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Effect of innovative bioproducts on the performance of bioformulated earthen plasters

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ABSTRACT

Bioconsolidation includes biotechnologies mainly based on precipitation of chemical compounds produced by microbial metabolism, that can be used as biotreatment or bioformulation. Bioformulation of cement-based materials has been a common research practice, though its application to earthen-based building materials is still limited. Although earth mortars are vulnerable to water damage, they are nowadays often used as plasters on new constructions but also for the retrofitting of existent buildings. Their reversibility and compatibility with old substrates make them also interesting for conservation interventions, namely when applied to protect archaeological structures as sacrificial renders. In this study, an innovative bioproduct based on cellular extracts of *Escherichia coli*, cultured in Lysogeny broth (LB) medium supplemented with iron, was developed and used as a component for earth mortars' bioformulation. An additional earth mortar bioformulated with LB medium as kneading liquid was also tested. Bioformulated mortars presented a very distinct porous structure, with a significant decrease on mechanical properties. Nevertheless, they also showed a decrease on thermal conductivity, a slight consolidative effect and a significant decrease on vulnerability towards water. Apart from the possible use as mortar air entraining agents, the obtained results show the interest on further studies on the use of nontoxic iron-based bioproducts for earthen mortars optimization.

Keywords: air entraining agent; bioconsolidation; clayish earth mortar; physical performance; porous structure; water absorption; vulnerability to water

1. Introduction

Bioconsolidation of building materials is a technique based on the use of bacterial cultures supplemented with nutrients [1-3]. These studies started using bacterial cells that promoted microbial induced calcium-carbonate precipitation (MICP) processes for the biotreatment of degraded limestones in ancient monuments [4]. Due to the achievement of very positive results showing efficiency, compatibility, and reversibility, bioconsolidation largely expanded to most of the commonly used construction materials. Based on the results of several studies on different construction materials [5-11], bioconsolidation can increase cohesion and decrease liquid water absorption without considerably affecting water vapor permeability. Therefore, precipitation of minerals can occur at the surface layer, when the bioproducts are applied as a surface treatment (biotreatment) or embedded all over the material, when the bioproducts are used as kneading liquid on the production of mortars, concretes, bricks or blocks (bioformulation).

Biotreatment is a sustainable technique that has been widely used in buildings´ material conservation or for the improvement of pre-casted building materials. This technique has been applied to a wide variety of building materials such as limestone [4,5,12], gypsum plaster [13], ceramic bricks [6,7,14], cement mortars [8,15-17] or earth-based mortars [18], leading to a significant reduction of the water absorption rate.

Bioformulation, in turn, creates a more in-depth effect because bacterial cells are a component of the building material mixture, as a raw material, working as an additional binder agent. In this case, precipitated minerals help to bind the solid particles, forming a more cohesive material, similarly to the effect that termites produce on mound soil [19,20]. As such, common binders, as cement, lime or clay, might be replaced (at least partially) or added by this type of bioproducts that promote biomineralization (formation of a mineral due to the activity of a biological system). Nevertheless, the fresh state characteristics of the bioformulated building materials have to be adequate, to be workable, and their mechanical and physical properties have to address requirements defined for each type of application of those building materials. That is the case of a bioformulated plastering mortar that has to present adequate workability while fresh and has to keep enough adhesion to the surface of the wall when applied. It is noteworthy that in cases where bacteria and binder are used together, bacteria cells should be compatible with the high alkalinity of some binder matrices [21], such as limes.

Bioformulation has been also applied to a wide variety of building materials such as sand [7,8,22], earth-based blocks [10,23-25] or cement mortars [26-28], increasing durability by enhancing mechanical resistances and decreasing water absorption. Table 1 lists some studies where bioformulation was used for the improvement of earth-based blocks and sand, reducing water absorption and increasing material consolidation.

Over the years, different bacterial species have been used in MICP processes, based on their metabolic ability to precipitate calcium-carbonate (Table 1). For this reason, cells from the *Bacillus* genus have been largely used due to its urease activity and ability to precipitate $CaCO_3$ [29-31]. The precipitation of calcium-carbonate is dependent on the environmental/experimental conditions [29], namely on the concentration of calcium and dissolved inorganic carbon, pH, availability of nucleation sites for mineral growth, and presence of urea [21]. Combination of the previous factors with the most adequate bacterial species may lead to a controlled enhanced benefic precipitation of calcium-carbonate in building materials. Parallel to the utilization of calcium-carbonate precipitation for repair or improvement of building materials, iron mineralization through iron-oxide precipitation may also be used for these purposes (Table 1). Iron biomineralization is a process less toxic than the calcium-carbonate mineral one since no toxic metabolites are produced and iron oxide minerals have a longer lifetime when compared with calcite crystals [1].

