgado Rodrigues, 2007; Sierra-Fernandez et al., 2017) and, therefore, considered risky with high probability of causing undesirable permanent effects.

Studies on biotreatment of building materials are becoming increasingly common, due to their importance in the current scenario of conservation of historical constructions in the world. Biotreatments have been mainly reported for the conservation of historical materials, such as limestone (Rozenbaum et al. 2014; Perito et al. 2014; Micallef et al. 2016), earth-based blocks (Stabnikov et al. 2016), cement mortar (Choi et al. 2017; Snoeck et al. 2018) and concrete (Kim et al. 2013). Biotreatment of ceramic bricks (or tiles) is still limited. Nevertheless, Sarda et al. (2009) and Raut et al. (2014) used microbial induced calcite precipitation (MICP), to improve the properties of bricks with cultures of *Sporosarcina pasteurii* (previously classified as *Bacillus pasteurii*). This bacterium has been used as urease producer and is able to precipitate calcite in the presence of urea under alkaline conditions. In both studies bricks were immersed in bacterial culture with urea and $CaCl_2$ and subsequently tested for water absorption capacity (by immersion). The biotreatments reduced the water absorption capacity when compared to control bricks immersed in tap water. Other investigations based on MICP have been described and reached the same conclusions (Dhami et al. 2012).

The use of biotreatments in conservation of historic buildings will enhance the durability of exposed materials and constructions as a whole, preventing their replacement and demolition, while ensuring compatibility and reversibility. Problems related to the depletion of natural resources are minimized, and the impact of residues on the environment reduced. As mentioned before, different methods of application have been tested, namely by spraying (Rozenbaum et al. 2014; Perito et al. 2014; Micallef et al. 2016; Stabnikov et al. 2016; Snoeck et al. 2018), by brushing (Micallef et al. 2016) and by immersion (Kim et al. 2013; Stabnikov et al. 2016; Choi et al. 2017). Micallef et al. (2016) compared the spraying and brushing methods to treat limestone with cultures of *Bacillus subtilis*, *Sporosarcina pasteurii* and *Lysinibacillus sphaericus,* concluding that spraying was the most practical and recommended application method for the biotreatment of more extensive surfaces of the built heritage. Comparison of the water absorption efficiency of ceramic bricks treated with commercial ethyl silicate (tetraethylorthosilicate, TEOS) by immersion and brushing revealed that the former was more effective than the later (Franzoni et al., 2014). Regarding the aesthetic point of view, brushing reached better results than immersion.

The most appropriate/efficient application technique depends on the type of construction material and on the treatment product. For instance, Franzoni et al. (2015) studied the effects of different application methods (brushing, poulticing and immersion) on limestones using diammonium hydrogen phosphate and a saturated solution of limewater applied by poulticing to produce hydroxyapatite. The results of this study showed that brushing was the most appropriate application method, with improvements in mechanical, physical and chromatic properties, without causing major microstructural changes (pore size distribution and water transport properties).

Although treatment by poulticing and immersion resulted in the enhancement of the mechanical properties, significantly altered the microstructure of the samples. Comparison of the consolidation effectiveness of surface treatment of carbonated stones by three commercial consolidators using different application methods was reported by Pinto and Delgado Rodrigues (2011). The authors concluded that: (1) the different consolidation methods determines the amount of absorbed product and, consequently, the degree of strengthening; (2) treatment by capillary was the most reliable procedure; and (3) the immersion time was an important parameter, since it has a strong influence on the total amount of product absorbed by the stone.

Deterioration of ceramic bricks and tiles on built heritage, but also in works-of-art-like sculptures, occurs mostly due to moisture ingress, by absorption of water through porous structure of ceramic materials (Sarda et al. 2009), by fissures, or by water permeation (Raut et al. 2014). Consequently, the durability of ceramic elements can be significantly improved by the application of adequate agents that will stop or slow down degradation without significantly changing the original properties of the material.

Most biotreatments reported on the literature are based on calcium mineralization. Nevertheless, MICP process presents some drawbacks: i) the process produces ammonium and ammonia that are toxic to humans and to the environment; ii) the high pH requirement that may promote corrosion of some construction materials; iii) the calcium biomineral responsible for consolidation is brittle, breaking with time; and iv) its high costs. Thus, new types of bioproducts and biotreatments, cost-effective, with a lower environmental impact, less toxic and, if possible, contributing to circular economy ought to be utilized to overcome the problems associated with MICP (Wang et al. 2018; Sarda et al. 2009; Sanchez et al. 2018). In this way, in the present work the efficacy of an innovative non-toxic/non-polluting biological product produced by *Escherichia coli* cells on the protection of ceramic brick was evaluated. The effect of different application methods, by either dropping, spraying, brushing, poulticing or by capillarity was investigated. Testing was conducted for one year to assess the durability of the biotreatments. Surface hardness and water drop absorption, as well as microstructure and composition characteristics on the surface, were analyzed by several techniques, namely by Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) and X-ray powder Diffraction (XRD) and compared with untreated (without the bioproduct) brick samples. Visual observation of the color was also included.

2. Materials and methods

2.1. Ceramic bricks samples

Red fired ceramic bricks from Cerâmica Torreense, Lda. with size 222 mm x 106 mm x 70 mm and classified as category II, HD based on EN 772-1+A1 (2015) were used. The dry density and the water absorption were measured in eight brick specimens according to EN 772-13 (2000) and EN 772-21 (2011), respectively.

Each brick was cut into similar samples of size 40 mm x 40 mm x 22 mm. A water-cooled diamond blade was used to cut the brick specimens. The cut surface was clean and smoothed but not vitrified, so that the absorption capacity and density were not affected. Only the cut surfaces were tested. Four brick samples were prepared for each bioproduct application method, including samples left untreated and taken as control, in a total of twenty-four samples. Samples of ceramic bricks were leveled and fixed with plasticine on the bottom surface and placed in Petri dishes before biotreatment to avoid surface treatment slipping with the slope of some samples.

2.2. Bioproduct production and rheology

The new bioproduct was produced using *Escherichia coli* BL21(DE3) cells from NZYTech. *E. coli* BL21(DE3) is a facultative anaerobic Gram-negative, non-sporulating, non-pathogenic bacterium (supplier's MSDS, According to Regulation (EC) 453/2010), and therefore it is safe to manipulate using standard microbiological practices.

The bioproduct was prepared as described by Parracha et al. (2019). Briefly, *E. coli* cells (NZYTech) harboring the plasmid pET21-c (Novagen) were grown on a nutritionally rich medium, Lysogeny broth medium (LB, 10 g/dm^3 tryptone, 5 g/dm^3 yeast extract, 10 g/dm^3 NaCl, pH = 7.0) containing 100 μg/cm^3 of ampicillin and 1.39 g/dm^3 of acidic iron (II) sulfate ($FeSO_4.7H_2O$), at 37 ºC with orbital shaking at 220 rpm. Cells were harvested by centrifugation at 8,000 g at 4 ºC, for 10 min, achieving a yield of 3-4 g/dm^3 (wet weight). For this work and to simulate easy handling and packaging conditions at a construction working site, the cell pellet was subsequently lyophilized to remove the aqueous solution and stored in Falcon tubes wrapped in aluminum foil for protection against light. The lyophilized bioproduct had a silky powder appearance. Before use, the bioproduct was freshly prepared by resuspension of the lyophilized powder in tap water (0.7 g/cm^3), that could, again, be easily performed in a working site.

The viscosity of the bioproduct suspension was assessed. The rheological measurements were carried out using a Bohlin Gemini HR^{nano} rotational rheometer, with parallel plates geometry (20 mm of diameter and a gap of 500 μm) to measure the viscosity growth curve as a function of the shear rate, applying an ascending ramp between 50 and 300 s^{-1}. A solvent trap was used in all measurements to minimize evaporation. The environmental conditions of the laboratory were controlled at 62 ± 5 % relative humidity (RH) and temperature of 20 ± 2 ºC.

