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Effect of surface biotreatments on construction materials

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Abstract

Surface treatment technology is instrumental to construction material conservation and more specifically to preventing decay and improving durability. Surface treatments help protect and consolidate the built heritage against material damage, reducing repair and replacement costs. This study assessed the effect of two eco-friendly healing agents, one generated by iron-enriched Escherichia coli and the other by mixed microbial cultures that metabolise glycerol, a biodiesel processing by-product, to produce polyhydroxylalkanoates. Healing was monitored by measuring the water drop absorption rate in cement mortar, air lime mortar, ceramic brick, limestone, adobe and compressed earth block. The agents tested lengthened water absorption times in all the materials studied, confirming their efficacy as external repair treatments for construction materials.

Keywords: Eco-friendly bioproduct; Consolidation; *Escherichia coli*; Mixed microbial culture; Microbial induced precipitation; Water absorption; Architectural heritage conservation.

1. Introduction

The outdoor surfaces of the built heritage are continually exposed to air pollution and weathering, not to mention frequent extreme events associated with global climate change. The surfacing materials in place in such structures differ with construction type and date. Stone, earthen mortar and adobe are often found in archaeological sites. Ceramic brick and tile, lime mortar and stone commonly appear in Roman sites dating back more than 2000 years, for instance. Those three materials were routinely used to surface structures up to the first half of the twentieth century, after which new buildings began to be clad in cement-based mortars and concrete. Although ceramic brick and tile continued to be used while lime mortars disappeared, all three are elements essential to the culture and economics of today's built heritage (Figure 1).

Figure 1. Envelope materials in the Portuguese built heritage: A, earthen mortar at Roça do Casal do Meio, a pre-historic site near Sesimbra; B, ceramic brick masonry ceiling in Palácio Vale Flor, Lisbon; C, concrete façade on Gulbenkian Foundation headquarters, Lisbon

Construction material porosity governs the degree of natural and accidental surface ageing and decay. Hence the significant role of consolidation in performance and durability. Even in exposed concrete, one of the least porous built heritage surfacing materials, porosity is a key to service life [1, 2].

Surface treatment is a cost-effective approach to improving construction material quality and durability. Inorganic substances such as sodium silicate, tetraethoxysilane and other nano-silica based components are being used to induce a substantial decrease in water absorption of concrete and cement mortars [3-

8]. Despite concerns around their poor fire resistance and limited service life, organic substance-based treatments are also applied for their effective interaction with and concomitant protection of cementitious substrates [9, 10]. Chandra et al. [11] reported improved water resistance in Portland cement mortar surfaces treated with eco-friendly products such as cactus extract.

Although sodium and potassium silicates have been applied to stone elements to lower the risk of salt damage due to soluble carbonate salt formation, their effect has been observed to be limited due to silica gel formation and their slow in-air reactivity [12]. Fluorosilicates have been seen to raise strength [13], adversely impacting compatibility with old materials and therefore construction material conservation. Acrylates have been shown to bond well and to be highly resistant to ageing [14-16], while the application of barium and calcium hydroxides to limestones has also yielded promising results [17]. Calcium oxalate, another option, has been ruled out due to the toxicity associated with recurrent exposure [18]. All the treatments mentioned are characterised by shallow penetration.

Tetraethoxysilanes (TEOS) are the consolidants most widely used in stone, including limestone, conservation [12, 19, 20]. Some researchers [21-23] have nonetheless contended that TEOS-based products exhibit poor chemical and mechanical compatibility with calcareous substrates and in some cases low durability and effectiveness. Phosphate-based treatments such as calcium phosphate have been proposed in the last 20 years as limestone consolidation agents that elude some of the drawbacks attributed to tetraethoxysilanes, such as lengthy curing times and short-lived water repellence [24, 25]. Nanolime consolidation efficacy and physical compatibility with coarse porous calcareous materials have been verified [26]. More recently, biodegradable polymers (polylactic acid and polyhydroxybutyrate) have been used as limestone consolidants with promising results [15]. Both Borsoi et al. [26] and Taglieri et al. [27] showed the efficacy of nanolimes for treating air lime mortars. Graziani et al. [28] reported the beneficial effect of an ammonium phosphate solution on porous limestone consolidation, while Arizzi et al. [29] analyzed the efficacy of calcium hydroxide nanoparticle sprays for improving mortar carbonation and compactness.

In general, the treatment of ceramic (fired clay-based) construction material surfaces with acrylic polymers [30, 31] and other synthetic materials such as methyl silicone resin, silicone spray and alkyl-alkoxy-silanes [32] proved to raise water repellence only slightly. Sarda et al. [33] and Raut et al. [34] reported better results with *Sporosarcina pasteurii* urease-induced biocalcification of brick masonry. Earthen (unfired clay) based construction materials have been successfully waterproofed with silane-siloxane [35], chitosan biopolymer [36] and carrageenan [37]. Tests with nanosilica sprays, in contrast, revealed no significant post-application change in water absorption [38]. Whilst some market tetraethoxysilanes (Silbond 40, Funcosil SAE 300E) have been found to be promising potential consolidants, others (Conservare OH100 and Funcosil Antihygro) have been observed to be detrimental [39]. The effects of a selection of surface treatments on construction materials are summarised in Table 1.

