PARRACHA JL, PEREIRA AS, VELEZ DA SILVA R, ALMEIDA N, FARIA P. (2019), Efficacy of iron-based bioproducts as surface biotreatment for earth-based plastering mortars. Journal of Cleaner Production 237, 117803.
https://doi.org/10.1016/j.jclepro.2019.117803

Efficacy of iron-based bioproducts as surface biotreatment for earth-based plastering mortars

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ABSTRACT

The use of bacteria for the enhancement of construction materials has been a focus of study in the past few years. Microbial cells have been applied to consolidate the surface of different materials. Earthen plasters are eco-efficient building products that can be applied on new buildings but also used to protect cultural heritage structures, with several advantages. They are based on abundant, affordable and easy to obtain materials, but are vulnerable to water. New eco-efficient bioproducts were developed based on *E.coli* cultures supplemented with iron, that can be obtained as an industrial by-product. They were applied in laboratory on an earth plaster surface and the biotreatment efficacy

was assessed. The main objective was to improve the durability of the plastering mortar when exposed to water. An optimized *E.coli*-iron based surface biotreatment strongly increased the plaster resistance to water with the application of only 0.0625 mL/cm^2 of E.coli++Fe leading to a significant increase of water absorption time in comparison with similar plasters where the same amount of water was applied. Moreover, all biotreatments produced an increase of resistance to water absorption creating a slight waterproofing effect, ensuring compatibility. Their loss of efficacy with time guarantees reversibility of the intervention. The results show the interest to perform an in-depth study on the microstructure of biotreated earth mortars to understand the biotreatment effect.
Keywords: bioconsolidation; biomineralization; protection; compatibility; reversibility; eco-efficiency

1. Introduction

The use of bacteria for the enhancement of construction materials has been a focus of study in the past few years. With applicability to a wide variety of construction materials, from concrete (Khaliq and Ehsan, 2016; Achal et al., 2011; Wiktor and Jonkers, 2015) and cement mortars (Ersan et al., 2015; Sierra-Beltran et al., 2014), ceramic clay bricks (Raut et al., 2014; Sarda et al., 2009; Dhami et al., 2012) and earthen bricks (Dhami and Mukherjee, 2015) to limestone (Le Métayer-Levrel et al., 1999; Jroundi et al., 2010a; Jroundi et al., 2010b; Jimenez-Lopez et al., 2008) and gypsum plasters (Jroundi et al., 2014), it is a sustainable technique with great improvements on the properties of these materials (Achal et al., 2015). Construction-related biotechnologies have been summarized by Ivanov et al. (2015).

Bacterial cells are most commonly used to precipitate calcium-carbonate on the surface or on the matrix of materials through a biological response mainly denominated as microbially induced calcium carbonate precipitation (MICP). Two major types of applications rely on MICP: biotreatment and bioformulation. Biotreatment is a superficial treatment where bacterial culture is applied at the surface of the material. Bioformulation occurs when microbial cells are integrated on the matrix of the material, for instance as kneading liquid of concrete and mortars, acting as an additional binder, connecting the particles that constitute the material. Bioconsolidation may involve both techniques.

MICP biotreatments and bioformulations have been widely tested in cementitious materials: in concrete (Khaliq and Ehsan, 2016; Achal et al., 2011), to improve compressive strength and to protect the reinforcement; or in cement mortars (Ersan et al., 2015; Sierra-Beltran et al., 2014), improving water absorption resistance and compressive strength. Limestones, specially from ancient monuments, have been treated with bacterial cells that inhabit the stone or by adding new exogenous bacteria, depositing calcium-carbonate on the surface of the limestone (Jroundi et al., 2010). Both types of applications have shown great improvements. Cardoso et al. (2016) tested the durability of sand columns biotreated with *Sporosarcina pasteurii* cultivated in NH_4-YE medium, pH 9.0. The biotreatment was applied and then specimens were daily feeding with urea-calcium medium (0.5 M urea, 0.5 M $CaCl_2$, 2 g $(NH_4)_2SO_4$, 10 g NH_4Cl, 2.12 g $NaHCO_3$ in 1:10 diluted culture medium) for 10 days once a day. The top 5 cm of the specimens were cut, and water submersed for more than one month. About 3 cm of the bottom of the sample crumbled, while the upper 2 cm remained intact, showing the potential utility of the application of such biotreatment for the improvement of sand columns vulnerability to liquid water.

Besides MICP, iron oxide producing microorganisms also have potential and have already been tested in soils but, to the authors knowledge, not yet in construction materials. Naeimi et al. (2014) performed several tests with different iron-based bioproducts in sand, achieving similar results to the ones obtained with calcium-based bioproducts.

The effect of microbial activity on earth bricks and ceramic bricks has also been investigated, by mixing the brick constituents with bacteria cells or by treating the surface with bacteria (Dhami and Mukherjee, 2015; Raut et al., 2014). Despite the very promising results, further studies are necessary, particularly in more porous building materials like earth-based ones. Building with earth is an ancient technique that has regrown in the last decades especially due to eco-efficient concerns. The combination of these eco-friendly techniques, biotreatment and bioformulation, with low embodied energy earthen construction could bring excellent developments in a sustainable point of view (Porter et al., 2018).

Utilization of earth-based mortars as a main construction material dates at least from the Neolithic (Bruno et al., 2010), used to fill branch structures for sheltering. Earthen plasters also exist in cultural heritage properties, such as archaeologic remains. Also, earthen plasters are sometimes applied as sacrificial coatings to protect old earthen-based structures. Compatibility of the intervention and its reversibility are important aspects to be considered (van Hees et al., 2016).

Earth mortars are mainly constituted by sand and clayish earth. Both these components are easily assessible and no heavy industrial process is necessary for their preparation. Some earth plasters include vegetal fibers, reducing thermal conductivity but eventually intensifying their biosusceptibility, which seems to be directly influenced by surface roughness (Santos et al., 2017).

A high adsorption and desorption capacity in comparison with other plasters and gypsum boards used indoors (Faria and Lima, 2018) are one of the main advantages of earth mortars used as plasters. This ability leads to the control of relative humidity in living places, improving the comfort and even contributing to control health problems such as asthma and allergies (Lima et al., 2016a).

On the other hand, unstabilized earth mortars are vulnerable to liquid water and can suffer significant damage. Even though earth mortars can achieve the required mechanical and adhesive strengths to be applied as plasters, the same cannot be achieved regarding their resistance towards water. When in contact with water, erosion rapidly occurs and, if saturation is reached, earth mortars regain plasticity. Nevertheless, vulnerability to liquid allows them to be reused for the same purpose (Lima et al., 2016a), as a dynamic plaster that can be applied on different masonries (Santos et al., 2019).

In order to achieve higher durability, the additions of low binder contents, such as gypsum, lime or cement, and natural products, such as oils, fats or fibbers, have been used in the formulation of earth mortars (Lima et al., 2016a; Lima et al., 2016b; Eires et al., 2017).

Several studies have been performed in order to understand how different methods of earth mortar stabilization may affect their characteristics, specially using chemical binders (Gomes et al., 2018).

Although stabilizers might improve the durability of earth mortars, some lead to dissolution or strongly decrease of their main strengths: contribution to control indoor humidity, acting as a humidity buffer, and capacity to be reused after new kneading with water (Faria and Lima, 2018).

Another possibility to improve earth plasters and other earthen products durability to water and weathering is the application of surface treatments.

Stazi et al. (2016) tested 8 different types of earth mortar stabilization on commercially available products: 4 additions on formulation (barley straw, silicon nano-particles, organic derivates of silicon and limestone aggregates admixed with fatty acids and synthetic polymers) and 4 surface treatments (silicon nano-particles, titania and silica nano-particles, silane-siloxane and beeswax). Earth mortars with additives reached slightly lower compressive strengths than control mortars. Contact angle test showed that even if some improvements might be obtained by including additives on formulation, a good impermeability could only be obtained with the use of surface treatments. From the drip erosion test, only mortars where silicon nanoparticles or organic derivatives of silicon were used as additives and mortars with surface treated with silicon nano-particles or silane-siloxane showed no surface erosion.

Besides chemical stabilization, natural and eco-friendly forms of improvement and stabilization of earth mortars have also been a focus of study. As an eco-friendly construction material, earth mortars should be improved without discarding their sustainable nature.

Aguilar et al. (2016) studied the use of chitosan, a biopolymer obtained from shells of shrimp and other crustaceans, as an admixture and as a superficial treatment for earth mortars. Chitosan-earth mortars showed an increase of almost 100% on both compressive and flexural strengths, when compared with reference mortars. Contact angle in chitosan-earth mortars reached an average value of about 70º. Mortars' surface treated with chitosan presented a higher contact angle, reaching an average value of about 90º. Contact angle in reference earth mortars was nonexistent. On the drip erosion test, erosion was lower on mortars formulated with chitosan. The best improvements were obtained for chitosan treated surface earth mortars, with practically no erosion being observed, while earth mortars without treatment suffered instant degradation.

Other eco-friendly bioproducts, alkali activated and nanoproducts have been studied as treatments but for earthen blocks such as adobe. Camerini et al. (2019) tested the effect of a ternary treatment based on SiO_2 nanoparticles, $Ca(OH)_2$ nanoparticles and hydroxypropyl cellulose, dispersed in ethanol and water for the consolidation of earthen masonry (adobe) and achieved very positive results and an impressive reduction on weight loss by wet-dry cycles. Nakamatsu et al. (2017), testing carrageenan as a biotreatment of adobe, increased water impermeability and resistance to erosion by water drops. In some cases, the efficacy of those treatments is compared to more traditional ones, such as ethyl silicate. La Russa et al. (2019), applying ethyl silicate, potassium silicate, potassium hydroxide and nanolime by immersion and soaking of different earth samples simulating adobe, assessed their efficiency against dissolution in water. Results of weight loss due to contact with water suggested that the efficacy is related to the main characteristics of the earth used. Nanolime and NaOH solution showed very poor performance, while ethyl silicate and KOH were efficient. Therefore, the latter alkaline treatment has been studied as an alternative to ethyl silicate (Elert et al., 2019).