2.3. Biotreatment methods

Brick samples were kept in laboratory conditions before and during the biotreatment, at 59 ± 8% RH and a temperature of 21 ± 3 °C. In each biotreatment, 2 cm^3 (1.25 dm^3/m^2) of bioproduct were applied on a 40 mm x 40 mm cut surface of brick samples. Different application methods were studied and compared with untreated samples: by dropping, spraying, brushing, poulticing, and absorption by capillarity. Dropping and capillarity were tested since they are easily applied in controlled laboratory conditions and, although difficult to apply on construction sites, they can be used for the consolidation and protection of works-of-art-like sculptures. Poulticing using a medical gauze method is currently used by conservators to treat works of art and similar heritage elements. Both spraying and brushing techniques are easily applied on site. Spraying is particularly useful when using low viscosity bioproducts, rapid and feasible, to treat all type of surfaces, either vertically or horizontally, facilitating the acceptance by conservators and other construction professionals. Compared with spraying, brushing is more time consuming and when the surface to treat presents low cohesion, particles can be brushed away.

2.3.1. Dropping

Dropwise application (0.110 cm^3/drop) up to 2 cm^3 of the bioproduct was performed according to Figure 1a, using a micropipette on the cut test surface of each sample, placed horizontally, following a 9-point grid scheme with two sequential applications.

2.3.2. Spraying

The bioproduct suspension was transferred to a spray container. Spraying occurred perpendicularly at a distance of 8-10 cm of the surface of specimen surface that were placed horizontally (Figure 1b). A total of 15 jets were sprayed to apply the 2 cm^3 of bioproduct, each of them fully covering the cut surface of the sample.

2.3.3. Poulticing by an impregnated gauze

A cotton medical gauze square (200 mm x 200 mm, folded into 40 mm x 40 mm pieces) was placed in a Petri dish and impregnated dropwise with a micropipette following a 9-point grid with bioproduct. The Petri dish with the gauze was flipped over the test cut surface of the sample and kept in direct contact for 8 h (Figure 1c).

2.3.4. Brushing

Before the first application, the dry brush with soft white bristles (brush with 40 mm length and 20 mm width) was soaked in the bioproduct. The bioproduct was applied by brushing of the test cut surface of samples until all bioproduct was transferred to the brick sample, moistening the brush between each brushstroke (Figure 1d). Brushing was done from side to side of the sample, always in the same direction.

2.3.5. By capillarity

The bioproduct was transferred to a Petri dish and the brick sample was placed on top, with the test cut surface to be biotreated facing down. After 8 h the sample was turned upside-down with the treated surface facing upwards (Figure 1e).

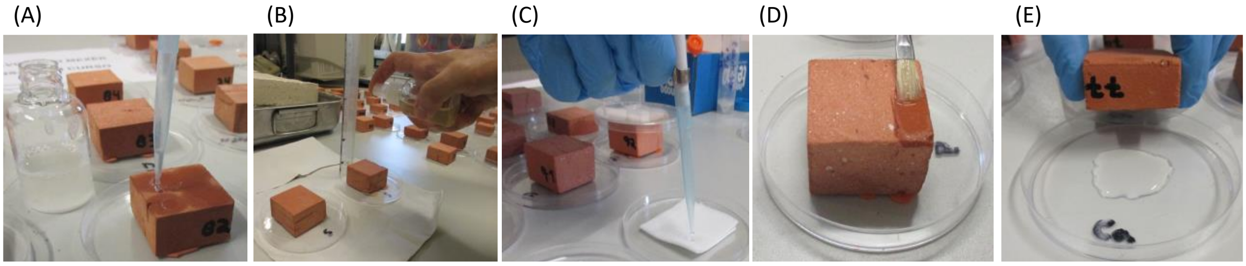

Figure 1 – Application of the bioproduct by: (A) dropping; (B) spraying; (C) poulticing by an impregnated gauze; (D) brushing; and (E) capillarity.

2.4. Characterization

2.4.1. Visual observation

One of the most relevant criteria in the choice of a protective product for conservation of building materials and works of art is the aesthetic, which cannot be significantly affected. Therefore, the test surface of all samples was visually observed, before and after biotreatments and drying, using a Pantone Kodak color scale.

2.4.2. Water drop absorption capacity and data analysis

The effectiveness of the proposed biotreatments was evaluated by measuring the time for a drop of water to be absorbed by the treated surface of the brick samples. The water drop test was carried out in accordance with RILEM II 8b (1980) and Parracha et al. (2019). This test consisted in releasing a drop of water (~0.1 cm^3) on each corner of the biotreated surface, with a fixed distance of 1.5-2.0 cm between the micropipette tip and the tested surface and recording the time until each water drop was completely absorbed. Water absorption capacity may differ in different areas of the same tested surface, due to natural heterogeneity and porosity of ceramic brick samples. For this reason, care was taken to perform the water drop test at 4 test points in each treated surface of the

4 replicates. Seven testing campaigns were performed at different time frames using the same brick samples: after 2 days, 1 week (7 days), 1 month (30 days), 3 months (92 days), 6 months (183 days), 9 months (275 days) and 12 months (365 days), under hygrothermal conditions of 22 ± 5 °C and 58 ± 10% RH.

The Z-score statistical method was used to reduce the dispersion of the data presented in the water drop absorption test and to exclude data that deviated too far from the mean value. The Z-score for each data point set of a sample was determined according with the equation:

$$Z = \frac{(x - \overline{x})}{SD}$$

where x is the absorption time of a water drop (one of the four measurements per sample), $\overline{x}$ is the mean value for the four biotreated replicates (4 × 4 experimental data points) and *SD* is the standard deviation of the experimental data points of the four replicates of each biotreatment.

For data analysis, a Z-score of 1.2 (or 120%) of the standard deviation was used, that according with the Z tables ("Cumulative from mean", "Cumulative" and "Complementary cumulative") represents 77% of the data, and in fact resulted in the inclusion of 75% of the experimental data (a maximum of 4 points were excluded).

For each campaign the coefficient of variation (*CV*), also known as relative standard deviation (*RSD*), defined as the ratio between the standard deviation (*SD*) and the mean value ($\overline{x}$), was calculated.

The incremental absorption time percentage relative to the control (t^*) for each campaign/application method was defined by the following equation:

$$t^* = \frac{(\overline{t} - \overline{t}_C)}{\overline{t}_C} \times 100$$

where $\overline{t}$ and $\overline{t}_C$ are the mean time for a drop of water to be absorbed by treated and control samples, respectively.

The experimental data was further analyzed using the software IBM SPSS Statistics (version 24). Two-way analysis of variance (ANOVA) and Tukey-Kramer post hoc test were performed. The significance level was set at $P < 0.05$.

2.4.3. Surface hardness

For conservation of historic materials, such as bricks, it is important that the water ingress is reduced but the mechanical characteristics should not significantly increase so that no mechanical incompatibility is created between the treated surface and the subjacent brick. To assess that effect, surface hardness analysis was performed according to ASTM D2240 (ASTM, 2000) using a PCE-DD-A Shore A durometer, with a scale between 0 and 100. Surface hardness was evaluated after 13 months of treatment, at 12 different points of the treated surface of each specimen. The test was performed only after the end of the water absorption campaign to be sure not to damage the surface of the biotreated samples along the experimental campaign. The surface hardness final value was calculated as the mean value.

2.4.4. SEM-EDS and XRD analysis

The morphological characteristics of biotreated bricks was analyzed by SEM. For comparison only samples treated by dropping, after 2- and 13 months of applications were investigated. Composition was assessed by EDS coupled to SEM. Data was collected using two variable pressure scanning electron microscopes: a VP-SEM-EDS HITACHI 3700 N coupled with an energy-dispersive X-ray spectrometer BRUKER Xflash 5010 SDD, operated at 20 kV in low vacuum and Backscattering Electrons mode, and a VP-SEM-EDS Thermo Fisher Phenom ProX with an EDS detector, operated at 15kV in low vacuum and Backscattering Electrons mode.