Table 1. Effect of several surface treatments of cement-based, lime-based and earth-based materials, stone and ceramic on water absorption

Material	Treatment	Water absorption decrease (%)	Reference
	Cement-based materials		
Cement paste	Nano-SiO_2	1-20	[4]
	Tetraethoxysilane	2-33	
Cement mortar	Cactus extract	83	[11]
	Nano-SiO_2	5-8	[3]
	Tetraethoxysilane	29-50	
	Silane with TiO_2 and SiO_2	10	[8]
Concrete	Tetraethoxysilanes	400-700	[7]
	Stone		
Limestone	WackerOH100	59	[19]
	Paraloid B-72	110	
	Paraloid B-44	79	

	Tetraethoxysilanes	163	
	Polyhydroxybutyrate	40	[15]
	Poly-L-lactide	90	
	Paraloid B-72	80	
	Hydroxyapatite	2	[24]
	Tetraethoxysilane	100	
	Hydroxyapatite	-1	[25]
	Tetraethoxysilanes	3	
	Nanolime	37-42	[26]
Lime and earth renders and plasters			
Air lime mortar	Nanolimes	60	[27]
		40	[26]
	$Ba(OH)_2 \cdot 8H_2O$	35700	[40]
	$Ca(OH)_2$	0	
Earth mortar	Nanosilica dispersions	3	[38]
	Silbond 40 in a 1:1 solution with ethanol	82	[39]
	Conservare OH100	30	
	Funcosil SAE 300E	65	
	Funcosil Antihygro with Funcosil SAE 300E	72	
	Derivates of silicon	-16	[35]
	Silane/Siloxane	100	
	Fatty acids + Synthetic polymers	28	
	Aqueous beeswax emulsion	39	
Ceramic			
Ceramic tile	Paraloid B-72	28-99	[31]
	Paraloid B-72	95-100	[30]
	Tetraethoxysilane	75-99	
	Polidimetilsiloxane	98-100	
Ceramic brick	Methyl silicone resin	8	[32]
	Silicone dispersion solution	9	
	Alkyl-alkoxy-silaneoligomer	18	
	Sporosarcina pasteurii + OptU	48.9	[34]
	Sporosarcina pasteurii + Nutrient Broth	19.9	
	Sporosarcina pasteurii + Brain Heart Infusion	44.6	[33]
	Sporosarcina pasteurii + Nutrient Broth	14.0	

A review of the literature revealed that a number of exposed heritage construction materials have been surface treated with different products, which appears to be a promising approach, particularly where the products used are eco-friendly, affordable, compatible and effective. The pursuit of innovative bioproducts compatible with existing materials and able to improve surface quality by consolidating incipient cracks and porous substrates would appear to be an ecologically and economically beneficial strategy. Healing, contributing to external repair, reducing the cost of maintaining aged materials and enhancing construction industry sustainability would be among the advantages. This study explored innovative eco-friendly bioproducts produced by *Escherichia (E.) coli* and mixed microbial (MMC) cultures using spent glycerol, an industrial waste, as a substrate, to generate surface treatment bioproducts able to reduce water absorption in the construction materials normally found on exposed built heritage surfaces.

2. Materials and methods

2.1. Experimental materials

The healing effect of the bioproducts tested in this work was firstly tested on samples of adobe, compressed earth blocks (CEB) and air lime mortar. These are common materials of architectural and even archaeological heritage, that have low resistance to water and, therefore, with increased needs of consolidation to improve its durability. Due to the encouraging results obtained with the aforementioned construction materials, the study was extended to other, more resistant, construction materials, also very frequent on architectural heritage, such as ceramic brick, limestone and cement mortar. The last-mentioned construction material, although commonly used since the 20th century, is nowadays showing significant repair needs.

Both air lime and cement mortars were produced with a siliceous sand from Abrantes, Portugal with a bulk density of 1.39 kg/dm^3 and a fineness modulus of 3.33.

Cement mortar was produced with a cement CEM II/A-L-32.5 N [41], from Secil Group, Portugal, with a loose bulk density of 1.18 kg/dm^3. The mortar was formulated with a cement:sand mass proportion of 1:1.9, that corresponds to a volumetric proportion of 1:3. The lime mortar was produced with a CL90-S powder hydrated air lime [42], from Lusical, Lhoist Group, Portugal, with a loose bulk density of 0.36 kg/dm^3 for air lime. The mortar was formulated with an air lime:sand mass proportion of 1:1.5, that corresponds to a volumetric proportion of 1:3. For both mortars, 40 mm x 40 mm x 160 mm samples were produced in metallic molds and cured for one year at laboratory conditions before specimens were prepared. Fired ceramic bricks from Cerâmica Torreense, Portugal, classified as category II, HD, based on EN 771-1 [43] were used. The limestone was from a quarry from Sesimbra Region, Portugal. Cubic specimens with 40 mm x 40 mm x 40 mm were cut from all previous materials by sawing with a diamond saw blade.

The adobe was non-industrially produced in Oficinas do Convento, with 70 mm x 160 mm x 320 mm, with earth from Herdade da Adua, Alentejo, Portugal. The earth used for its production is characterized in Table 2 and for production a plastic mix of earth and water was just molded. Compressed earth blocks (CEB), with 90 mm x 140 mm x 295 mm, were produced with two local earths from Monte de Caparica, Portugal: 50 % of a silty earth without coarse aggregates and 50 % of coarse sandy soil. The earths had a very low content on clay and, therefore, the CEB were stabilized with the addition of 5% of Portland cement CEM II/A-L-32.5 N [41], from Secil Group. For CEB production a manual press was used, filled with the moistened mix of earth and binders. No humid curing to optimize hydration was performed to achieve a low strength CEB. The CEB characterization was performed by Ribeiro et al. [44]. The adobe and CEB samples were cut with 40 mm x 65mm x 65 mm by sawing with a diamond saw blade. All samples were dried for 24 h in an oven at 60 °C before tested (Figure 2).