Despite already been tested in earth-based materials, to the authors knowledge the bioconsolidation effect, both by biotreatment or bioformulation, has been rarely studied in earth plastering mortars. This eco-efficient construction material may be further improved by using an eco-friendly new biotechnology. Furthermore, earthen-based materials have mostly been bioconsolidated using MICP technology (Porter et al., 2018), with no reports on the application of bioproducts that involve iron precipitation processes.

The present study focused on the development and optimization of a new bioproduct based on iron biomineralization using *Escherichia (E.) coli* cells grown in a rich culture medium supplemented with iron. The produced iron-based bioproducts were then tested on samples of an earth plastering mortar as a surface consolidant biotreatment. As unstabilized earthen materials are highly vulnerable to liquid water, the main objective of this research study was to improve the durability of the plastering mortar when exposed to water, while trying to maintain the plaster ecological and technical advantages.

2. Materials

2.1. Earth-based mortar

The earth mortar samples used in this research study were produced with a ready-mixed plastering product composed by clayish earth, siliceous sand and cut oat fibbers, from Embarro company (Portugal and Spain). The exact proportions of each component are not available. The clayish earth was extracted from the same clay quarry as the one used by Lima et al. (2016a), in Algarve, south region of Portugal. This ready-mixed product has been used and extensively studied by Faria et al. (2016) and particle size distribution, determined by dry sieving, and X-ray diffractograms are presented in Figures 1 and 2.

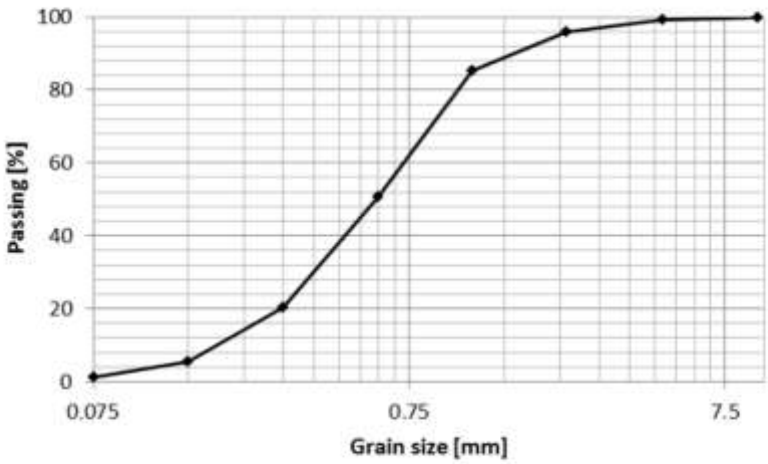
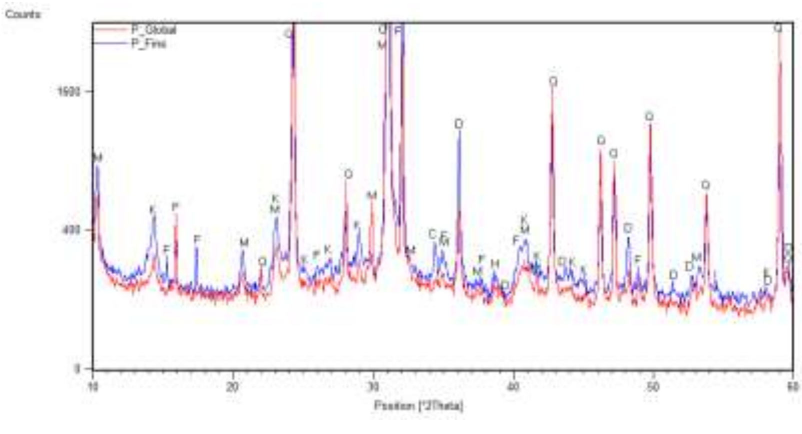


Figure 1 – Dry particle size distribution of the ready mixed product (Faria et al. 2016)



Q – quartz; F – K-fedspar; D – dolomite; M- illite; K – kaolinite; C – calcite; H – hematite.

Figure 2 – X-ray diffractograms of global and fine samples of the ready-mixed product (Faria et al. 2016).

The X-ray diffractograms show the presence of calcite ($CaCO_3$) and hematite (Fe_2O_3), among other compounds, in the earth mortar composition. These two constituents may be used by bacterial cells inhabiting the earth mortar or by the ones added with the biotreatment, supporting the bioconsolidation process.

Cubic earth mortar samples (40 mm × 40 mm × 40 mm) were cut from larger prismatic samples (40 mm × 40 mm × 160 mm) and used to test the selected biotreatments. The original prismatic samples were prepared utilizing a Putzmeister MP25 mixing and pumping equipment. The loose bulk density of fresh mortars, determined in accordance with EN 1015-6 (1999), was 1.17 kg/dm^3. For each selected biotreatment, three cubic earth mortar samples' replicates were tested.

2.2. Iron-based bioproducts and treatments

The bioproducts were produce using *E.coli* BL21(DE3) from NZYTech (Portugal), a well-characterized microbiological organism not known to consistently cause disease in immunocompetent adult humans and that presents minimal potential hazard to laboratory personnel and environment using standard microbiological practices (Biosafety in Microbiological and Biomedical Laboratories, 2009).

Bacteria cells transformed with plasmid pET21-c (Novagen) were cultured in LB (Lysogeny broth) medium, a nutritionally rich medium (10 g of tryptone, 5 g of yeast extract and 10 g of NaCl.pH = 7.0) containing 100 µg/mL of ampicillin, overnight at 37 ^{o}C with orbital shaking at 220 rpm. This culture was then used to inoculate 1 L of fresh LB medium (LB with 100 µg/mL of ampicillin) and grown in the same conditions. At an $OD_{600\ nm}$ of ~0.5, the culture was supplemented with 1 mM (bioproduct labeled as E.coli+Fe) or 5 mM (bioproduct E.coli++Fe) of acidic iron (II) sulfate ($FeSO_4.5H_2O$) and incubated for 3 h.

The production of the bioproduct does not generate any waste, since the whole microbial culture is used; it is not a high-energy or chemicals-intensive process. If industrial wastes, from iron and steel industries, are used as the iron source, the process will contribute to a circular economy. Contrarily to MICP, iron biomineralization does not produce toxic metabolites and the products (iron oxides minerals) are found in nature as raw materials or industrial wastes or by-products, and have a longer life-time than calcite crystals that are brittle (Ivanov and Stabnikov, 2016).

The effect of iron mineralization on the biotreatment was evaluated by adding iron ($FeSO_4 \cdot 7H_2O$) to bacterial cultures; two different iron concentrations were analyzed, 2 mmol/dm^3 and 5 mmol/dm^3. The effect of the presence of a protein from the ferritin family (Dps), that catalyzes fast iron biomineralization (fast production of iron oxides inside the protein inner cavity in the form of mineral) was also assessed. In this case *E.coli* Bl21(DE3) cells hosting the overexpression vector harboring the gene that codifies Dps protein from *Marinobacter hydrocarbonoclasticus* was grown as described by Penas et al., (2019).

This study consisted in two different phases. Firstly, four biotreatment products (LB, LB+Fe, E.coli+Fe, E.coli+Fe+Dps) were applied to test samples and compared with two types of controls (Control, H_2O) (Figure 3 and description in the next section). This was called as 1st phase and the details are described in the following section 3.2.1. For each treatment, three earth mortar replicates were used. This 1st phase consisted in the screening of experimental conditions to establish optimal conditions, that were subsequently used in the 2nd phase. Based on the output results of the 1st phase, biotreatments and testing conditions were optimized, namely, application method, volume applied and concentration of iron supplement, to be assessed on the 2nd phase. Here, nine triplicates of cubic earth mortar samples (plus Control) were tested (Figure 3).

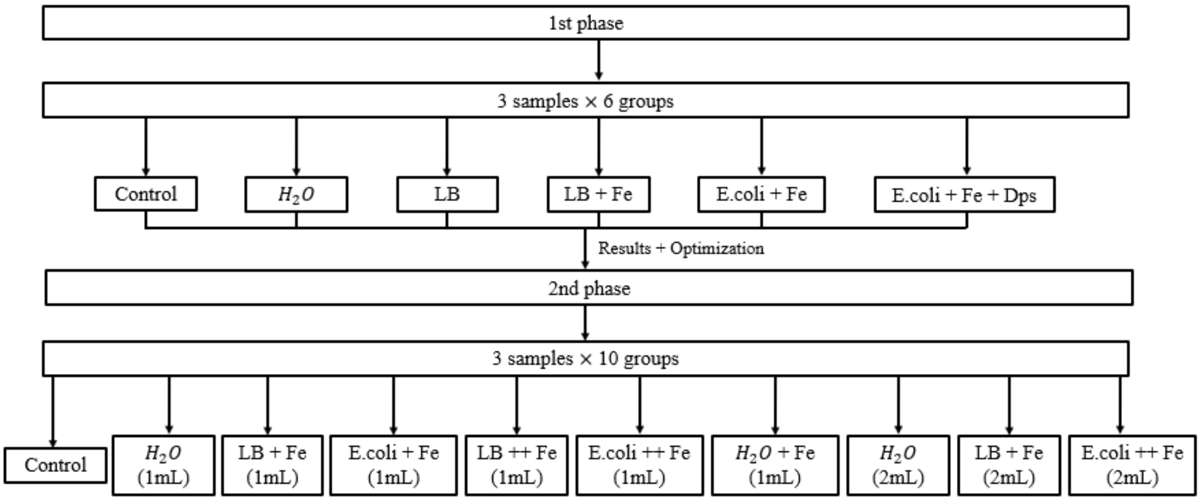


Figure 3. Application scheme of biotreatments to earth-based mortar samples.

All treatment products used in this study were applied as liquids in the upper surface of each selected sample (40 mm x 40 mm), in order to assess improvements obtained on surface hardness, surface cohesion and resistance to water absorption.