X-ray powder diffraction (XRD) was used to identify the presence of crystalline material using a benchtop RIGAKU MiniFlex II X-ray diffractometer with Cu K(alpha) radiation operating at 30 kV and 15 mA, wavelength of 1.5406 Å and scanning between 20° and 80° 2*θ*, by the LAQV-REQUI*M*TE Analytical Laboratory services. Due to the presence of organic material, samples were not previously dried.

3. Results and discussion

3.1. Characterization of materials

The results for the characterization of the ceramic bricks samples are shown in Table 1, including the technical information for standard bricks provided by the manufacturer (Cerâmica Torreense, 2009).

Table 1 - Characterization of the ceramic masonry bricks

Characteristics	**Standard**	**Datasheet** (Cerâmica Torreense, 2009)	**Experimental ± SD**
Dimensions (mm^3)	EN 772-16 (2011)	222 x 106 x 70	40 ± 0.9 x 40 ± 1.0 x 22 ± 3.3
Water absorption by immersion (%)	EN 772-21 (2011)	8.70	6.95 ± 1.30
Dry density (kg/m^3)	EN 772-13 (2000)	2030	2143 ± 64
Compressive strength (N/mm^2)	EN 772-1+A1 (2015)	32.2	ND

ND − not determined

The experimental results for water absorption and dry density characterization of ceramic bricks samples differ from the datasheet information probably due to the fact that cut specimens were analyzed and compared with larger brick units.

The bioproduct suspension presented a light gray color (RGB 179, 182, 175 – Pantone 413C) (left panel on Figure 2). The rheology curve of the apparent viscosity as a function of the shear rate (right panel on Figure 2) displayed two dispersions phases and a transition between shear thickening and thinning regimes at a shear rate at around 50 s^{-1}. This trend is similar to the ones previously reported for motile *E. coli* cultures (Gachelin et al., 2013; López et al., 2015). At low shear rates, a plateau was detected followed by an increase (shear thickening) up to ~0.040 Pa·s. According with López et al. (2015) this behavior is observed for diluted cell suspensions. The maximum viscosity was obtained at a shear rate around 90 s^{-1}. For shear rates higher than 130 s^{-1} the viscosity decreased with the applied shear rate (shear thinning). Thus, the bioproduct suspension tested in this study can be classified as a non-Newtonian fluid with viscosity lower than 1, adequate for applications as a surface treatment agent.

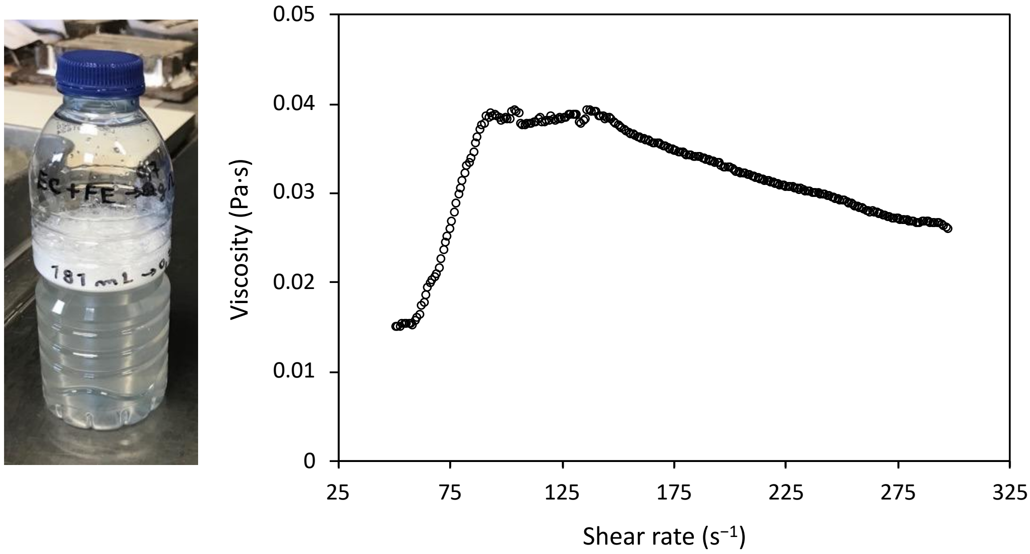

Figure 2 – Color (left) and viscosity curve (right) of the bioproduct suspension with tap water.

Whilst the viscosity of fluids is dependent on many parameters, such as the rheometer, applied shear rate and temperature, cell status and density, cell viability and possible leakage of intracellular components (namely DNA, proteins and lipids), can also affect the viscosity of active suspensions (Saintillan, 2018). The *E. coli* bacterium behaves as a pusher reducing the apparent viscosity, presenting viscosity values close to those of water (Gachelin et al., 2013).

No change in color and/or gloss could be visually observed on the surface of biotreated bricks, when compared with the control.

3.2. Water drop absorption capacity

Five different methods were chosen for the application of the bioproduct: dropping, spraying, poulticing by an impregnated gauze, brushing, and capillarity. The water absorption capacity of surface treated bricks is presented in Figure 3, while the relative incremental time for absorption of a water drop obtained for the different application methods is presented in Figure A.1 (supplementary material).

Analysis of Figure 3 shows that all biotreatments, with the exception of poulticing, were efficient, retarding water absorption by brick samples. Poulticing did not reach promising results and was statistically identical to the control (untreated) samples. This result was probably due to the small volume (2 cm^3) of bioproduct used, that was not enough to saturate the gauze, impeding the proper transfer of the bioproduct to the brick samples.

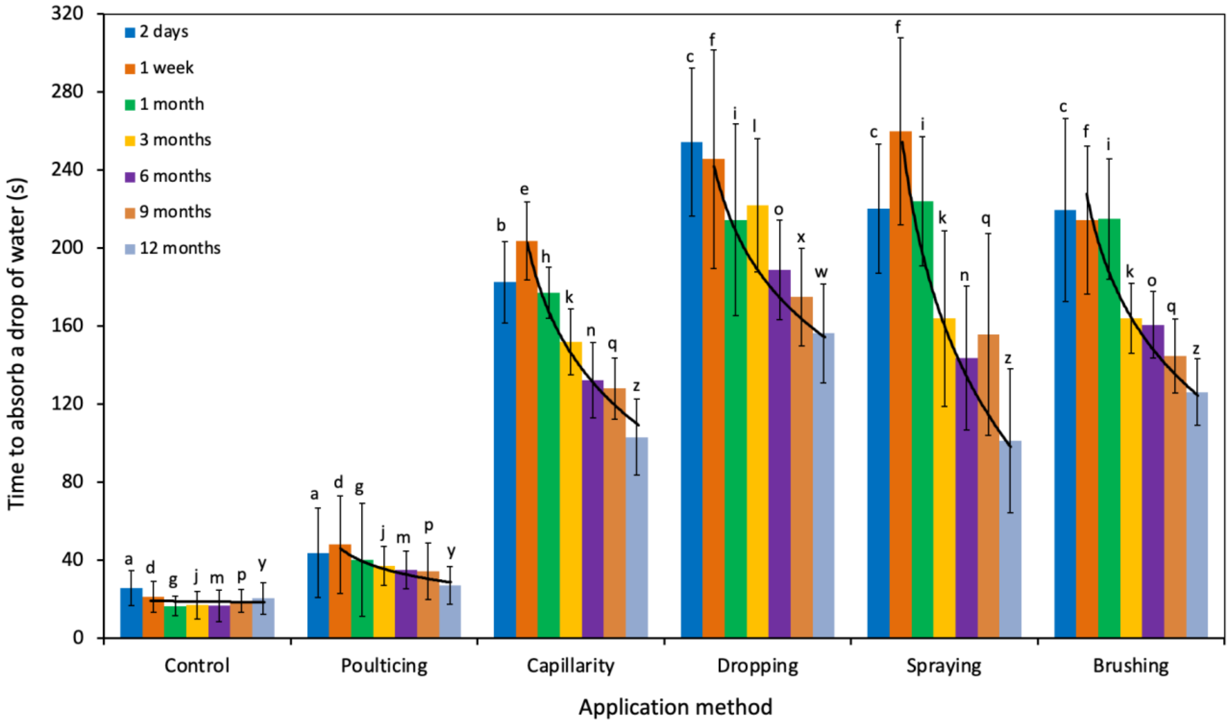

Figure 3 – Water absorption capacity of surface biotreated bricks using different application methods. Analysis of differences of data for each application method over time by two-way analysis of variance (ANOVA) with mean values comparison using Tukey-Kramer post hoc test. Vertical error bars indicate the mean absorption time (± standard deviation) of a drop of water. The different letters indicate a statistically significance difference for $P < 0.05$. Solid lines on top of each dataset represent a logarithmic decay fit to the experimental mean data points for each application method over time.