Table 2. Adobe earth characterization [45]

Earth constitution				**Atterberg limits**		
Gravel (%)	**Sand (%)**	**Silt (%)**	**Clay (%)**	**Liquid limit (%)**	**Plastic limit (%)**	**Plasticity index (%)**
1.5	50.6	17.9	30.0	26.47	21.76	5

Figure 2. Materials samples before surface treatment. From left to the right: air lime mortar, cement mortar, limestone, ceramic brick, adobe and CEB.

Except for adobe blocks and CEB that would disintegrate when immerged in water, materials were characterized by water absorption after 24 h immersion in tap water, based on EN 772-21 [46]. All materials were characterized by dry bulk density, based on the dry mass and geometrical dimensions. Results are presented in Table 3.

Table 3. Water absorption after 24 h immersion and dry density of the materials

Material	Cement mortar	Limestone	Air lime mortar	Ceramic brick	Adobe	CEB
Water absorption (%)	7.8 ± 0.2	3.7 ± 0.2	12.1 ± 0.1	10.3 ± 0.2	-	-
Dry density (kg/dm^3)	1.95 ± 0.2	2.38 ± 0.7	1.60 ± 0.3	2.00 ± 0.5	1.67 ± 0.6	1.81 ± 0.6

For each construction material triplicate specimens were prepared and tested. To simulate degraded surfaces, a cut surface of samples (40 mm x 40 mm) of all materials was treated, with exception of adobe and CEB samples, for which a mold surface was treated since the cut surface was very irregular. Samples were located on a test room one week before biotreatments to establish uniform laboratory conditions (20 ± 2 °C and 40 ± 5% relative humidity).

2.2. Bioproducts

The bioproducts used as treatment agents were divided into two different groups. The first group included products obtained from *Escherichia coli* BL21(DE3) cultures [47]. Several *E. coli*-based bioproducts were produced and tested for their waterproofing effect when applied as a surface treatment. The effect of different experimental conditions was also assessed, namely, supplement of bacterial culture with iron (5 mmol/dm^3 $FeSO_4{\cdot}7H_2O$), processing such as centrifugation and resuspension, storage conditions of bioproduct suspension (4 °C, −20 °C) and application method (capillarity and dropping). A second group of products was obtained using waste biomass from mixed microbial cultures (MMC) for polyhydroxyalkanoates production process, grown in tap water plus crude glycerol (biodiesel by-product) [48]. MMC whole cells suspensions (here designated MMC) and after sonication for disruption of cell walls (MMC_S) suspensions were used as bioproducts to treat the surface of the different construction materials. Effect of MMC-based bioproducts concentration was also evaluated: low (1), medium (2) and high (3) concentration, respectively. For comparison, different control samples were prepared: i) untreated; ii) specimens in which the bioproduct was replaced by the same volume of tap water; iii) samples treated with the same volume of aqueous iron solution; and iv) samples treated with the culture medium supplemented with iron used to growth *E. coli* cells. A summary of all biotreatments and controls is presented on Table 4.

Table 4. Description and short names of bioproducts/biotreatments and controls

Treatment	**Short name**
Controls	
Control (no treatment)	Control
Reference (tap water)	H_2O
5 mM Fe solution in water	H_2O +Fe
Luria Broth with 5 mmol/dm^3 Fe solution	LB+Fe
E. coli bioproducts	
E. coli culture (2.0 g/L)	EC
E. coli culture supplemented with 5 mmol/dm^3 Fe	EC+Fe
E. coli culture supplemented with 5 mmol/dm^3 Fe applied to samples by capillarity	EC+Fe (↑)
E. coli culture supplemented with 5 mmol/dm^3 Fe after centrifugation and resuspension in water	EC+Fe (?)
E. coli culture supplemented with 5 mmol/dm^3 Fe stored at 4 °C for 48 h	EC+Fe (4°C_48h)
E. coli culture supplemented with 5 mmol/dm^3 Fe stored at −20 °C for 48 h	EC+Fe (-20°C_48h)
MMC-glycerol bioproducts	
MMC grown with crude glycerol, low concentration (0.39 g/L)	MMC_Gly_1
MMC grown with crude glycerol, medium concentration (0.59 g/L)	MMC_Gly_2
MMC grown with crude glycerol, high concentration (1.18 g/L)	MMC_Gly_3
MMC grown with crude glycerol after sonication, low concentration (0.25 g/L)	MMC_Gly_S1
MMC grown with crude glycerol after sonication, medium concentration (0.38 g/L)	MMC_Gly_S2

MMC grown with crude glycerol after sonication, high concentration (0.76 g/L)	MMC_Gly_S3

As the bacterial cultures were applied to the materials directly, they continued to generate polyhydroxyalkanoates after application. In the absence of controlled growth conditions, however such subsequent PHA production was insignificant.

2.3. Bioproduct surface treatment and test method

Due to the large number of variables (bioproducts and construction materials) some materials were not treated with all bioproducts. Most bioproducts were applied onto the surface of construction materials by dropping into a 3 x 3 grid of 9 addition points in a surface of 40 mm x 40 mm in a total of 2 cm^3 of each bioproduct suspensions using a pipette. The exception was EC+Fe bioproduct, where the solution was also applied by capillarity. For the last method, 2 cm^3 of bioproduct were transferred to Petri dishes and samples were placed on top of the liquid with the test surface facing down; when all bioproduct was absorbed samples were turned upside down.