2.2.1. 1st phase

Before the 1st phase, a simple test was performed to gauge the appropriate volume of liquid biotreatment to be applied on the surface of each mortar, without risk of disintegration. Different volumes of water were applied to remnants from the cutting of prismatic samples, leading to a volume of 1 mL to be applied with a micropipette.

As stated before, four biotreatment products and two controls were treated in parallel (Figure 3):

- Control – non-treated earth mortar samples;
- H_2O – treatment of earth mortars with water;
- LB – biotreatment with LB medium;
- LB+Fe – biotreatment with LB medium supplemented with iron;
- E.coli+Fe – biotreatment with *E.coli* culture supplemented with iron;
- E.coli+Dps+Fe – biotreatment with *E.coli* culture expressing Dps, supplemented with iron.

Besides testing the effect of each biotreatment, percolation and dissipation at the surface and in-depth were also assessed. The biotreatments and water were applied on a central point of the samples surface, concentrating the biotreatment at the application point and surrounding area. After application of each treatment, samples were left to dry for 72 hours and fed with 1 mL of nutritive LB medium, or water, in the case of H_2O control group. Refeeding in the same conditions was performed daily for four consecutive days, according to the calendarization presented in Table 1.

At day eleven, a first experimental testing campaign was performed. After that, the same biotreatments were re-applied on the earth mortar samples and refed once more after 72 h. Then, two more experimental testing campaigns were conducted after the application of this second biotreatment (Table 1). Beside the experimental testing campaigns mentioned in Table 1, a 4th experimental testing campaign was performed 115 days after the last biotreatment, i.e. 130 days after the first biotreatment.

Table 1. Calendarization of the 1st phase.

1 Application of 1st biotreatment	**2**	**3**	**4** 1st day feeding	**5** 2nd day feeding	**6** 3rd day feeding	**7** 4th day feeding
8 5th day feeding	**9**	**10**	**11** 1st Testing	**12**	**13**	**14**
15 Application of the 2nd treatment	**16**	**17**	**18** Single day feeding	**19**	**20**	**21** 2nd Testing
22	**23**	**24**	**25** 3rd Testing			

2.2.2. 2nd phase

As stated before, and based on the results obtained in the 1st phase, the biotreatments were ameliorated and the following testing parameters were adjusted: application method, applied volume and iron concentration.

In the 1st phase, the application method of the biotreatment on a central point of the sample surface caused some damage. Because of that, in the 2nd phase all biotreatments were applied with a micropipette throughout the entire surface area, instead of being applied on a single central point. The treatments that obtained better results in the 1st phase were now tested in higher volume (2 mL application) and with higher concentrations of iron (five times more concentrated).

For this 2nd phase, ten different triplicates of earth mortar samples were tested with the following treatments:

- Control – non-treated samples;
- H_2O (1mL) – “treatment” with 1 mL of water;
- LB+Fe – biotreatment with 1 mL of LB medium supplemented with iron;
- E.coli+Fe (1mL) – biotreatment with 1 mL of *E.coli* culture supplemented with iron;
- LB++Fe (1mL) – biotreatment with 1 mL of LB medium supplemented with five times more concentrated iron;
- E.coli++Fe (1mL) – biotreatment with 1 mL of *E.coli* culture supplemented with five times more iron;
- H_2O+Fe (1mL) – treatment with 1 mL of water supplemented with iron;
- H_2O (2mL) – treatment with 2 mL of water;
- LB++Fe (2mL) – biotreatment with 2 mL of LB medium supplemented with five times more iron;
- E.coli++Fe (2mL) – biotreatment with 2 mL of *E.coli* culture supplemented with five times more iron.

The treatment procedure used in the 2nd phase was very similar to the second treatment applied on the 1st phase. After application of the bioproducts and controls, the samples were left to dry for 72 hours, and then one feeding was applied. Feeding was performed using the same volumes of the treatments. Treatment groups H_2O (1mL), H_2O+Fe (1mL) and H_2O (2mL) were fed with water while the biotreatment groups were fed with LB medium.

Testing was performed four days after the feeding and another 65 days after treatment application – the latter to assess the short-term durability of the treatment.

3. Methods

3.1. Visual and microscopic observation

Samples were visually observed for color change detection, film layer and eventual cracking. A binocular microscope was also used.

3.2. Surface hardness

Surface hardness test was performed according to ASTM D22240 (2000) using a PCE Shore A durometer, applicable to soft and rubber-like materials. It was evaluated for each treated surface of cubic earth mortar samples (40 mm x 40 mm) in a grid of 12 different points along the surface (Figure 4a).

All tests were performed on laboratory-controlled conditions with a temperature of 18-21 ºC and a relative humidity of 46 ± 5%.

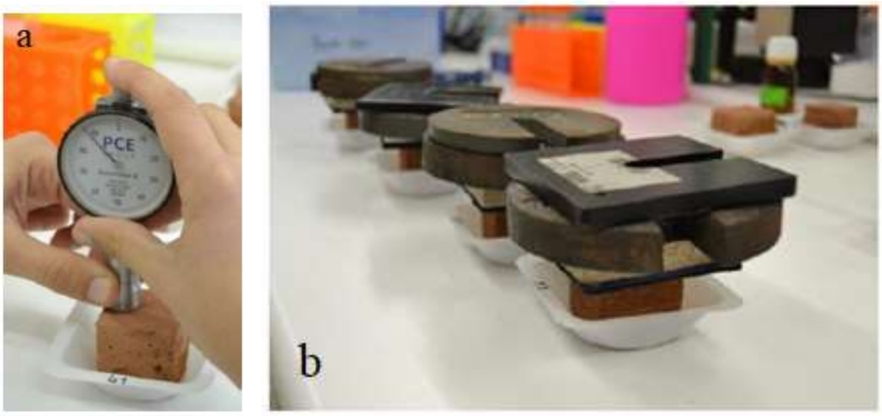


Figure 4. Surface hardness test (a) and surface cohesion test procedure (b).

3.3. Ultrasound transmission test

Ultrasound transmission test was performed according to EN 12504-4 (CEN, 2004) using a Proceq Pundit Lab equipment. The results from this test allows to evaluate the compactness of the sample and, therefore, the thickness of the applied biotreatment. The ultrasound test was performed in a direction perpendicular to the surface of the biotreatment application.

3.4. Surface cohesion

The surface cohesion test procedure was performed based on the method described by Drdácký et al. (2014) and adapted to earth plasters by Faria et al. (2016).

An adhesive tape was cut in pieces of 50 mm x 50 mm, weighted and placed on the treated surface of each sample, that was covered by a neoprene tissue. A weigh of 1.5 kg was positioned on the top of the tissue for 5 minutes (Figure 4b), allowing to perform the test with an equal applied pressure to all samples. Then, the weight and the tissue were removed, and the adhesive tape was peeled from the surface and weighted. The mass increase expresses the sample surface lack of cohesion. All weightings were performed on a scale with a precision of 0.0001g.

3.5. Water drop test

The water drop test is a simple test that allows to observe the behavior of the tested plastering mortars towards water ingress. It simulates the contact with water that can occur indoors in a plastered wall, although horizontally to include gravity force and increase the absorption rate. In this test (Figure 5a), a drop of water of 100 μL (0.1 cm^3) was dropped (height of 2 cm) on the surface of each sample and the time until the drop of water was totally absorbed by the sample was measured (Figure 5b). The whole procedure was video recorded. The surface was also visually observed to assess the degradation that the water drops produced in the samples.

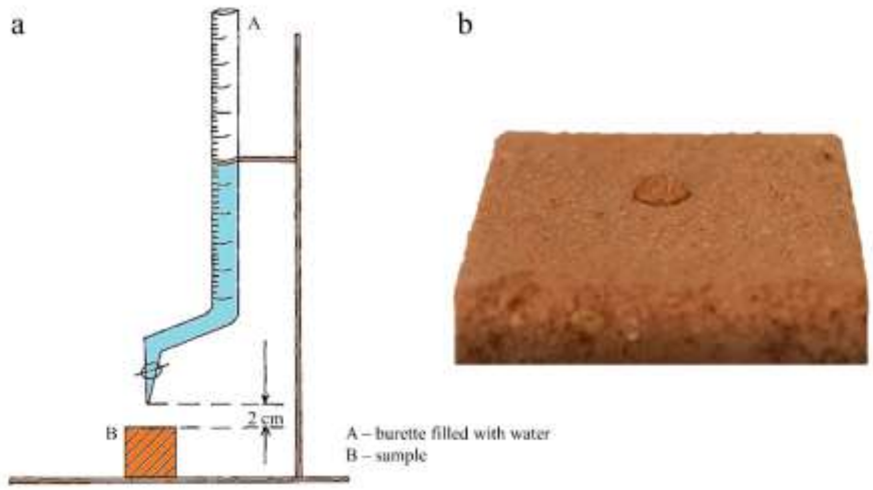


Figure 5. Scheme of the water drop test (a), adapted from RILEM (1980), and drop of water applied on a sample surface, previously treated with a bioproduct, during the water drop test (b).

4. Results and discussion

4.1. 1st phase

The plastering earth mortar has a reddish color, due to the presence of iron minerals in the clayish earth, very common in earth composite materials (Daoudi et al., 2017). No film layer, cracking or color change was visually detected on the treated surface of all samples.

Due to the application method used on the 1st phase, the surface of some samples was damaged on the central application point of the treatment, presenting a slight concavity.

Figure 6 presents the results for the surface hardness test. Here "1st Treatment" and "2nd Treatment" designations refer to the experimental testing campaigns performed 72 hours after biotreatments application (treatment + feeding).