Examination of the variance (ANOVA) with mean values comparison using Tukey-Kramer post hoc test at a level of significance of 0.05 for the four most encouraging application methods (all but poulticing) detailed information can be gathered. While for the second (one week) and third (one month) time points tested there was no significant statistical difference for the biotreatments by dropping, spraying and brushing, for the remaining timespan those could be observed.

From 3 months, biotreated samples by spraying could be clustered with capillarity. Biotreatment by brushing did follow the same profile over time, except for the 6-month-old sample. The results for the dropping application method were the most promising ones, presenting one of the highest efficiencies towards water absorption resistance and a slower decay rate of the biotreatment. The greatest improvement was observed one month after the biotreatment of brick samples by dropping, spraying and brushing, achieving averaged incremental times of absorption on around 1200% relative to the untreated control ceramic brick (Figure A.1, supplementary material).

As just described, the application procedure affected the water resistance mechanism, which was probably related with the manner the bioproduct was distributed over the surface. For instance, the application by dropping would locally concentrate the bioproduct at each point of application. A more in-depth absorption would also explain the slowest decay on the water resistance effect after one year of biotreatment. For the timespan tested, the improvements achieved by spraying and brushing were similar. The results suggest that the bioproduct was transferred all over the surface by both techniques, creating a relatively thin layer. A thinner layer of bioproduct would cause dispersion of the biofilm, more susceptible to dryness and dehydration, producing a less effective treatment. The efficacy of the biotreatment by capillarity was lower than the aforementioned techniques. Absorption of the bioproduct by this technique likely produced a more uniform layer, covering the whole surface area, possibly altering the microstructure of the material. Diffusion of oxygen may have been blocked impairing the development of a biofilm (and its activity) and diminishing the effect of the biotreatment. This is supported by obtaining lower coefficients of variation (Table A.1, supplementary material). Whilst hard to apply on buildings conservation, application by capillarity can be appropriate for conservation of some ceramic works-of-art.

García-González et al. (2020) have studied the healing effect on ceramic bricks of a bioproduct of the same type, although with different composition. For the current work, after cell growth, the liquid medium (composed by the rich nutrient LB medium and extracellular substances from the cellular metabolic activity) was discarded by centrifugation and lyophilization. The cellular powder was then resuspended in tap water before use, whereas in the referred study the whole cellular culture was applied as a treatment agent immediately after growth. Even though, the most significant effect observed by García-González et al. (2020) reached an enhancement in water resistance of about 180%, much lower than the one observed in the present work.

Considering the decay on the water absorbance capacity from one week up to 12 months of biotreatment (solid line on top of each dataset in Figure 3), three different groups could be observed. Biotreated samples by poulticing presented the slowest decay of the effectiveness, while sprayed ones decayed fastest. Samples treated by capillarity, dropping and brushing presented an intermediate rate of decay of water absorption capacity. The weakening of the water absorption resistance may be explained by cell death (and lysis) due to desiccation/dehydration and starvation that would negatively affect the biofilm. The observed decay proves the reversibility of the biotreatment, regardless of the technique used to apply the bioproduct.

Control samples presented a slight decrease on the time taken to absorb a water drop from day 2 to 6 months, although within the experimental error. The absorption time varied between 26 s after 2 days and 17 s at 6 months and showed a small increase at 9 and 12 months, reaching 19 s and 21 s, respectively (Table A.1, supplementary information).

The high coefficient of variation determined for all experimental points (Table A.1, supplementary information) can be explained by the high heterogeneity of the brick samples (Franzoni et al. 2014 and Barnat-Hunek and Smarzewski 2015), some with visible cracks due to heterogeneous firing and, eventually, different porosity (Whittemore, 1981 and Cultrone, 2004).

Overall, the results indicate that the biotreatment was quite efficient, remaining active throughout the time period in study (12 months). In addition, the biotreatment showed important characteristics such as reversibility (decay of the efficiency over time, observed in all cases) and compatibility with ceramic bricks. These reversibility and compatibility characteristics were also found by Parracha et al. (2019), when studying the efficacy of iron-supplemented *E. coli* cultures as a surface bioproduct for earth-based plastering mortars.

Regardless of the method, the application of the bioproduct enhanced the resistance to water ingress, protecting ceramic bricks. In fact, the efficiency of the biotreatment was greater than the ones described by Sarda et al. (2009), Raut et al. (2014) and Dhami et al. (2012), all based on MICP. In the first two studies bricks were treated with the bioproduct by immersion and after 28 days their water absorption capacity decreased 45-49%. About the same effect was reported for rice husk ash bricks and fly ash bricks (Dhami et al., 2012).

3.3. Surface hardness

The results of the surface hardness (shore A) test on biotreated ceramic bricks by the different application methods after 13 months show that, within the experimental error, all biotreated samples, regardless of the method of application, presented a surface hardness similar to the control untreated samples and equal to 97 ± 2 shore A. This result indicates that the biotreatment did not affect the surface hardness, demonstrating to be compatible with fired bricks and, therefore, adequate to be used as a conservation technique. Although it is a simple test, to authors knowledge it has not been applied yet to assess surface hardening by biotreatments of bricks. Parracha et al. (2019) applied the same method to assess the hardness by *E. coli*-based biotreatments on the surface of an earth plaster. Albeit the measured hardness was higher for bricks samples, as expected, the influence of the biotreatments on surface hardness was also insignificant.

3.4. SEM-EDS and XRD analysis

SEM-EDS and XRD analysis was performed to assess the effect of the biotreatment on the microstructure of bricks.

Samples with 2 days and 13 months after the application of the bioproduct by dropping were chosen as they exhibited the highest water barrier effect (Figure 3). Characteristic SEM photomicrographs are presented on Figures 4 and 5.

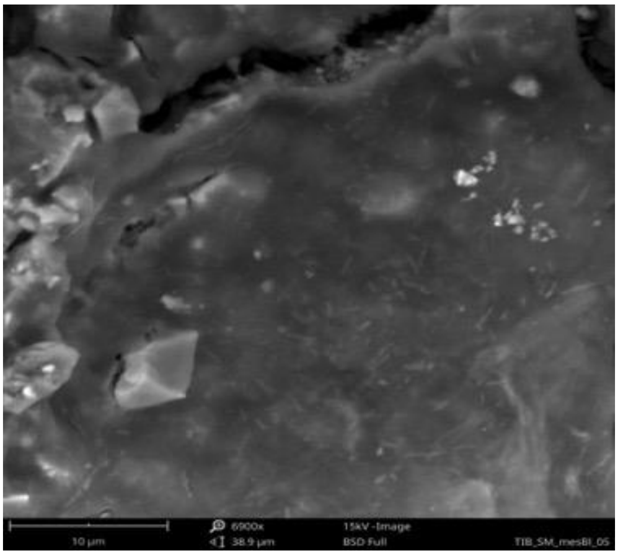

Figure 4 – SEM photomicrographs of brick samples 2 days after biotreatment by dropping, performed at low vacuum in backscattering electrons mode.