Five days after the treatment, the healing effect was assessed by testing for water droplet absorption,, which consisted in measuring the time in seconds needed for a droplet dripped onto the surface of the sample to be completely absorbed, as attested by the loss of sheen (Figure 3). This test allows evaluating the permeability variation of biotreated surfaces by monitoring the rate of absorption of a 0.1 cm^3 drop of water, that is, the time required for a material to fully absorb a water drop under open air conditions. The absorption period of time was video-recorded. The surface of all treated samples was observed visually and compared with the different control samples in order to assess color change.

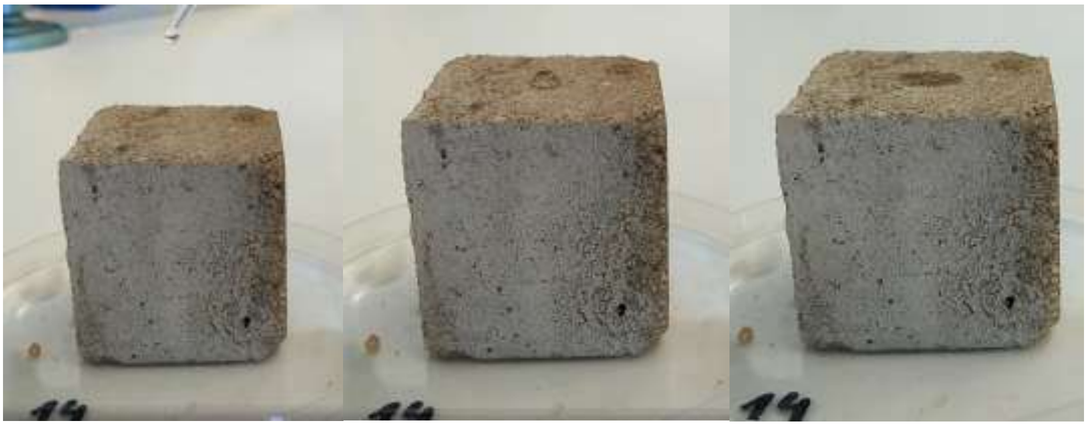

Figure 3. Water drop absorption test

The effect of the most efficient of each biotreatment was further characterized by a variable pressure scanning electron microscope (VP-SEM-EDS) HITACHI 3700N coupled with an energy-dispersive X-ray spectrometer BRUKER Xflash 5010 SDD operated at 20 kV in high vacuum and Secondary Electrons mode.

3. Results and discussion

3.1. Water drop absorption for adobe, CEB and air lime mortar samples

In a first approach, only adobe, CEB and air lime mortar samples were biotreated and compared with controls, to uncertain the best experimental variables. As such, the effect of iron in the *E. coli* products and the sonication step and concentration on the MMC-based bioproducts were tested in these three types of construction materials. The treatments did not alter the aesthetics color of the materials. The results of the waterproof effect are graphically represented in Figure 4.

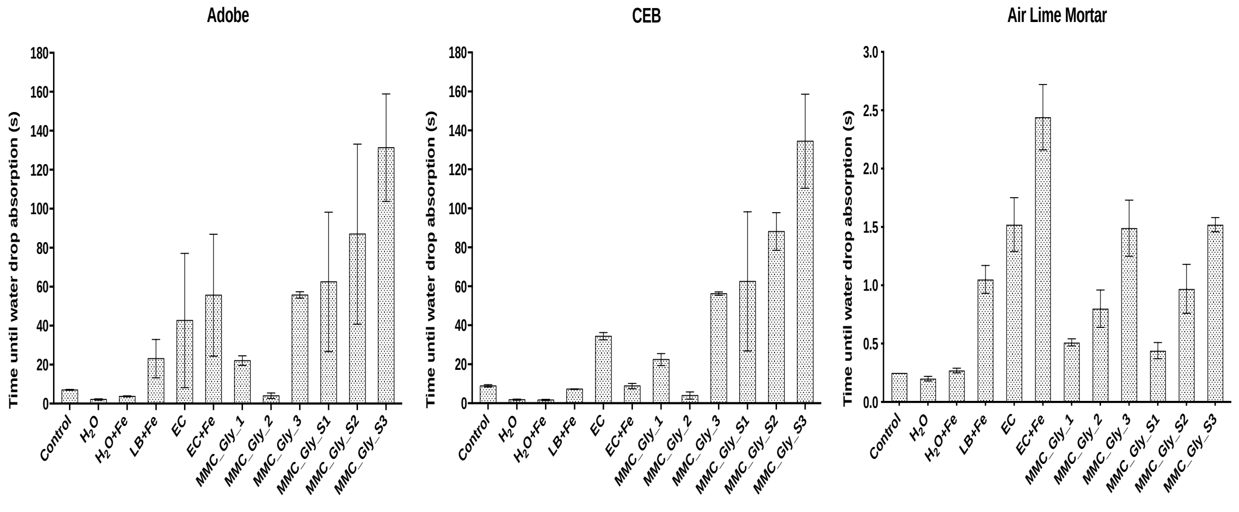

Figure 4. Water drop absorption time of surface treated adobe, CEB and air lime mortar