Earth plasters are known for their improved environmental performance in comparison to common binder plasters [32]. However, when in contact with liquid water, earth mortars regain their plasticity. Therefore, for application where contact with water may occur, there is a crescent need to enhance the resistance of earth-based mortars to weathering [33-36]. Several research studies have been performed with the objective of increasing earth plasters durability, though natural products are hardly recommended for this purpose [37,38]. Using iron-based bioproducts, earth mortars' optimization might be achieved either as a surface treatment of previously applied earth plastering mortars [18] or using the bioproducts on their formulation. Earth mortars are naturally pigmented by the earth particles, mainly the finer ones (clay and silt) and, therefore, a slight change of color that iron oxides could cause would not be problematic.

This study aims at assessing the influence of an innovative bioproduct based on an iron supplemented *Escherichia (E.) coli* culture grown in a rich medium used as kneading liquid, replacing water, on the formulation of earth-based plastering mortars.

Table 1. Literature review on biotreated and bioformulated earth-based blocks, soil and sand

Reference	Bioproduct	Material	Treatment	Main results
Bernardi et al. [24]	*Sporosarcina pasteurii* ATCC 11859 grown in ammonium-yeast extract	Sandy earth blocks and cement or NHL5 stabilized blocks	Bioformulation; blocks were fed with urea-calcium medium, in an average of 3 times/day. Blocks were fed 21, 42 and 84 times during 7, 14- and 28-days curing	Similar compressive strengths (with low confining stress of ~10 kPa) for bioformulated blocks and blocks stabilized with cement or lime
Dhami and Mukherjee [23]	*Bacillus megaterium* cultivated in NBU medium	Earthen blocks	Bioformulation	40% reduction in water absorption and a decrease in linear expansion, resulting in a more stable earth-based block
Mukherjee et al. [10]	*Bacillus megaterium* cultivated in NBU medium, pH 8.0	Earth-cement blocks	Bioformulation and subsequent curing by spraying with NB medium for 28 days, after which blocks were dried for more than 30 days	33.6% reduction on water absorption (tested by immersion of blocks in water for 24 h) and 10% increase on wet compressive strength
Ivanov et al. [39,40]	*Bacillus sp.* VS1 grown in culture medium containing $CaCl_2$ and urea or with iron ore and organic waste	Soil	Biotreatment	Iron-based biosoil: compressive strengths not as high as the calcium-based biosoil but significantly reduced water permeability; precipitate with a gel-like appearance instead of the crystal appearance of the calcium-based biosoil
Achal et al. [22]	*Sporosarcina pasteurii* grown in CSL or NBU media, pH 6.5	Sand columns	Biotreatment; feeding with Corn steep liquor or NB used to feed	10 days after treatment, control samples presented a flow rate of 2.6 mL/min while samples treated with Corn Steep Liquor and NB were totally obstructed after 8 days and 10 days, respectively
Cardoso et al. [8]	*Sporosarcina pasteurii* cultivated in NH_4-YE medium, pH 9.0	Sand columns	Biotreatment and then 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
Dhami et al. [7]	*Bacillus megaterium* in NBU medium	Sand columns	Biotreatment. Sand columns were fed twice a day for 10 days (∼50 mL of medium/day for the first 5 days and ∼30 mL for the second 5 days)	Control and bacteria treated samples presented an average initial flow rate of 15 mL/min. After 7 days feeding, biotreated samples were totally obstructed while control samples still exhibiting a flow rate of 12.3 mL/min after 10 days feeding
Dhami et al. [41]	*Bacillus cereus* grown in NBU medium	Sand columns	Biotreatment	Control sand: 12.15% water absorption, 25.3% total porosity. Biosand: 8.84% water absorption, 19.2% total porosity

Notation: NB – nutrient broth growth medium; NBU – NB medium supplemented with 2% urea and 25 mM $CaCl_2$; CSL – Corn steep liquor; *Sporosarcina pasteurii* was formerly known as *Bacillus pasteurii*

2. Materials

The earth mortars used in this study were produced with a pre-mixed plastering mortar product commercialized by Embarro company (Portugal and Spain) and previously studied by Faria et al. [42]. This mortar is formulated with clayish earth, siliceous sand and cut oat fibers, though the exact proportions of each component are not available. The clayish earth was extracted from a quarry in the South of Portugal [43]. The particle size distribution and the X-ray diffractogram of the pre-mixed plastering product are shown in Figures 1 and 2. The clay is mainly illitic, with calcite ($CaCO_3$) and hematite (Fe_2O_3). Both these constituents may be useful for the bioconsolidation process through bioformulation [25]. The loose bulk density of the pre-mixed product determined following EN 1097-3 [44], was 1.47 kg/dm^3.