SEM analysis of brick samples 2 days after the biotreatment (Figure 4) showed the formation of a gelatinous biofilm, heterogeneously distributed over the treated surface. Embedded rod-shaped cells were visible with apparent lengths ranging from 0.9 to 1.9 µm, with an average length around 1.7 µm, typical of *Escherichia coli* (Obeng et al., 2018). Cracking of the biofilm was also observed probably due to dehydration or adherence of cells to uneven areas of the surface of ceramic bricks. A biofilm, albeit thinner, was also observed by García-González et al. (2020) on bricks biotreated with the bioproduct first described by Parracha et al. (2019).

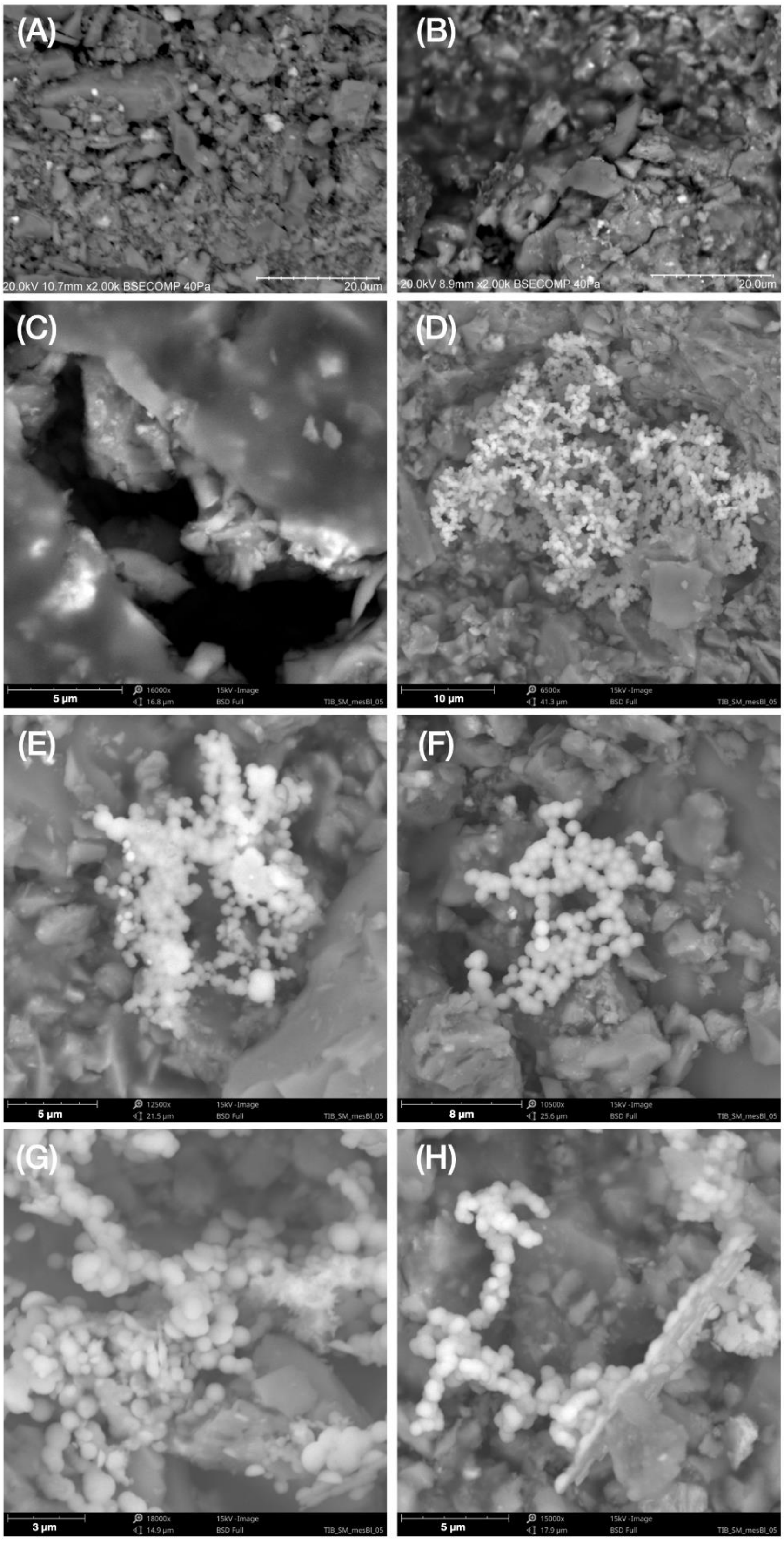

Figure 5 – SEM micrographs of brick untreated control (A) and 13 months after biotreatment (B-H) samples at different brick surface regions and magnifications, performed at low vacuum in backscattering electrons mode.

The biofilm was still visible on SEM images after 13 months of biotreatment (Figure 5 B and C). However, SEM images of these samples at higher resolution were strikingly different, revealing the formation of large aggregates of particles with different shapes and sizes (Figure 5 D to H). Most of these particles exhibited spherical or quasi-spherical shapes (Figure 5D to F, and H), although disc-shaped (Figure 5G) and irregular ones (Figure 5E) were also observed.

The aggregates observed seemed to appear on depressions of the surface, where the biofilm was thicker. SEM analysis of the untreated control sample (Figure 5A), kept for 13 months on the same experimental conditions did not reveal any of these structures, demonstrating that the bioproduct played a major role in the formation of the aggregates/precipitates.

In the 13 months-old biotreated bricks samples, bacterial cells embedded in the biofilm were no longer visible (Figure 5C). That may be explained by cell death due to prolonged starvation, desiccation and subsequent degradation and release of intracellular organic material. The aggregates observed are consistent with amorphous silica precipitates formed by a biosilicification process, induced/catalyzed by amino acids, peptides and proteins released from bacterial cells. Biosilicification occurs in nature, at around neutral pH and ambient conditions, mostly by diatoms, sponges and plants and is still not fully understood (Patwardhan and Clarson, 2002, Patwardhan and Clarson, 2003, Ramanathan et al., 2011a, Abdelhamid and Pack, 2020). Contrarily, chemical synthesis of silica is well known and occurs in acidic or basic media, and/or presence of solvents, high temperatures and pressures (Greenwood and Earnshaw, 1984, Pell et al., 2004, Hyde et al., 2016, Raza et al., 2018). *In vitro* biopolymerization of silica has been extensively investigated using organosilicon precursors, namely TEOS (tetraethylorthosilicate) and TMOS (tetramethylorthosilicate), and different amino acids or peptides, either in water or in ionic liquids, frequently in the presence of salts (Konhauser et al., 2004, Ramanathan et al., 2011a, Ramanathan et al., 2011b). These biomolecules function as catalysts/scaffolds for spontaneous biosilica formation. Varying the concentration of silica precursors and biomolecules in the reaction, amorphous biosilica nanostructures with different morphologies (spherical, rod-shaped, plates, and sizes (0.3 to 0.7 μm)) have been reported (Ramanathan et al., 2011a). In the present work, particles with different morphologies were observed and are attributed to the presence of different amounts of biomolecules, probably due to the local heterogeneous distribution of cells throughout the biofilm, which creates specific conditions (salt concentration, pH, viscosity, among others) for precipitation and polymerization. Silica precursors, likely from brick clay and sand, had to be mobilized and converted into amorphous silica. Beside amino acid, peptides and proteins, lipids and polysaccharides play a role on the reaction (Xue et al., 2015). Functional groups of these biomolecules mediate the formation of an organic-inorganic hybrid material bonded through ionic interactions and hydrogen bonds (Patwardhan and Clarson, 2003). The cellular organic matrix creates the appropriate scaffold for nucleation and growth of the silica mineral in a favorable biological environment (Beloin et al., 2008). For instance, while the mineral shown in Figure 5F was mainly composed by spherical particles with an average diameter of 0.9 μm, ranging from 0.6 to 1.4 μm, the aggregates in Figure 5G were predominantly constituted of disc-shaped particles with non-uniform sizes (varying between 0.5 and 1.0 μm). Fusion of biosilica particles was detected on Figure 5H, reminiscent of the deposition of mineral lamellae on bio-sintering processes (Konhauser et al., 2004, Wang et al., 2012). Since the surface hardness of 13 months-old biotreated samples were similar to the control one (97 ± 2 shore A), one can conclude that the formation of amorphous biosilica aggregates did not significantly affect the mechanical properties of biotreated bricks.