Analysis of Figure 4 reveals that the efficacy of each biotreatment is dependent on the construction material. In the tested conditions, the EC+Fe bioproduct applied by dropping was most efficient in air lime mortar, while for adobe and CEB, the best results were obtained with the sonicated MMC-base product at higher concentration (MMC_Gly_S3). In all three materials, the sonicated MMC-based products presented a better water barrier effect, that can most probably be explained by the release of the cellular content, namely carbon-based polymers as polyhydroxyalkanoates, produced as intracellular granule inclusions, that were able to form a biofilm on the surface of biotreated samples. When, assessing the effect of increasing concentrations of bioproduct, the highest concentration was beneficial in all materials. Thus, when adobe and CEB samples were treated with the most concentrated bioproduct MMC_Gly_S3, the water drop was totally absorbed in an average time of 133 ± 26 s (131 s and 135 s, respectively, versus 7 ± 0.2 s and 9 ± 0.6 s for the untreated control samples), representing an improvement of 1763 % for treated adobe and 1411 % for CEB. When adobe and CEB samples were treated with the medium concentrated bioproduct (MMC_Gly_S2) the absorption time was around 88 s and when the most diluted bioproduct was applied (MMC_Gly_S1), the absorption time was 63 ± 36 s. Based on these results, one can conclude that the behavior of these two types of materials towards the MMC-derived bioproducts was similar, and can be explained by the fact that both are earth-based materials with similar mechanical and durability properties. The consolidation effect was less expressive when the *E. coli* bioproducts were used to treat the surface of adobe and CEB samples. In the case of CEB, the most efficient treatment was with EC, presenting an absorption time of 34 ± 2 s.

For air lime mortar samples the time for absorption of a drop of water was much smaller than for the previous materials, 2.4 ± 0.3 s for the EC+Fe bioproduct (876% improvement when compared with the untreated control), and for treatments with EC or MMC_S at high concentration, around 1.5 ± 0.2 s. In fact, for treated lime mortar the bioconsolidation effect was almost two order of magnitude smaller than the maximum observed for the earthen-based materials. As the density of the three materials is similar, the difference may be due to the composition, namely the lamellar clayish particles that exist in adobe and CEB.

3.2. Water drop absorption for cement mortar, limestone and ceramic brick

In a second experimental campaign, the consolidation effect of bioproducts was evaluated in cement mortar, limestone and ceramic brick. In addition to the *E. coli* bioproducts used before, the EC+Fe bioproduct (*E. coli* cells cultured with 5 mmol/dm^3 of iron sulfate) was stored at 4 °C and at −20 °C for 48 h before application, to study the stability of the bioproduct during storage, for construction and conservation site utilization. The application mode was also studied, capillarity and dropping with a micropipette. In parallel, to analyze the role of LB medium in the observed waterproof effect, LB medium present in the EC+Fe bioproduct was removed by centrifugation and replaced by the same volume of tap water. For the MMC bioproducts, only the highest concentration was tested for both sonicated and non-sonicated biomass. Also, for these materials, the surface color did not changed. Results are presented in Figure 5.

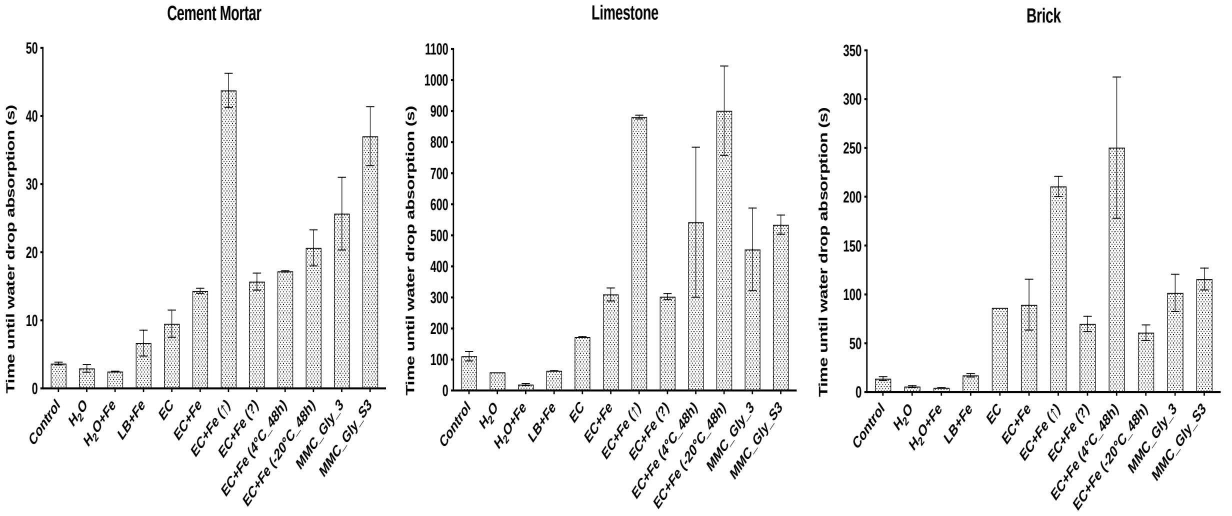

Figure 5. Water drop absorption time of surface treated cement mortar, limestone and brick

Analyzing the water barrier effect of treatments with *E. coli* derived bioproducts, one can conclude that the presence of iron contributes to a more efficient biotreatment, as observed before. The most promising results were obtained when the EC+Fe bioproduct was applied by capillarity to cement mortars and limestone samples. Even with ceramic brick, this treatment showed a water absorption time similar, within the experimental error, to the EC+Fe stored at 4 °C for 48 h. This could be due to a more homogeneous distribution of the bioproduct over the treated surface. In all tested samples, the presence of LB culture medium did not seem to contribute to the consolidation (EC+Fe versus EC+Fe?). In some cases, the storage process improved the efficacy of the biotreatment. That was the case of limestone samples biotreated with the EC+Fe product kept at 4°C for 48 h before application (EC+Fe (4°C_48h)), in which an improvement of 75% in comparison with the samples treated with the EC+Fe applied immediately after production of the bioproduct (EC+Fe). A more significant effect was also observed in ceramic brick samples, with water absorptions times 180 % higher. For cement mortar samples, the storage at 4 °C did not seem to affect the consolidation (17 ± 0.1 s versus 14 ± 0.4 s, for EC+Fe (4°C_48h) and EC+Fe, respectively). When exposed to a cold shock, bacterial cells cope with the stress, producing a set of specific proteins, increasing the membrane permeability and decreasing its fluidity, and reducing the efficiency of several metabolic processes, promoting cellular aggregation and biofilm formation [49, 50]. The water resistance enhancement observed in biotreated limestone and brick samples could be due to the alteration of bacterial physiology. When the bioproducts were stored at −20 °C for 48 h, the absorption time was slightly increased in treated cement mortars (21 ± 2.7 s versus 14 ± 0.4 s for EC+Fe) and about 191 % higher for limestone samples (901 ± 144 s). No significant changes were observed for brick samples. The freezing-thaw process promotes cell lysis, releasing the cellular content, that can enhance the waterproof effect of biological products.