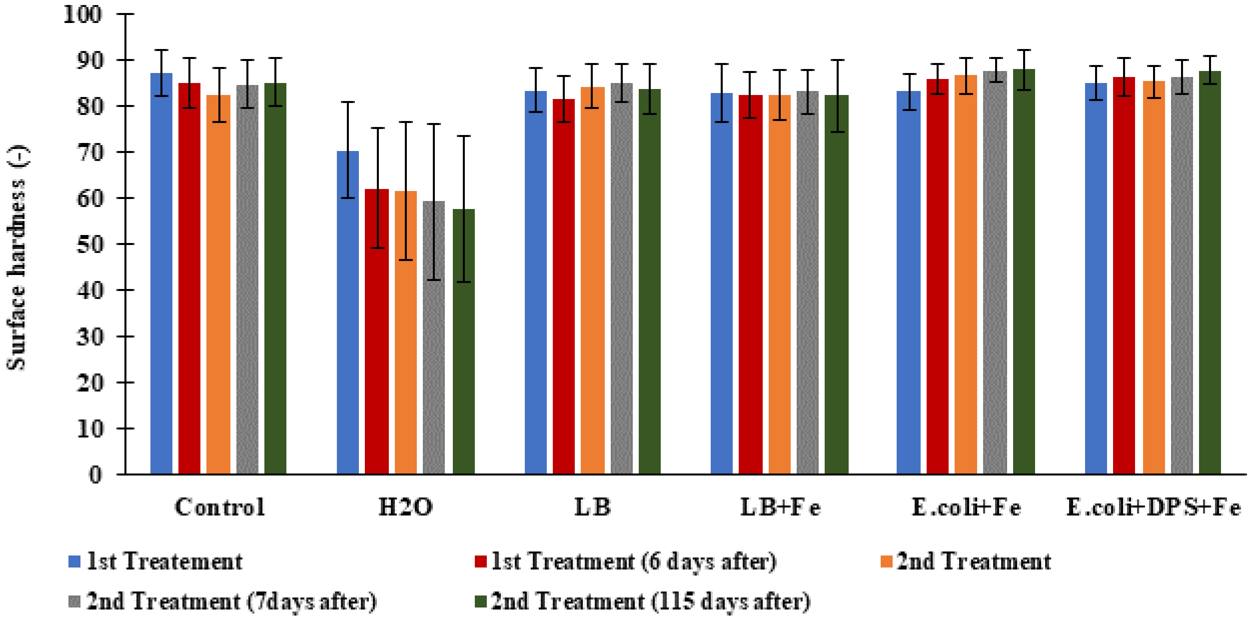


Figure 6. Surface hardness for the 1st phase.

Through analysis of Figure 6, it can be concluded that when compared with control samples, biotreated earth mortars presented no significant improvements on surface hardness, that proves compatibility of the biotreatment (van Hees et al. 2016). Within the experimental error, all treatments did not affect the surface hardness of samples, reaching values similar to control samples.

Results obtained for samples treated with H_2O, although with a very high standard deviation, demonstrate that such application led to a significant decrease of the surface hardness. Since all the applied bioproducts are aqueous suspensions, in comparison with the H_2O group results, a consolidation effect was created on the biotreated surfaces. This behavior is similar to the one obtained in previous studies for different building materials (Raut et al., 2014; Dhami et al., 2012; Cardoso et al., 2016), decreasing liquid water absorption without considerably affecting water vapor permeability.

The results of the ultrasound transmission test are presented in Figure 7. It is expected an increase in the ultrasound propagation velocity if the biotreatment resulted in an in-depth consolidation. If the biotreatment only reached the surface layer, without great impregnation, the results for the biotreated samples should be identical to the control ones.

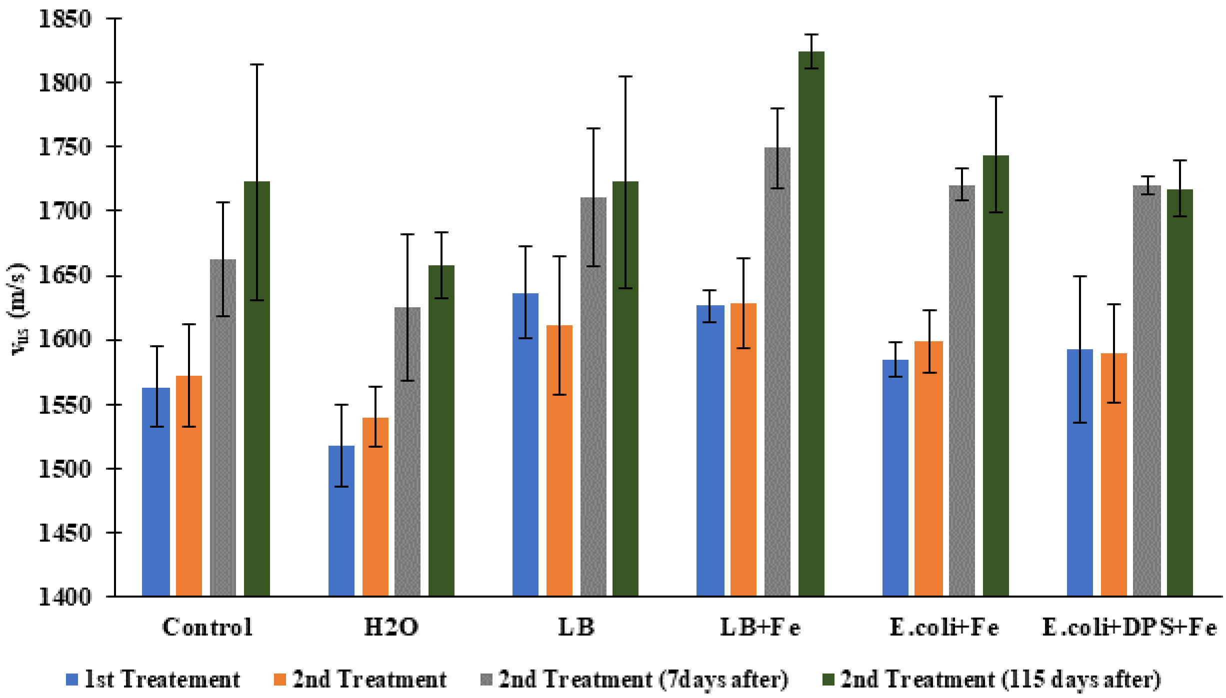


Figure 7. Ultrasound propagation velocity for 1st phase.

It can be observed that all biotreated samples presented higher ultrasound propagation velocity than control groups (H_2O and Control). Therefore, and following the same trend as surface hardness results, a slight improvement in compactness was observed for bioproducts treatment groups. As Figure 7 presents, an increasing ultrasound propagation velocity was obtained for all tested samples with time. This behavior may be explained due to a higher adsorption of water vapor by the earth mortar samples. In fact, a higher relative humidity in the laboratory when the test was performed

(45% to 55% RH) is most likely the reason for the increase between “2nd Treatment” and “2nd Treatment (7 days after)” results.

All adhesive tapes from the surface cohesion tests were re-attached to a white paper sheet (Faria et al., 2016). It was noticed a pattern on the adhesive tapes of the treated samples: a halo of loose particles around the application point had been formed, derived from the application method (Figure 8) and corresponding concavity on samples surface central application point.

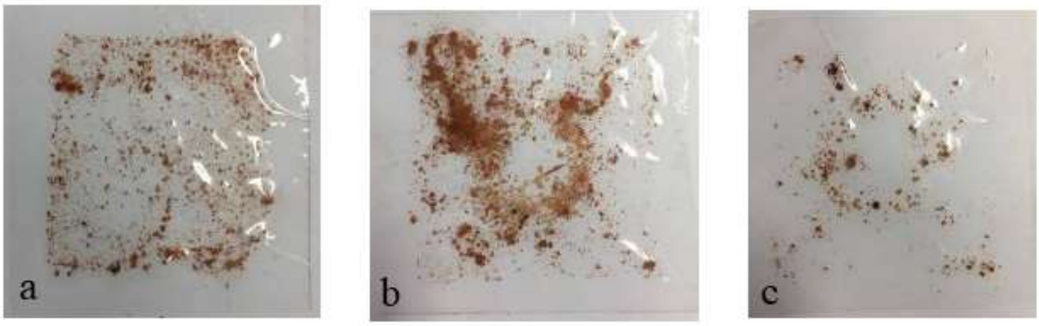


Figure 8. Surface cohesion adhesive tapes from 1st phase: (a) Control; (b) H_2O and (c) E.coli+Fe.

The obtained results for surface cohesion test are shown in Figure 9. It can be observed a very distinct pattern between the control and biotreated samples, with advantages for the latter. H_2O group samples presented almost the double mass loss than the other samples. E.coli+Fe group continue to show better results, with the lowest mass loss. Treatments LB and LB+Fe produced similar effects, while the presence of *E.coli* cells seems to result in a greater cohesion. The presence of the enzyme Dps, that catalyzes the formation of iron oxides inside its inner cavity, did not seem to have a positive impact, which could be attributed to the fast removal (oxidation) of ferrous iron in solution by the enzyme, reducing its availability (Penas et al, 2019).

After the second treatment performed in optimized experimental conditions, the biotreated samples presented no great improvements and the H_2O control group had similar behavior. For biotreated samples, the mass loss did not significantly increase, revealing a possible consolidation effect.