SEM-EDS was performed for local determination of chemical elements in brick specimens, 2 days and 13 months after biotreatment (by dropping) and control samples (Figure 6).

In all SEM-EDS spectra (Figure 6) a strong silicon (Si) signal was detected. Besides Si, Al and O were identified in all samples, as well as a less intense Fe peak, components of the brick material. The carbon peak present in the EDS spectra of biotreated samples is associated with the presence of organic material from the bioproduct.

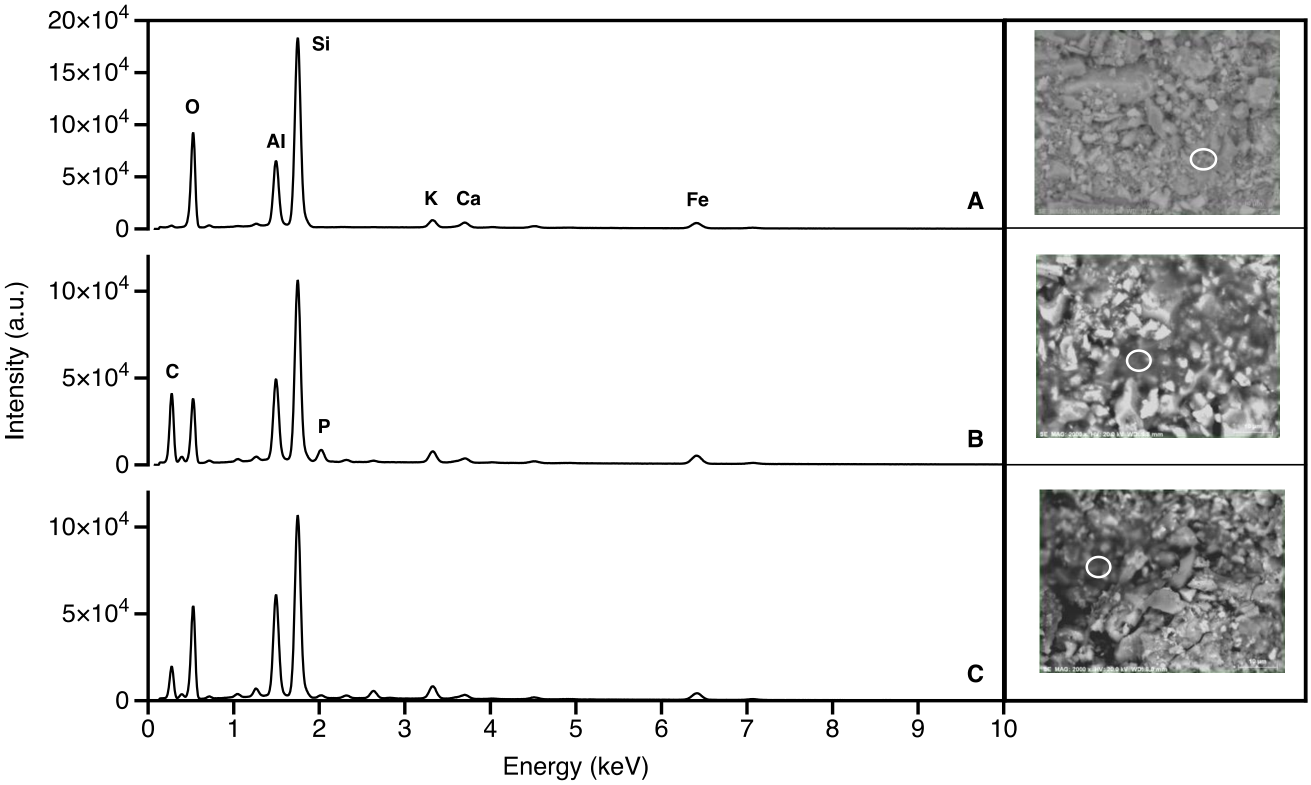

Figure 6 – Comparison of SEM images (right panel) and corresponding of EDS spectra (left panel) of untreated control brick samples (A) versus 2 days (B) and 13 months (C) after biotreatment by dropping. The open white circles depict the area analyzed on each SEM image.

To infer about the nature of the observed structures, XRD analysis of a 16 months-old-treated brick sample was performed and compared with the untreated control (Figure 7 and Table 2 of the supplementary material). As expected, the predominant crystalline mineralogical phase was quartz, from the brick material. Other mineral phases were also found, probably of hematite, mullite and feldspars, although accurate identification was difficult due to the much lower intensity of the later relative to the quartz signal. No significant differences were observed between the biotreated and untreated bricks samples, confirming the non-crystalline nature of biosilica structures identified by SEM.

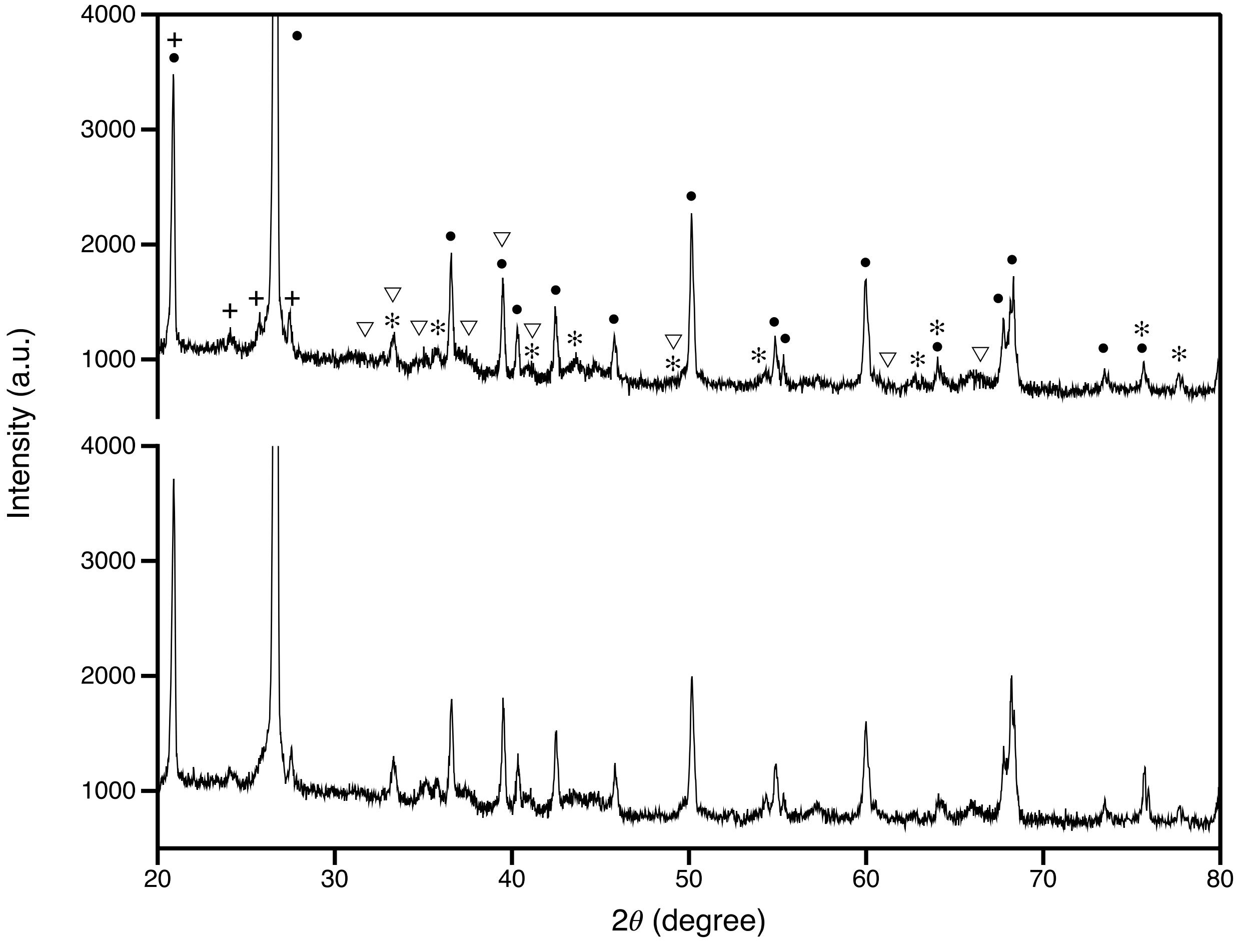

Figure 7 – XRD diffractograms of brick untreated control (top) and after 16 months of biotreatment by dropping (bottom). Crystalline mineralogical phases detected: quartz (●), hematite (✻), mullite (▽) and Feldspars (+).