None of the storage temperatures tested induced repair more effectively than any other. -Whilst cement mortar and limestone exhibited higher performance after product storage at -20 °C, consolidation was more effective in brick after 4 °C storage. Under both conditions, however, the effect on water absorption was greater than when *E. coli* was applied directly with no prior product storage.

Capillarity-based application yielded better results than dripping treatments onto the horizontal surface of the samples.

As for treatment of the three construction materials with the MMC bioproducts, sonicated ones were more effective than non-sonicated, with an increase of about 25 %. As mentioned before, release of intracellular components enhances the consolidation effect.

3.3. Summary of results of most effective treatments

Figure 6 presents the most efficient treatments for each tested material. A summary of all results is presented in Table S1 on the supplementary data.

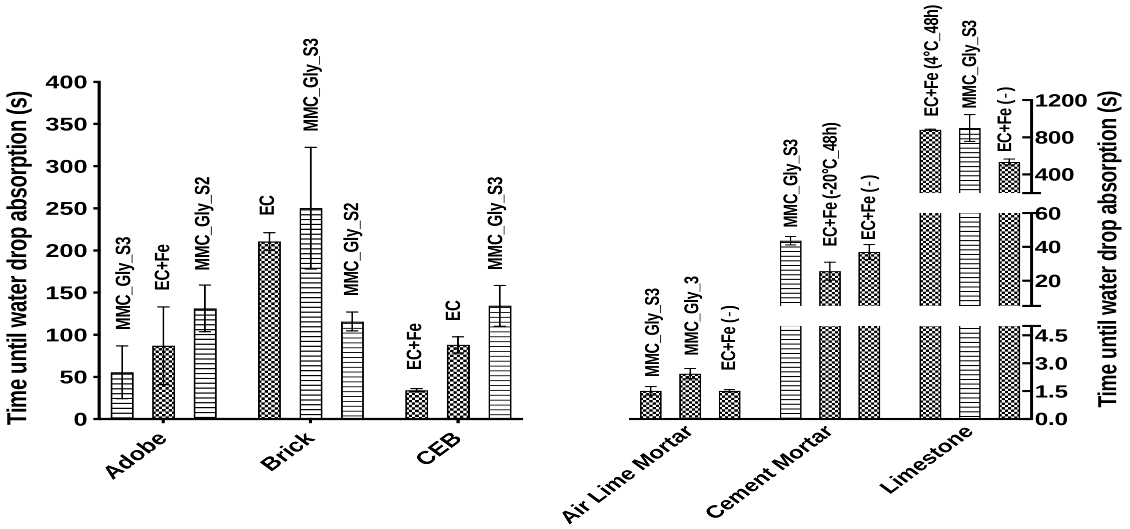

Figure 6. A synthesis of the three most effective surface treatments for the six materials tested

3.4. SEM-EDS results

The most efficient biotreatments, i.e., those that retarded water droplet absorption the longest, were further analyzed on an EC+Fe drip-treated brick sample and an MMC_Gly_S3-treated adobe sample under a variable pressure scanning electron microscope coupled to an energy dispersive X-ray spectrometer (SEM-EDS). As expected, aluminum and silicon were the predominant elements in the brick sample (Figure 7), whilst the detection of carbon and oxygen denoted the presence of organic matter. The sodium, potassium, chloride and phosphorus identified would have been sourced from the LB culture medium used to grow *E. coli* cells. The micrograph in Figure 7 shows that the carbon was not distributed uniformly, but located primarily in pits in the construction material, a circumstance consistent with the development of a surface crack-sealing biofilm. Such distribution might also explain the wide standard deviation in water absorption times in some samples. Element distribution in the MMC_Gly_S3-treated adobe samples (Figure 8) was similar to that observed for the EC+Fe-treated brick material. The exception was the absence of sodium and chloride, for the MMC_Gly_S3 did not contain the medium used to grow *E. coli*. Figure 8 reveals the uneven distribution of carbon in the adobe also, where it was located in a manner compatible with the possible existence of a biofilm.