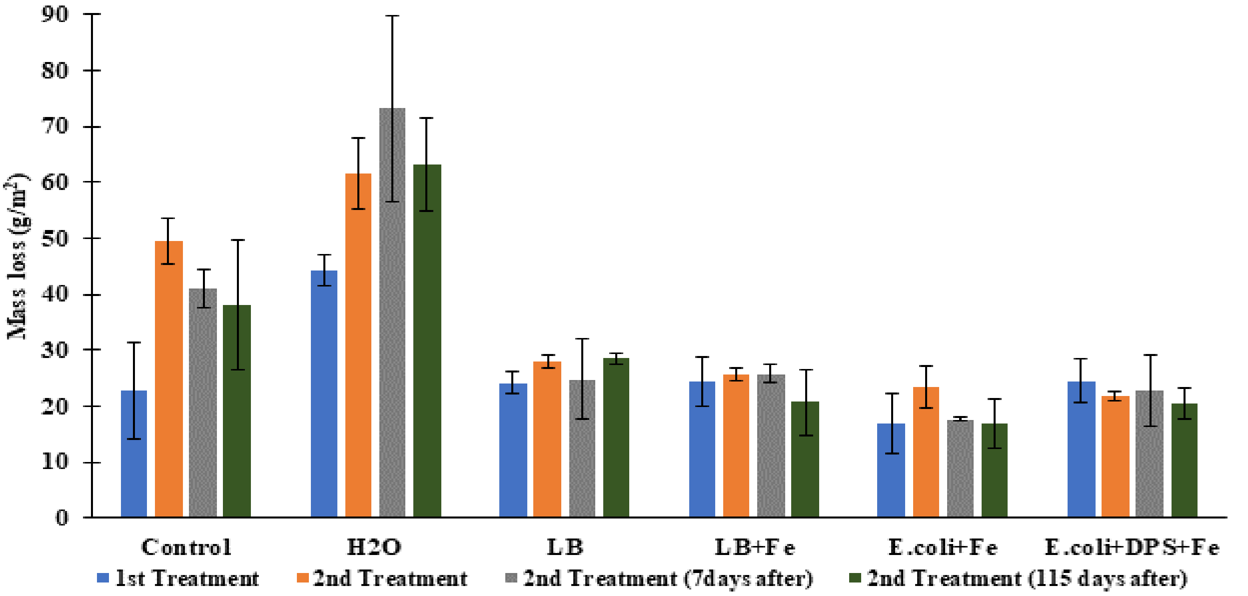


Figure 9. Mass loss due to lack of surface cohesion for 1st phase.

The water drop test exhibited the most evident results, especially after application of the second treatment. Significant differences were observed between the mortar treated groups (Figure 10).

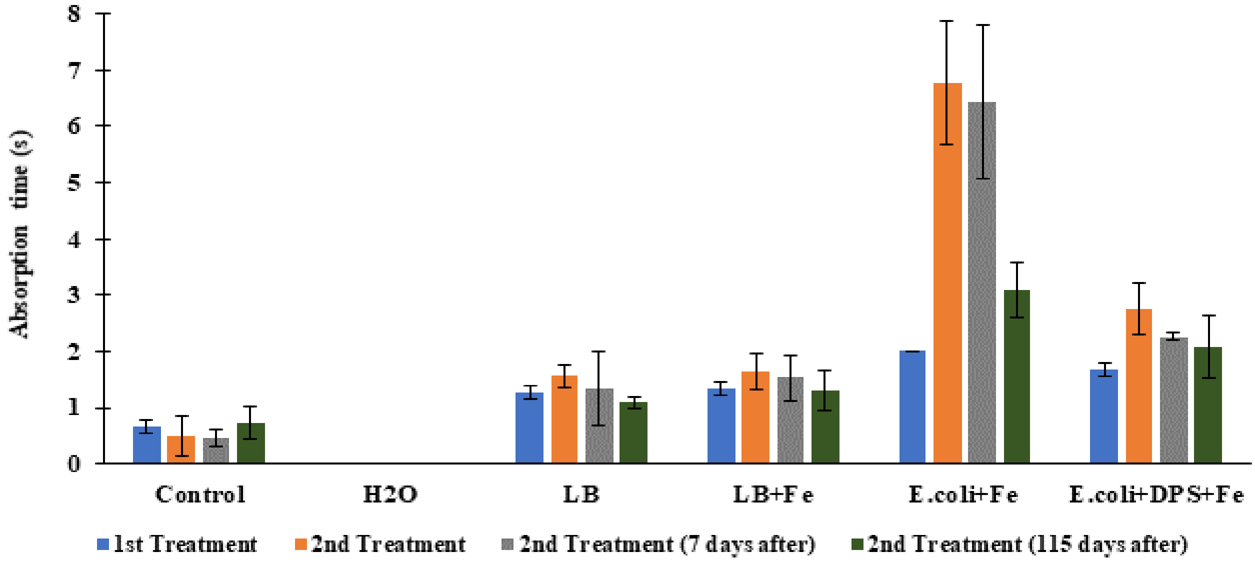


Figure 10. Water drop absorption time for 1st phase.

As it was expected, H_2O group samples presented lower absorption time values than the Control, untreated, group samples. While Control samples showed an absorption time of approximately 0.5 s, on the H_2O samples the water drop was instantly absorbed. After application of the first treatment all biotreated samples showed an increase on the water absorption time.

The water drop test performed after the second treatment showed that E.coli+Fe biotreated samples had an increase of more than 850% when compared to control samples. All biotreatments showed increases on water absorption drop time but the previous was by far the most noteworthy.

Tests performed 115 days after the treatment showed no significant difference when compared with other treatment, with exception of E.coli+Fe group, where time to water absorption considerably decrease but was still the highest. This behavior might be explained by the consecutive tests that damage the treated surface (Nakamatsu et al., 2017), that might be more explicit on the treatment that showed higher improvements. Nevertheless, these are very promising results that prove the durability of the treatments, as well as the reversibility of interventions, as the treatments ultimately loss their efficacy with time. The compatibility factor is also achieved since most commonly used bacterial cultures inhabit earth (Dhami, 2015; Porter et al., 2018) which contain iron-based materials.

4.2. 2nd phase

As mentioned before, after analysis of the results obtained for 1st phase, some parameters were adjusted for the 2nd phase. This 2nd phase started before the experimental campaign at 115 days of the 1st phase and, therefore, observation from this stage were not taken into consideration.

The 2nd phase treatments were performed taking into account the following considerations:

- The application method of the treatment was changed due the reasons mentioned above; instead of application on a central point, the bioproduct was applied to fully cover the surface.
- As no great differences were observed on the results from the "LB" group and "LB+Fe" group, "LB" biotreatment was not used on this 2nd phase but the effect of a higher iron concentration was analyzed.
- The use of Dps did not led to improved results in comparison with E.coli+Fe group. As an iron scavenger protein, Dps stores the iron, decreasing iron availability, which probably led to the decrease of the consolidation effect. Thus, E.coli+Fe+Dps group was not used in this 2nd phase.

- To infer about the effect of the amount of bioproduct on the properties of mortars, the applied volume was doubled to 2 mL. However, while treatments were applied, difficulties were felt related to the application of a larger volume of treatment on samples' surface.

4.2.1. Visual and microscopic observation and surface hardness

As in the 1st phase, no film layer, cracking or color change on the samples treated surface was assessed by visual observation. No alteration nor degradation due to the application method was observed this time (Figure 11a). Binocular microscope observation allowed to see the sand grains within the clayish matrix and the very porous structure of the earth plaster (Figure 11b).

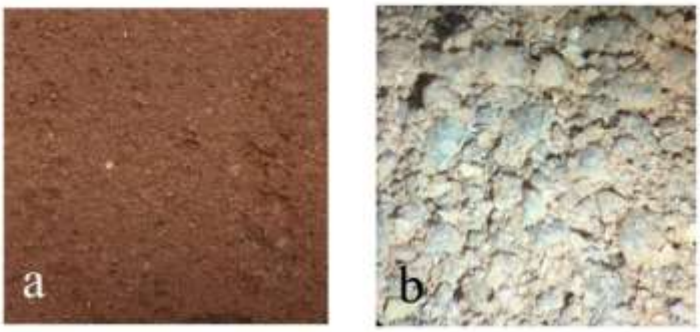


Figure 11. E.coli++Fe sample surface observation: (a) visual; (b) by binocular microscope.

The results of the surface hardness test are shown in Figure 12. The same 12 points grid used on the 1st phase was used here.

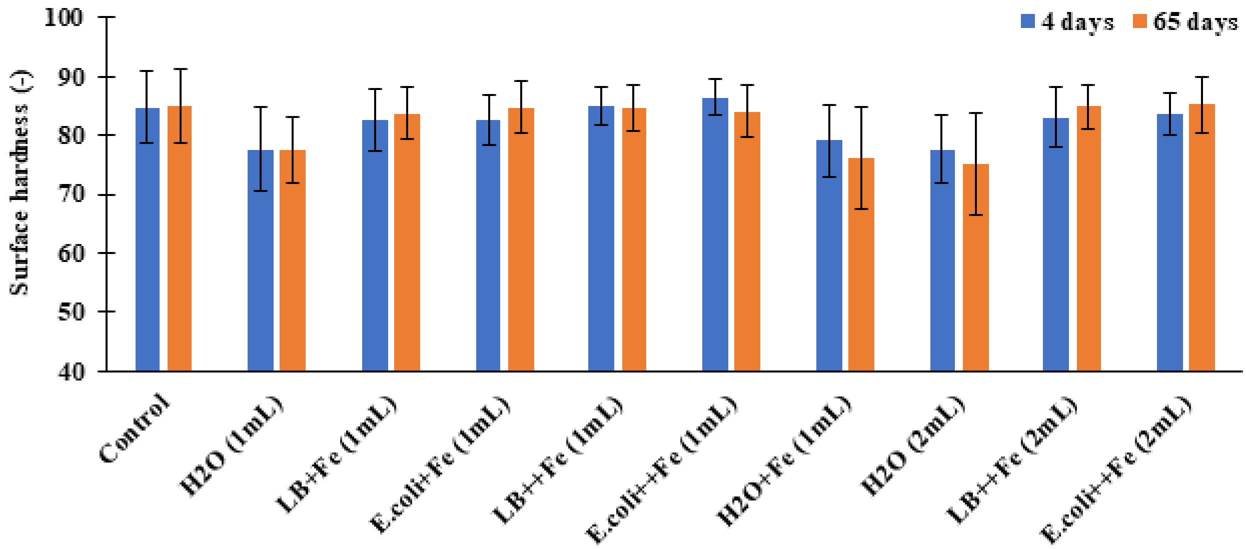


Figure 12. Surface hardness for 2nd phase.

In line of what was observed in the 1st phase, samples “treated” with water presented a slight decrease in surface hardness. All biotreated samples showed surface hardness results in the same range as the control samples (untreated mortar, therefore not damaged due to the application of a liquid), supporting the idea that with the application of the biotreatment on the surface, its hardness is maintained, assuring compatibility.