4. Conclusions

In this study a microbial based bioproduct used to protect the surface of fired bricks was investigated. Comparison of the efficacy and durability of five different application methods monitored for about one year after the biotreatment was performed. The results presented in this work refer to the effect of a single application of a bioproduct reconstituted by resuspending a dried powder with tap water for easier in site handling.

A significant reduction in water ingress and, consequently, a longer material durability, important for conservation of both fired brick architectural heritage and works-of-art conservation, was achieved for four of the application methods; poulticing was ultimately deemed not efficient. The importance of the application technique on the effectiveness and durability of the biotreatment was demonstrated. The best results were obtained when the bioproduct was applied by dropping, spraying and brushing, techniques that can easily be performed *in situ* for buildings conservation.

Besides the water repellent effect, the bioproduct has relevant characteristics, such as reversibility and compatibility with bricks. After one week of biotreatment, the water barrier effect started to decay. Nevertheless, at the end of the experimental campaign, 12 months after the application of the bioproduct, the effect was still promising, with incremental absorption times around 660%, 515% and 400% higher than the control, for the brick samples biotreated by dropping, brushing and spraying, respectively.

No change of appearance, in color and/or gloss could be visually observed. The bioproduct did not interfere on the surface hardness, regardless of the method of application, ensuring mechanical compatibility with the original materials.

SEM analysis of 2 days-old biotreated brick samples showed the formation of a gelatinous biofilm concentrated on depressions of the treated surface, filling the pores of the material. After 13 months of biotreatment bricks exhibited amorphous silica aggregates formed by biosilicification, induced or catalyzed by amino acids, peptides, proteins and other intracellular material released from bacterial cells. XRD analysis of treated bricks showed that the biotreatment process did not affect the crystalline structure of the brick material.

Overall, these results demonstrate that this biotreatment process is attuned with conservation principles of protection without a transformative effect on the original material. To our knowledge, this process represents a novel type of biomineralization so far never observed in biotreated construction materials such as masonry bricks and can represent an alternative to MICP. Further studies are needed to explore the use of microbial induced biosilicification on the conservation of construction materials.

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References

Abdelhamid, M.A.A., Pack, S.P., 2020. Biomimetic and bioinspired silicifications: Recent advances for biomaterial design and applications. Acta Biomaterialia. Available online. https://doi.org/10.1016/j.actbio.2020.05.017

Alvarez de Buergo, M., Fort, R., Gomez-Heras, M., 2004. Contributions of scanning electron microscopy to the assessment of the effectiveness of stone conservation treatments. Scanning 26, 41–47. https://doi.org/10.1002/sca.4950260107

Barnat-Hunek, D., Smarzewski, P., 2015. Increased water repellence of ceramic buildings by hydrophobisation using high concentration of organic solvents. Energy and Buildings 103, 249–260. https://doi.org/10.1016/j.enbuild.2015.06.048

Beloin, C., Roux, A., Ghigo, J.M., 2008. *Escherichia coli* biofilms, in: Romeo, T. (Ed.), Bacterial biofilms, Curr. Top. Microbiol. Immunol., 322, pp. 249–289. https://doi.org/10.1007/978-3-540-75418-3_12.

Choi, S.-G., Wang, K., Wen, Z., Chu, J., 2017. Mortar crack repair using microbial induced calcite precipitation method. Cem. Concr. Compos. 83, 209–221. http://dx.doi.org/10.1016/j.cemconcomp.2017.07.013

Cultrone, G., Sebastián, E., Elert, K., de la Torre, M.J., Cazalla, O., Rodriguez-Navarro, C., 2004. Influence of mineralogy and firing temperature on the porosity of bricks. J. Eur. Ceram. Soc. 24, 547–564. http://dx.doi.org/10.1016/S0955-2219(03)00249-8

Dhami, N., Reddy, M., Mukherjee, A., 2012. Improvement in strength properties of ash bricks by bacterial calcite. Ecol. Eng. 39, 31–35. http://dx.doi.org/10.1016/j.ecoleng.2011.11.011

Doehne, E., Price, C.A., 2010. Stone conservation: An overview of current research, in: Lucke, A., Hohenegger, B., Bohn, C.N. (Eds.), Research in conservation. The Getty Conservation Institute, Los Angeles, 2nd edition, pp. 54–57.

EN 772-1: 2011 + A1 2015, Methods of test for masonry units - Part 1: Determination of compressive strength. CEN, Brussels.

EN 772-13:2000, Methods of test for masonry units - Part 13: Determination of net and gross dry density of masonry units (except for natural stone). CEN, Brussels.

EN 772-16:2011, Methods of test for masonry units - Part 27: Determination of dimensions. CEN, Brussels.

EN 772-21:2011, Methods of test for masonry units - Part 21: Determination of water absorption of clay and calcium silicate masonry units by cold water absorption. CEN, Brussels.

Franzoni, E., Pigino, B., Leemann, A., Lura, P., 2014. Use of TEOS for fired-clay bricks consolidation. Mater. Struct. 47, 1175–1184. http://doi.org/10.1617/s11527-013-0120-7

Franzoni, E., Sassoni, E., Graziani, G., 2015. Brushing, poultice or immersion? The role of the application technique on the performance of a novel hydroxyapatite-based consolidating treatment for limestone. J. Cult. Herit. 16, 173–184. https://doi.org/10.1016/j.culher.2014.05.009

Gachelin, J., Miño, G., Berthet, H., Lindner, A., Rousselet, A., Clément, E., 2013. Non-Newtonian viscosity of *Escherichia coli* suspensions. Phys. Rev. Lett. 110, 268103. https://doi.org/10.1103/PhysRevLett.110.268103

García-González, J., Pereira, A.S., Lemos, P.C., Almeida, N., Silva, V., Candeias, A., Juan-Valdés, A., Faria, P., 2020. Effect of surface biotreatments on construction materials. Constr. Build. Mater. 241, 118019. https://doi.org/10.1016/j.conbuildmat.2020.118019

Greenwood, N.N., Earnshaw, A., 1997. Chemistry of the elements, in: Greenwood, N.N., Earnshaw, A., (Eds.), Elsevier, 2nd edition, pp. 328–366. https://doi.org/10.1016/C2009-0-30414-6

Hyde, E.D.E.R., Seyfaee, A., Neville, F., Moreno-Atanasio, R., 2016. Colloidal silica particle synthesis and future industrial manufacturing pathways: a review. Ind. Eng. Chem. Res. 55, 8891–8913. http://dx.doi.org/10.1021/acs.iecr.6b01839

Kim, H.K., Park, S.J., Han, J.I., Lee, H.K., 2013. Microbially mediated calcium carbonate precipitation on normal and lightweight concrete. Constr. Build. Mater. 38, 1073–1082. http://dx.doi.org/10.1016/j.conbuildmat.2012.07.040

Konhauser K.O., Jones, B., Phoenix, V.R., Ferris, G., Renaut, R.W., 2004. The microbial role in hot spring silicification. AMBIO: A Journal of the Human Environment 33, 552–558. http://dx.doi.org/10.1579/0044-7447-33.8.552

López, H.M., Gachelin, J., Douarche, C., Auradou, H., Clément, E. 2015. Turning bacteria suspensions into superfluids. Phys. Rev. Lett., 115, 028301. http://dx.doi.org/ 10.1103/PhysRevLett.115.028301

Micallef, R., Vella, D., Sinagra, E., Zammit, G., 2016. Biocalcifying *Bacillus subtilis* cells effectively consolidate deteriorated Globigerina limestone. J. Ind. Microbiol. Biotechnol. 43, 941–952. https://doi.org/10.1007/s10295-016-1768-0.