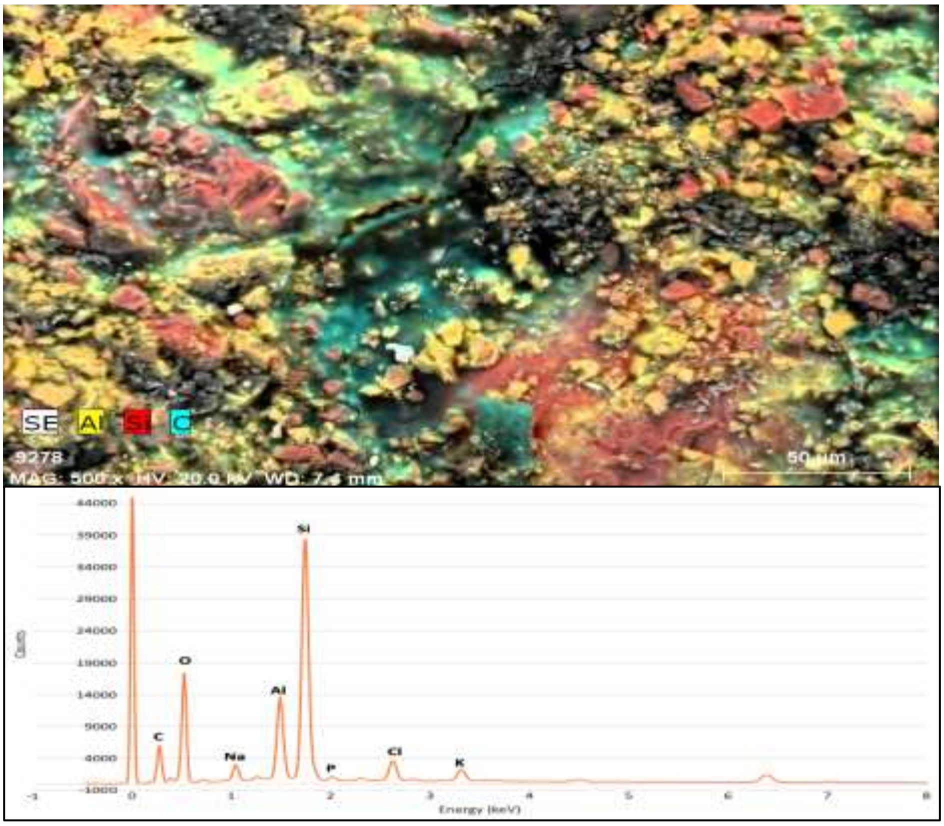

Figure 7. SEM-EDS micrograph (500x magnification) of an EC+Fe-treated brick sample with the EDS elemental distribution map (yellow=Al, red=Si and blue=C) and respective EDS spectrum

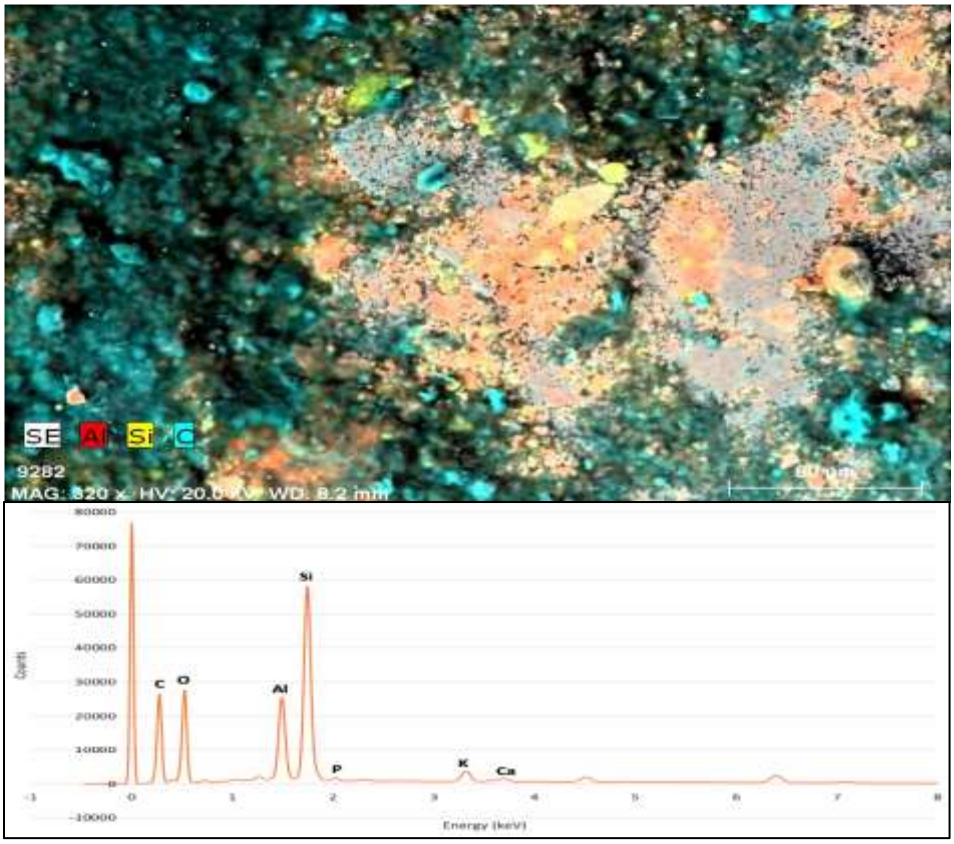

Figure 8. SEM-EDS micrograph (500x magnification) of an MMC_Gly_S3-treated adobe sample with the EDS elemental distribution map (red=Al, yellow=Si and blue=C) and respective EDS spectrum

3.5. Discussion

Comparison of the effect of surface treatments on water absorption and permeability of different construction materials to water is not straightforward, due to the diversity of matrix compositions used in different studies reported in the literature and the different test procedures used to assess it. Nevertheless, the water absorption capacity of various construction materials surface treated with different products were summarized in Table 1.