Globally, the surface hardness results obtained 65 days after the treatment did not present significant differences from the ones performed 4 days after the treatment. Some of the biotreated samples had their surface hardness decreased while others showed a slight increase, but no pattern was defined.

4.2.2. Ultrasound transmission

Regarding the 1st phase, the results of the ultrasound transmission test did not lead to significant conclusions, maybe due to low penetration depth of the treatments. For this 2nd phase, when 2 mL treatments were applied, a more in-depth consolidation might have been obtained, affecting the results of ultrasound transmission test.

Figure 13 presents the obtained results. Even if better results were obtained for LB++Fe (2mL) and E.coli++Fe (2mL) groups, almost all samples presented slightly higher ultrasound propagation velocity than the control samples. Control samples results were in the same range of the results obtained on the 1st phase, but all other biotreated groups registered higher values than the control. These results are somehow contradictory and are probably due to the hole created by the application method on the 1st phase, leading to misleading results; thus, the comparison is not possible.

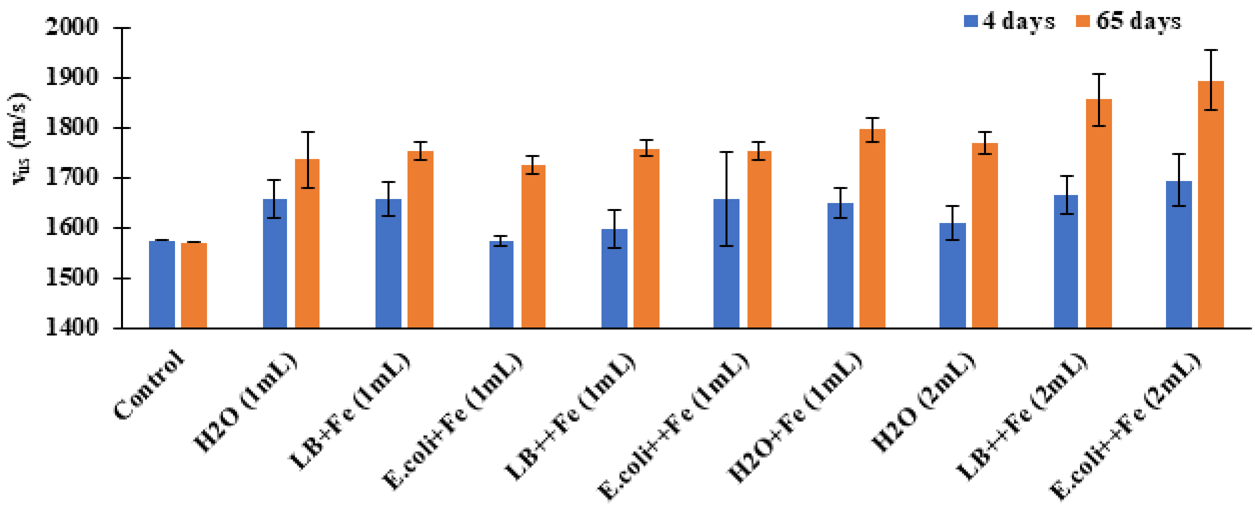


Figure 13. Ultrasound propagation velocity for 2nd phase.

Results for 65 days showed a slight increase in ultrasound propagation velocity in all samples, except for the control samples, that remain similar to the 4 days results. Control samples have shown an increase in ultrasound propagation velocity; those results could be justified by a slight increase in relative humidity (47% to 51%).

4.2.3. Surface cohesion

The obtained results for surface cohesion test are presented in Figure 14. Similar results to the 1st phase were observed: water "treated" samples presented lower surface cohesion and biotreated samples were in the same range of values for surface cohesion as control samples. As no concavity was visually observed, there were considerably less free particles on the samples' surface, being common to all samples tape a pattern of free particles distributed along the surface.

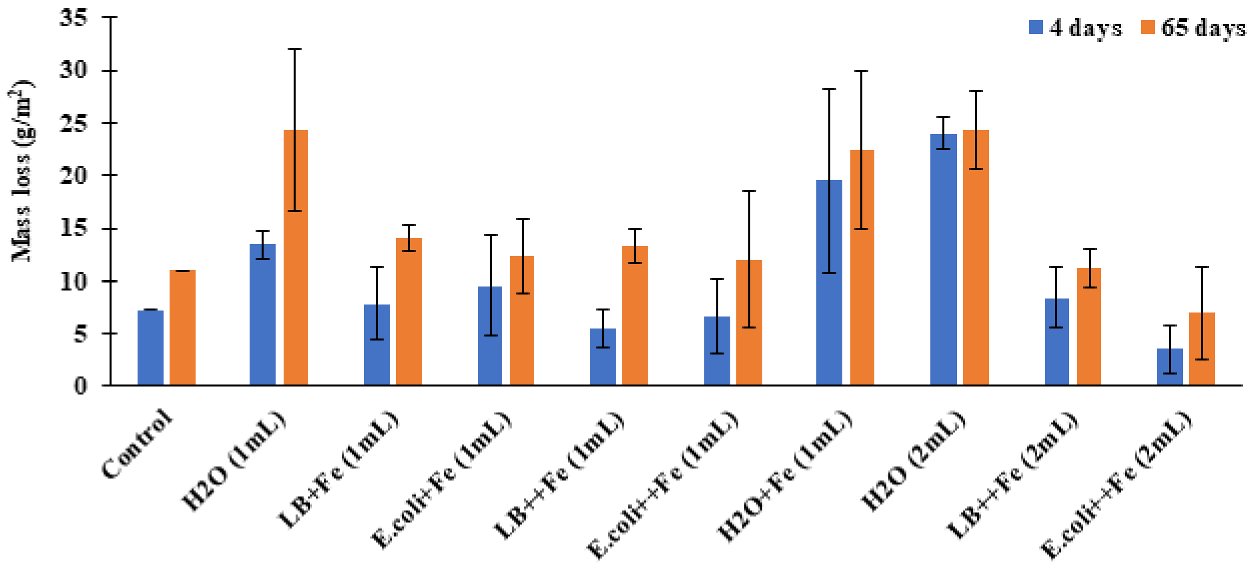


Figure 14. Mass loss due to lack of surface cohesion for 2nd phase.

It should be noted that E.coli++Fe (2mL) group presented, at both test ages, lower mass loss than the Control mortar. The consolidation effect obtained for this biotreatment is similar to the ones of the treatments based on native bacteria cells that inhabit the damaged limestone (Jroundi et al., 2010b) or with application of a bacterial *Myxococcus xanthus* culture to repair degraded limestone (Jroundi et al., 2010a).

Camerini et al. (2019) obtained increased surface cohesion for treated adobe samples and found that each component of the ternary treatment applied plays a major role for the consolidation process, that occurred due to the formation of calcium silicate hydrate (CSH), that reacts with silica and lime nanoparticles in the presence of water and HPC (ethanol solution of a commercial hydroxypropyl cellulose) and is the main responsible for the hardening process of cement (Ridi et al., 2011). In the present study, and when compared to the results obtained for surface hardness, an analogous effect occurs for surface cohesion with the biotreatment with higher iron concentration reaching higher surface cohesion. Since surface hardness and surface cohesion are related, the results support the suggestion that iron has an important role on the consolidating effect.

Higher mass loss was obtained in all samples in the test performed 65 days after treatment application. Even if these results can be attributed to a low durability of the treatment, the decrease in surface cohesion can be explained as degradation of the treated surface due to successive testing performed in each sample (for each phase), specially the surface hardness test (Nakamatsu et al., 2017; Camerini et al., 2019).

4.2.4. Water drop test

The results for the water drop test in this 2nd phase are presented in Figure 15. During feeding of samples, after treatment, before performing the water drop test, a resistance towards water absorption was evident on both E.coli++Fe (1mL) and E.coli++Fe (2mL) biotreatments. This slight waterproofing effect was later confirmed by the results obtained for these two groups (Figure 15).

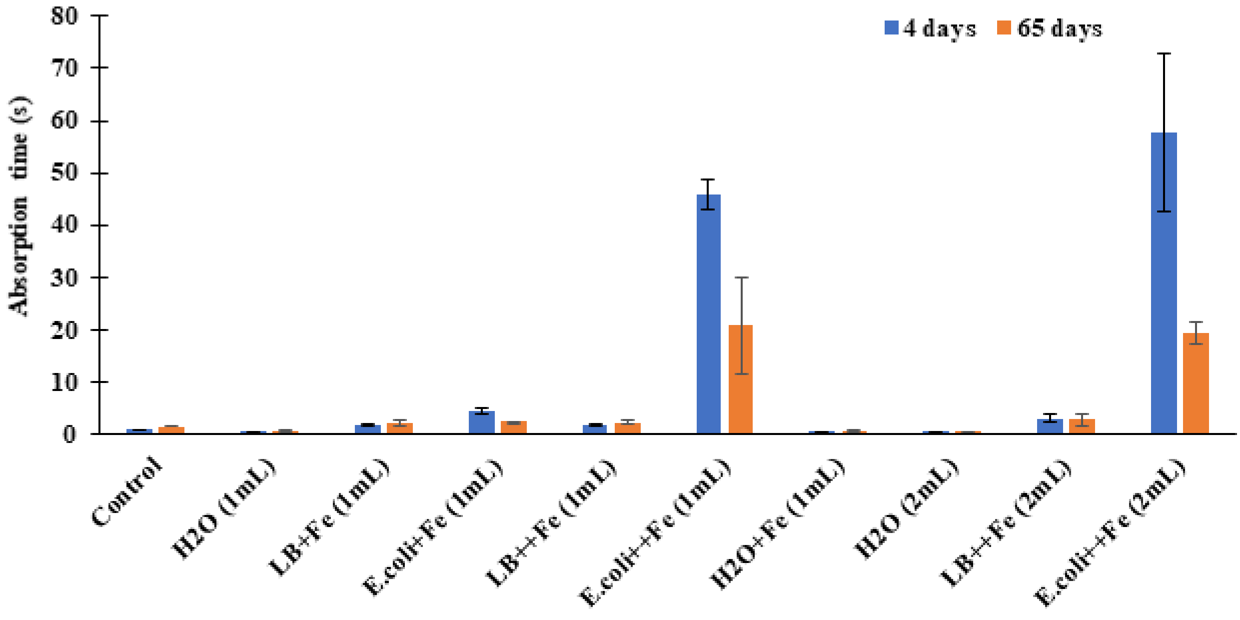


Figure 15. Water drop absorption time for 2nd phase.