Nehdi, M., M. A. Rahman. 2004. Estimating rheological properties of cement pastes using various rheological models for different test geometry, gap and surface friction. Cem. Concr. Res. 34, 1993–2007. https://doi.org/10.1016/j.cemconres.2004.02.020

Obeng, E.M., Brossette, T., Ongkudon, C.M., Budiman, C., Maas, R., Jose, J., 2018. The workability of *Escherichia coli* BL21 (DE3) and *Pseudomonas putida* KT2440 expression platforms with autodisplayed cellulases: a comparison. Appl. Microbiol. Biotechnol. 102, 4829–4841. https://doi.org/10.1007/s00253-018-8987-4.

Parracha, J.L., Pereira, A.S., da Silva, R.V., Almeida, N., Faria, P., 2019. Efficacy of iron-based bioproducts as surface biotreatment for earth-based plastering mortars. J. Clean. Prod. 237, 117803. https://doi.org/10.1016/j.jclepro.2019.117803.

Patwardhan, S.V., Clarson, S.J., 2002. Silicification and biosilicification. Silicon Chem. 1, 207–214. https://doi.org/10.1023/A:1021243810915.

Patwardhan, S.V., Clarson, S.J., 2003. Silicification and biosilicification. Part 7: Poly-L-arginine mediated bioinspired synthesis of silica. J. Inorg. Organomet. Polym. Mater. 13, 193–203. https://doi.org/10.1023/A:1026120309903.

Pell, L.E., Schiricker, A.D., Mikulec, F.V., Korgel, B.A., 2004. Synthesis of amorphous silicon colloids by trisilane thermolysis in high temperature supercritical solvents. Langmuir 20, 6546–6548. https://doi.org/10.1021/la048671o.

Perito, B., Marvasi, M., Barabesi, C., Mastromei, G., Bracci, S., Vendrell, M., Tiano, P., 2014. A *Bacillus subtilis* cell fraction (BCF) inducing calcium carbonate precipitation: Biotechnological perspectives for monumental stone reinforcement. J. Cult. Herit. 15, 345–351. http://dx.doi.org/10.1016/j.culher.2013.10.001.

Pinto A.P.F., Delgado Rodrigues, J., 2007. Stone consolidation: The role of treatment procedures. J. Cult. Herit. 9, 38–53. https://doi.org/10.1016/j.culher.2007.06.004.

Pinto A.P.F., Delgado Rodrigues, J., 2011. Consolidation of carbonate stones: Influence of treatment procedures on the strengthening action of consolidants. J. Cult. Herit. 13, 154–166. https://doi.org/10.1016/j.culher.2011.07.003.

Ramanathan, R., Campbell, J.L., Soni, S.K., Bhargava, S.K., Bansal, V., 2011a. Cationic amino acids specific biomimetic silicification in ionic liquid: A quest to understand the formation of 3-D structures in diatoms. PLOS ONE 6, e17707. http://dx.doi.org/10.1371/journal.pone.0017707.

Ramanathan, R., Bhargava, S.K., Bansal, V., 2011b. Biomimetic silicification to mimic 3D ornate diatom structures – a myth or a reality? In: Conference Chemeca 2011: Engineering a Better World, NSW, Australia.

Raut, S.H., Sarode, D.D., Lele, S.S., 2014. Biocalcification using bacilus pasteurii for strengthening brick masonry civil engeneering structures. World J. Microbiol. Biotechnol. 30, 191–200. http://dx.doi.org/10.1007/s11274-013-1439-5.

Raza N., Raza, W., Madeddu, S., Agbe, H., Kumar, R.V., Kim, K.H., 2018. Synthesis and characterization of amorphous precipitated silica from alkaline dissolution of olivine. RSC Adv. 8, 32651–32658. http://dx.doi.org/10.1039/c8ra06257a.

RILEM Commission 25 - PEM Protection et érosion des monuments, 1980. Matériaux et Constructions 13, 175–253. https://doi.org/10.1007/BF02473564.

Rozenbaum, O., Anne, S., Rouet, J.-L., 2014. Modification and modeling of water ingress in limestone after application of a biocalcification treatment. Constr. Build. Mater. 70, 97–103. http://dx.doi.org/10.1016/j.conbuildmat.2014.07.038.

Saintillan, D., 2018. Rheology of Active Fluids. Annu. Rev. Fluid Mech. 50, 563-592. https://doi.org/10.1146/annurev-fluid-010816-060049.

Sánchez, M., Faria, P., Ferrara, L., Horszczaruk, E., Jonkers, H.M., Kwiecien, A., Mosa, J., Peled, A., Pereira, A.S., Snoeck, D., Stefanidou, M., Stryszewska, T., Zajak, B., 2018. External treatments for the preventive repair of existing constructions: A review. Constr. Build. Mater. 193, 435–452. https://doi.org/10.1016/j.conbuildmat.2018.10.173.

Sarda, D., Choonia, H.S., Sarode, D.D., Lele, S.S., 2009. Biocalcification by *Bacillus pasteurii* urease: a novel application. J. Ind. Microbiol. Biotechnol. 36, 1111–1115. https://doi.org/10.1007/s10295-009-0581-4.

Sierra-Fernandez, A., Gomez-Villalba, L.S., Rabanal, M.E., Fort, R., 2017. New nanomaterials for applications in conservation and restoration of stony materials: A review. Materiales de Construcción 67, e107. http://dx.doi.org/10.3989/mc.2017.07616

Snoeck, D., Wang, J., Bentz, D.P., De Belie, N., 2018. Applying a biodeposition layer to increase the bond of a repair mortar on a mortar substrate. Cem. Concr. Compos. 86, 30–39. https://doi.org/10.1016/j.cemconcomp.2017.11.001.

Stabnikov, V., Ivanov, V., Chu, J., 2016. Sealing of sand using spraying and percolating biogrouts for the construction of model aquaculture pond in arid desert. Int. Aquat. Res. 8, 207–216. https://doi.org/10.1007/s40071-016-0136-z.

Cerâmica Torreense, 2009. Technical datasheet of ceramic bricks based on EN 771 standard (in Portuguese).

van Hees, R., Veiga, R., Slížková, Z., 2016. Consolidation of renders and plasters. Mater. Struct., 50, 65. https://doi.org/10.1617/s11527-016-0894-5.

Wang, X., Wiens, M., Schlobmacher, U., Jochum, K.P., Schröder, H.C., Müller, W.E.G., 2012. Bio-sintering/bio-fusion of silica in sponge spicules. Adv. Eng. Mater. 14, B4–B12. https://doi.org/10.1002/adem.201180059.

Wang, L., Wang, J., Xu, Y., Chen, P., Yuan, J., Qian, X., 2018. Novel surface treatment of concrete bricks using acid-resistance mineral precipitation. Constr. Build. Mater. 162, 265–271. https://doi.org/10.1016/j.conbuildmat.2017.12.019.

Xue, S.H., Xie, H., Ping, H., Li, Q.C., Su, B.L., Fu, Z.Y., 2015. Induced transformation of amorphous silica to cristobalite on bacterial surfaces. RSC Adv. 5, 71844–71848. https://doi.org/10.1039/c5ra13619a.