The results obtained in the present work, namely the treatments of cement mortar samples with bioproducts EC+Fe by capillarity (EC+Fe (↑)) and sonicated MMC at high concentration (MMC_Gly_S3) by dropping, with improvements of, respectively, 1096 % and 912 % of water absorption time versus untreated samples (Control) were more effective than cactus extract, nano-SiO_2 or tetraethoxysilane agents studied by Chandra et al. [11], Hou et al. [3] and Subbiah et al. [8].

For limestone samples, surface treatment by applying EC+Fe bioproduct by capillarity and MMC_Gly_S3 by dropping, increased their waterproof effect 694 % and 382 %, respectively, being more efficient than hydroxyapatite, tetraethoxysilane, polyhydroxybutyrate, poly-L-lactide and nanolime, applied in other studies [15, 19, 24-26].

Raut et al. [34] and Sarda et al. [33] used *Sporosarcina pasteurii* cultures to treat brick samples, achieving a decrease in water absorption up to about 49 %, less expressive than the result obtained with all the bioproducts from *E. coli* and MMC tested in the present work, which showed reductions from 341 % up to 1712 %.

Concerning adobe and CEB samples, all treatments were more efficient (Table S1) than other agents, previously used on earth plasters, as aqueous beeswax emulsion, fatty acids and silane [35, 38, 39]. Nevertheless, the most efficient treatments were obtained by applying the MMC_Gly_S3 bioproduct by dropping, with a water proofing effect enhanced by 1763 % in the treated adobe case and 1411 % in treated CEB samples.

Finally, comparing the effects of the studied bioproducts on air lime mortar samples, all treatments with *E. coli* and MMC bioproducts, showed higher waterproof effect (from 76 % up to 876 % water absorption reductions) than the obtained by nanolime treatments tested by Taglieri et al. [27] and Borsoi et al. [26]. However, Slížková et al. [40] obtained a greater waterprooding effect treating air lime mortar with barium hydroxide.

While for adobe and CEB materials the MMC_Gly_S3 bioproduct proved to be more effective (up to 1411-1763 % improvements), for all other construction material, EC-based bioproducts were preferable. For air lime mortar the efficacy of treatments was less expressive; that can be justified by the high pH of air lime mortars, around 9 after carbonation [51], that can affect the formation of a biofilm on the treated surface.

4. Conclusions

The *E. coli* and the MMC crude glycerol-based bioproducts did not change the aesthetic of the surfaces of the tested materials. The tested *E. coli*-based bioproducts produced delayed the time for water absorption, ; being the most efficient treatment the *E. coli* culture supplemented with iron bioproduct. This bioproduct kept its activity after refrigeration or freezing (4°C and -20°C) and, in some cases, conditioning enhanced the water barrier effectrelative to direct application of the *E. coli* culture immediately after substance generation. Neither storage condition was clearly better than the other, however, with performance depending on the material treated.

Treatments with MMC grown with crude glycerol bioproduct resulted in an increase of the time to water absorption in all tested material. The sonicated bioproduct achieved a greater healing effect when compared with the non-sonicated MMC ones. Higher concentrations of bioproducts improved the waterproof effects. When tested, the application method by capillarity optimized the effect in comparison to dropping on the horizontal surface of samples.

Both types of bioproducts are eco-friendly. The main goal of MMC cultivation was the production of polyhydroxyalkanoates, a biodegradable polymer candidate to replace most petrochemical-derived plastics. This process used non-sterile conditions for biomass growth, since mixed cultures, as opposite to pure cultures, were grown together with the utilization of a by-product of biodiesel manufacture as carbon substrate. Additionally, the room temperature operation and a simple medium for biomass growth also have a positive impact on process costs. A third advantage of this utilization is that only waste biomass was used, and it did not interfere with the main goal of the MMC cultivation that was the production of polyhydroxyalkanoates. The healing process proposed here using MMC could be integrated in an enhanced biorefinery, where the use of natural/renewable resources are preferred. In the case of *E. coli* even though the process for growing biomass would be more expensive, their wider application on most of the materials tested could counteract this fact. The utilization of iron for the EC+Fe production could be provided by an industrial waste.

In comparison with the results of water absorption decrease of surface treatments previously applied on the same type of materials, it seems that both the *E. coli*-based and the MMC crude glycerol-based bioproducts are significantly effective, what justifies the continuation of this line of research, searching for a detailed evaluation of repair effect and durability of the bioagents after aging.

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Appendix A. Supplementary data

The following table is Supplementary data to this article:

	Improved resistance against water ingress (%)		
Treatment	Adobe	CEB	Air Lime Mortar
H_2O	-70	-80	-20
H_2O+Fe	-46	-81	8
LB+Fe	227	-19	320
EC	505	287	508
EC+Fe	689	-1	876
MMC_Gly_1	213	152	104
MMC_Gly_2	-42	-56	220
MMC_Gly_3	691	532	496
MMC_Gly_S1	786	602	76
MMC_Gly_S2	1134	891	288
MMC_Gly_S3	1763	1411	508

	Improved resistance against water ingress (%)		
Treatment	Cement Mortar	Limestone	Brick
H_2O	-19	-47	-58
H_2O+Fe	-32	-82	-68
LB+Fe	82	-42	25
EC	160	56	525
EC+Fe	292	179	548
EC+Fe (↑)	1096	694	1424
EC+Fe (?)	328	173	405

EC+Fe (4˚C_48h)	370	389	1712
EC+Fe (-20˚C_48h)	464	713	341
MMC_Gly_3	601	310	636
MMC_Gly_S3	912	382	738

Table S1: Waterproof effect of different biotreatments on adobe, CEB, air lime mortar, cement mortar, limestone and brick samples, showing the time of water droplet absorption, expressed by seconds with standard deviation, and the water absorption decrease, expressed by percentage.

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