As the feeding was being applied, the LB medium was hardly absorbed by the earth mortar, leading to the conclusion that the bioproduct itself was creating a slight waterproofing effect and thus the feeding process could not be necessary.

Despite iron concentration being one of the main triggers for a higher waterproofing effect, the presence of *E.coli* cells is also fundamental. Only *E.coli* culture based biotreatments had a significant increase in resistance towards water absorption, as it was previously observed on the 1st phase.

Results from the test performed 65 days after the treatment application showed a significant decrease in the waterproofing effect of E.coli++Fe (1mL) and E.coli++Fe (2mL) treatments. Nevertheless, still significant improvements were achieved. Like the results obtained for surface cohesion, a tendency to affirm that the treatment is not very durable rises with the results obtained for this test. But again, eventual damage on samples' surface produced by previous tests repeated in each test phase may partially justify this decrease. At the same time, reversibility of the treatment intervention is assured (Van Hees et al., 2016), what will be important when this type of treatments is applied on cultural heritage earthen plasters, such as archaeologic remains.

When the top 5 cm of the sand columns specimens biotreated by Cardoso et al. (2016) were cut, and water submersed for more than one month, about 3 cm of the bottom of the sample crumbled, while the upper 2 cm remained intact. That shows the potential utility of the application of such biotreatment for the improvement of sand columns vulnerability to liquid water but also that penetration is limited even for that type of material. All biotreatments applied in the present study using a different microbial culture produced a slightly waterproofing effect, particularly when the iron concentration used to supplement microbial cultures was increased. In comparison to results of Cardoso et al. (2016) and La Russa et al (2019), the penetration of the biotreatments of the present study is considered to be only superficial.

4.2.5. Summary

A summary of the qualitative results of the tested treatments on the 2nd phase and the percentage of water drop absorption time increase in comparison to the control samples, where the same amount of water was applied, are presented in Table 2. Two milliliter application of E.coli++Fe bioproduct on the 40 mm x 40 mm surface of the earth plaster presented the most effective results, both for water absorption time reduction and surface cohesion, although half that quantity (1 mL) was also very efficient in terms of resistance to water absorption.

Table 2. Qualitative comparison of the tested treatments and percentage of water drop absorption time increase in comparison to similar water treated samples.

Comparison with control specimens	Surface hardness	Surface cohesion	Resistance to water absorption (water drop)	Absorption time (s)	
				4 days	65 days
H_2O (1 mL)	↓	↓	↓	0.2	0.5
LB+Fe (1 mL)	↓	↓	↑	1.7	2.3
E.coli+Fe (1 mL)	-	↓	↑	4.5	2.4
Lb++Fe (1 mL)	-	↓	↑	1.8	2.2
E.coli++Fe (1 mL)	↓	↓	↑	45.8	20.9
H_2O+Fe (1 mL)	↓	↓	↓	0.2	0.4
H_2O (2 mL)	↓	↓	↓	0.2	0.4
LB++Fe (2 mL)	-	↓	↑	2.9	2.8
E.coli++Fe (2 mL)	-	↑	↑	57.9	19.4

Notation: negative results in red; positive results in green; results equal to control in black.

The obtained results are very promising since degradation by water is one of the major weakness of earth mortars. Nevertheless, it is their vulnerability towards water that provide earth mortars the capability of being reusable: for example if one wants to change the texture of an earth plaster, one just need to wet it, namely with a sponge, and finish the surface differently; on the other hand, if one wants to remove an earth plaster, the material can be reused to produce another earthen product. Therefore, when compared to the results of the commercially available treatments studied by Stazi et al. (2016), or with chitosan studied by Aguilar et al. (2016), that can reach a complete waterproof effect, these iron-based biotreatments reach inspiring improvements but without affecting the future re-use of the earth plasters. Only E.coli++Fe (2 mL) presented an slight increase in surface cohesion and no increase was noticed for surface hardness. Nevertheless, it can be expected that, with further optimization, the production of an eco-friendly bioproduct capable of increasing surface hardness and surface cohesion without affecting compatibility of the biotreatment is still possible to achieve.

It should be also mentioned that the bacterium *E.coli* is a common inhabitant of the bowel flora of healthy humans and other mammals. It is one of the most intensively studied prokaryotes, applied in biopharmaceutical industry and biotechnology in general. The used *E.coli* strain has the advantages of fast multiplication, reaching high densities in inexpensive media and is approved by FDA (Food and Drug Administration) for human applications namely for production of recombinant.

Without feeding, in starvation (where nutrients are depleted) *E.coli* culture enters stationary phase, followed by a death phase, in which about 99% of the cell population dies (Farrell and Finkel, 2003). So, when handled properly, the bioproduct described in the present study, should not present any risk for human health. Moreover, after production of the bioproduct in laboratory, cells can be collected by centrifugation and freeze-dried by lyophilization. Therefore, it will be possible to mix it with water in the construction or conservation site, just before application, assuring easiness of commercialization, transport and application. The production of the bioproduct involves the use of affordable culture medium and is not time consuming (24 h to growth a batch of 4 L at 37 ºC), since *E.coli* has a high multiplication rate (Farrell and Finkel, 2003).

5. Conclusions

In this study, an iron-based bioproduct was produced and applied as surface biotreatment for earth mortars, being the consolidative and waterproofing effects of the bioproduct assessed through an experimental campaign.

The main conclusions of this study are the following:

- All the water-based treatments (H_2O (1mL), H_2O+Fe (1mL) and H_2O (2mL)) degraded the surface of the earth mortar samples, decreasing hardness, cohesion and water resistance. These water-based treatments were the only ones in which a decrease in water resistance was observed; all biotreated samples showed an increase in water resistance.
- No increase in surface hardness was noticed in comparison with control, untreated, samples and only the non-pathogenic E.coli++Fe (2mL) showed an increase in surface cohesion. The fact that no significant increase was obtained assures compatibility of the biotreatment, particularly important for interventions on cultural heritage.
- The best results were obtained for the increase of resistance to water absorption as all biotreatments created a slight waterproofing effect.
- *E.coli* culture based biotreatments were the ones achieving the best results, in particular when the iron concentration used to supplement microbial cultures was increased. In this case, the resistance towards water absorption, assessed through the water drop test after 65 days, increased significantly in comparison with the similar plasters were the same amount of water was applied.
- With the application of only 0.0625 mL/cm^2 of E.coli++Fe (1 mL in the 40 mm x 40 mm plaster surface) a very impressive increase of water absorption time was achieved, reducing by half the consumption of the bioproduct in comparison with the even more efficient 0.125 mL/cm^2 application.

With these positive results the authors are encouraged to perform an in-depth study on the microstructure of biotreated earth mortars to understand the biotreatment effect, namely on different plastering mortars based on different earths. On the other hand, as contribution to hygrometric conditions equilibrium is one of the greatest advantages of earth plasters when applied indoors, a hygroscopic test should be performed to assess the sorption-desorption behavior of these biotreated mortars and assess the influence of the biotreatments. To infer about the durability of the biotreatment, a long-term testing campaign of the plasters should also be foreseen.

Acknowledgments

The authors acknowledge the support of the Fundação para a Ciência e Tecnologia (FCT) through research project PTDC/EPH-PAT/4684/2014: DB-Heritage, Database of building materials with historical and heritage interest. Part of this work was supported by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). Nídia Almeida is supported by the Radiation Biology and Biophysics Doctoral Training Programme (RaBBiT-PD/00193/2012; UCIBIO-UID/Multi/04378/2019 and CEFETIC-UID/FIS/00068/2013) and by a PhD fellowship from FCT/MCTES (PD/BD/106034/2015). Acknowledgements are also due to Dr. António Candeias and Hercules Laboratory of Évora University for the VP-SEM-EDS observations, and to Eng. Vítor Silva for the experimental support.

References

Achal V, Mukherjee A, Reddy M (2011), Effect of calcifying bacteria on permeation properties of concrete structures. Journal of Industrial Microbiology & Biotechnology 38, 1229-1234. http://dx.doi.org/10.1007/s10295-010-0901-8

Achal V and Mukherjee A (2015), A review of microbial precipitation for sustainable construction. Construction and Building Materials 93, 1224-1235. http://dx.doi.org/10.1016/j.conbuildmat.2015.04.051

Aguilar R, Nakamatsu J, Ramírez E, Elgegren M, Ayarza J, Kim S, Pando M and Ortega-San-Martin L (2016), The potential use of chitosan as a biopolymer additive for enhanced mechanical properties and water resistance of earth construction. Construction and Building Materials 114, 625-637. https://doi.org/10.1016/j.conbuildmat.2016.03.218

ASTM D2240 (2000) Standard test method for rubber property – durometer hardness. American Standard. West Conshohocken, PA.

Biosafety in Microbiological and Biomedical Laboratories (2009), 5th Edition. https://www.cdc.gov/biosafety/publications/bmbl5/index.htm (accessed 22 February 2019).

Bruno P, Faria P, Candeias A and Mirão J (2010), Earth mortars use on pre-historic habitat structures in Southern Portugal. Case Studies. Journal of Iberian Archaeology 13, 51-67.

Camerini R, Chelazzi D, Giorgi R, Baglioni P (2019), Hybrid nano-composites for the consolidation of earthen masonry. Journal of Colloid and Interface Science 539, 504–515. https://doi.org/10.1016/j.jcis.2018.12.082

Cardoso R, Pedreira R, Duarte S, Monteiro G, Borges H and Flores-Colen I (2016), Biocementation as rehabilitation technique of porous materials. New Approaches of Building Pathology and Rehabilitation 6, 99-120. http://dx.doi.org/10.1007/978-981-10-0648-7_5

Daoudi L, Rocha F, Costa C, Arrebei N and Fagel N (2018), Characterization of rammed-earth materials from the XVIth century Badii Palace in Marrakech Morocco to ensure authentic and reliable restoration. Geoarchaeology 33, 529-541. https://doi.org/10.1002/gea.21671

Dhami N, Reddy M and Mukherjee A (2012), Improvement in strength properties of ash bricks by bacterial calcite. Ecological Engineering 39, 31-35. http://dx.doi.org/10.1016/j.ecoleng.2011.11.011

Dhami N, Mukherjee A (2015), Can we benefit from the microbes present in rammed earth? Rammed Earth Construction, Ciancio & Beckett (Eds.), Taylor & Francis Group, London, 89-92. http://dx.doi.org/10.13140/2.1.3968.0326

Drdácký M, Lesák J, Niedoba K and Valach J (2014), Peeling tests for assessing the cohesion and consolidation characteristics of mortar and render surfaces. Materials and Structures 45, 1947-1963. https://doi.org/10.1617/s11527-014-0285-8

Eires R, Camões A and Julali S (2017), Enhancing water resistance of earthen buildings with quicklime and oil. Journal of Cleaner Production 142, 3281-3292. https://doi.org/10.1016/j.jclepro.2016.10.141

Elert K, Bel-Anzué P, Monasterio-Guillot L and Pardo S (2019), Performance of alkaline activation for the consolidation of earthen architecture. Journal of Cultural Heritage. https://doi.org/10.1016/j.culher.2019.03.004

Erşan Y, Silva F, Boon N, Verstraete W, Belie N (2015), Screening of bacteria and concrete compatible protection materials. Construction and Building Materials 88, 196-203. http://dx.doi.org/10.1016/j.conbuildmat.2015.04.027

EN 1015-6 (1999), Methods of test for mortar for masonry. Part 10: Determination of dry bulk density of hardened mortar. CEN, Brussels.

EN 12504-4 (2004), Testing concrete. Determination of ultrasonic pulse velocity. CEN, Brussels.

Farrell MJ and Finkel SE (2003), The growth advantage in stationary-phase phenotype conferred by *rpoS* mutations is dependent on the pH and nutrient environment. Journal of Bacteriology 185(24), 7044-7052. https://doi.org/10.1128/JB.185.24.7044-7052.2003

Faria P, Lima J (2018). Earth plasters (in Portuguese). Argumentum, Lisbon.

Faria P, Santos T and Aubert J-E (2016), Experimental characterization of an earth eco-efficient plastering mortar. Journal of Materials in Civil Engineering 28(1), 04015085-1/9. https://doi.org/10.1061/(ASCE)MT.1943-5533.0001363

Gomes MI, Faria P and Gonçalves TD (2018), Earth-based mortars for repair and protection of rammed earth walls. Stabilization with mineral binders and fibers. Journal of Cleaner Production 172, 2401-2414. https://doi.org/10.1016/j.jclepro.2017.11.170

Ivanov V and Stabnikov V (2016), Construction biotechnology: Biogeochemistry, microbiology and biotechnology of construction materials and processes. Springer International Publishing. http://dx.doi.org/10.1007/978-981-10-1445-1

Ivanov V, Chu J and Stabnikov V (2015), Basics of construction microbial biotechnology. In F Torgal, J Labrincha, M Diamanti, C Yu and H Lee (eds), Biotechnologies and Biomimetics for Civil Engineering, 21-56. http://dx.doi.org/10.1007/978-3-319-09287-42

Jimenez-Lopez C, Jroundi F, Pascolini C, Rodriguez-Navarro C, Piñar-Larrubia G, Rodriguez-Gallego M and González-Muñoz M (2008), Consolidation of quarry calcarenite by calcium carbonate precipitation induced by bacteria activated among the microbiote inhabiting the stone. International Biodeterioration & Biodegradation 62, 352-363. http://dx.doi.org/10.1016/j.ibiod.2008.03.002

Jroundi F, Bedmar E, Rodriguez-Navarro C and Gonzalez-Muñoz M (2010a), Consolidation of ornamental stone by microbial carbonatogenesis. Global Stone Congress 2010, Alicante, Spain.

Jroundi F, Bedmar E, Rodriguez-Navarro C and Gonzalez-Muñoz M (2010b), Conservation of carbonate stone by means of bacterial carbonatogenesis: evaluation of in situ treatments. International Symposium on the Conservation of Monuments in the Mediterranean, Patras, Greece.

Jroundi F, Gonzalez-Muñoz M, Garcia-Bueno A and Rodriguez-Navarro C (2014), Consolidation of archaeological gypsum plaster by bacterial biomineralization of calcium carbonate. Acta Biomaterials 10, 3844-3854. http://dx.doi.org/10.1016/j.actbio.2014.03.007

Khaliq N, Ehsan M (2016), Crack healing in concrete using various bio influenced self-healing techniques. Construction and Building Materials 102, 349-357. http://dx.doi.org/10.1016/j.conbuildmat.2015.11.006

La Russa MF, Rovella N, Ruffolo SA, Scarciglia F, Macchia A, Licchelli M, Malagodi M, Khalilli F, Randazzo L (2019), Consolidation of earthen building materials: a comparative study. Archaeological and Anthropological Sciences. https://doi.org/10.1007/s12520-019-00821-8

Le Métayer-Levrel G, Castanier S, Orial G, Loubière J-F and Perthuisot J-P (1999), Applications of bacterial carbonatogenegis to the protection and regeneration of limestones in buildings and historic patrimony. Sedimentary Geology 129, 25-34. http://dx.doi.org/10.1016/S0037-0738(99)00029-9

Lima J, Faria P and Silva A (2016a), Earthen plasters based on ilitic soils from barrocal region of Algarve: Contributions for building performance and sustainability. Key Engineering Materials 678, 64-77. http://doi.org/10.4028/www.scientific.net/KEM.678.64

Lima J, Silva S and Faria P (2016b), Earthen plasters: adition of linseed oil and comparison to current plasters (in Portuguese). 1º Congresso de Ensaios e Experimentação em Engenharia Civil, Lisboa, Portugal.

Naeimi M (2014), Biocementation of Sand in Geotechnical Engineering. PhD thesis, Nanyang Technological University, Singapore

Nakamatsu J, Kim S, Ayarza J, Ramírez E, Elgegren M, AguilarR (2017), Eco-friendly modification of earthen construction with carrageenan: Water durability and mechanical assessment. Construction and Building Materials 139, 193–202. https://doi.org/10.1016/j.conbuildmat.2017.02.062

Porter H, Blake J, Dhami NK, Mukherjee A (2018), Rammed earth blocks with improved multifunctional performance. Cement and Concrete Composites 92, 36-46. https://doi.org/10.1016/j.cemconcomp.2018.04.013

Pena D, Pereira AS and Tavares P (2019), Direct evidence for ferrous ion oxidation and incorporation in the absence of oxidants by Dps from *Marinobacter hydrocarbonoclasticus.* Angewandte Chemie International Edition 58, 1013-1018. https://doi.org/10.1002/anie.201809584

Phue J, Lee SJ, Trinh L and Shiloach J (2008), Modified *Escherichia coli* B (BL21), a superior producer of plasmid DNA compared with *Escherichia coli* K (DH5α). Biotechnology and Bioengineering 101(4), 831-836. https://doi.org/10.1002/bit.21973

Raut S, Sarode D and Lele S (2014), Biocalcification using *B. pasteurii* for strengthening brick masonry civil engeneering structures. World Journal of Microbiology and Biotechnology 30, 191-200. http://dx.doi.org/10.1007/s11274-013-1439-5

Ridi F, Fratini E and Baglioni P (2011), Cement: a two thousand year nano-colloid. Journal of Colloid and Interface Science 357:255-264. https://doi.org/10.1016/j.jcis.2011.02.026

RILEM Commission 25 - PEM - Protection and erosion of monuments (1980), Test II.8b – Water drop absorption. Recommended tests to measure the deterioration of stone and to assess the effectiveness of treatment methods. Materials and Structures 13:175-253. https://doi.org/10.1007/BF02473564

Santos T, Nunes L and Faria P (2017), Production of eco-efficient earth-based plasters: Influence of composition on physical performance and bio-susceptibility. Journal of Cleaner Production 167, 55-67 https://doi.org/10.1016/j.jclepro.2017.08.131

Santos T, Faria P and Silva V (2019), Can an earth plaster be efficient when applied on different masonries? Journal of Building Engineering 23, 314-323 https://doi.org/10.1016/j.jobe.2019.02.011

Sarda D, Choonia H, Sarode D and Lele S (2009), Biocalcification by *Sporosarcina pasteurii* urease: a novel application. Journal of Industrial Microbiology & Biotechnology 36, 1111-1115. http://dx.doi.org/10.1007/s10295-009-0581-4

Sierra-Beltran M, Jonkers H, Schlangen E (2014), Characterization of sustainable bio-based mortar for concrete repair. Construction and Building Materials 67, 344-352. http://dx.doi.org/10.1016/j.conbuildmat.2014.01.012

Stazi F, Nacci A, Tittarelli F and Munafò P (2016), An experimental study on earth plasters for earthen building protection: The effects of different admixtures and surface treatments. Journal of Cultural Heritage 12, 27-41. http://dx.doi.org/10.1016/j.culher.2015.07.009

Van Hees R, Veiga R, Slížková Z (2016), Consolidation of renders and plasters. Materials and Structures 50, 65. https://doi.org/10.1617/s11527-016-0894-5

Wiktor V and Jonkers H (2015), Field performance of bacteria-based repair system: Pilot study in a parking garage. Case Studies in Construction Materials 2, 11-17. http://dx.doi.org/10.1016/j.cscm.2014.12.004