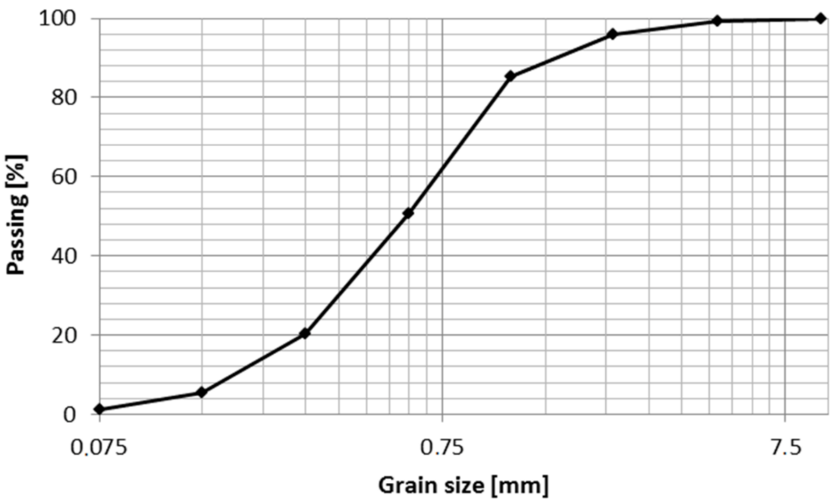

Figure 1. Particle size distribution of the pre-mixed plastering product [42]

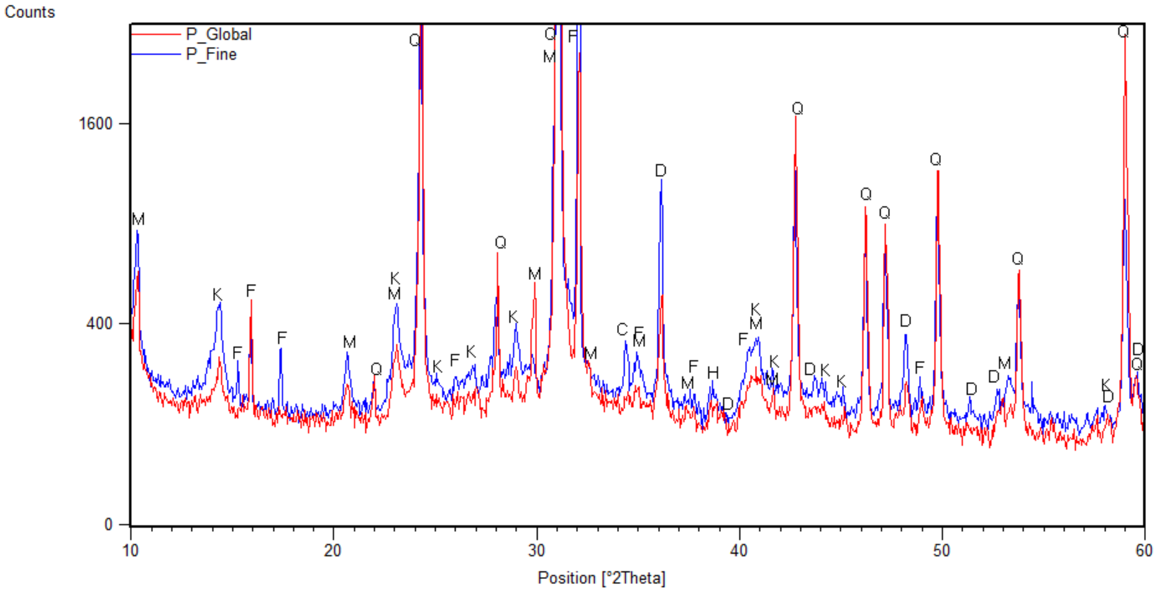

Figure 2. XRD of global and fine fractions of the pre-mixed plastering product [42]

Bioformulated mortars were prepared by adding a suspension of *Escherichia (E.). E. coli* cells cultured in Lysogeny broth medium (LB) supplemented with iron (E.coli+Fe), produced as in Parracha et al. [18]. This bacterial strain, *E. coli* BL21(DE3) from NZYTech (Portugal) is a research model, widely used to express recombinant proteins. It is classified as not hazardous, which do not cause disease in immunocompetent adult humans, presenting minimal potential hazard to laboratory personnel and the environment, complying with the current European Union Regulation (EC) 453/2010 [MSD] and Regulation (EC) 1272/2008 [CLP], as well as the current national legislation [45]. The LB medium contained 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl, with a final pH of 7.0.

To assess the effect of the culture medium, an additional earth mortar was formulated with LB as the kneading liquid (LB). For control, an earth mortar was formulated by replacing the bioproduct liquid suspension by tap water (Control).

All mortars were mechanically produced in laboratory following standard procedures of DIN 18947 [46], using a volumetric ratio of kneading liquid/dry product of 0.25 as suggested by the manufacturer, in order to obtain a workability considered adequate to ensure applicability. The kneading liquid (bioproducts or water) was firstly transferred to the mixing equipment recipient, followed by pouring the appropriate weight of pre-mixed product. The mixing process involved three steps: 1 min mixing, 5 min resting, and 30 s mixing. Mortars were characterized in the fresh state just after mixing. To assess if the mortars fresh state properties would be maintained over time, allowing to pre-batch the mortars before application, a portion of each mortar was sealed in a zipper plastic bag and tested in the fresh state after 72 h.

Two different types of samples were produced: prismatic earth mortar samples with dimensions 40 mm x 40 mm x 160 mm; and a layer of earth mortar applied on ceramic hollow brick to simulate a plaster, with dimensions of approximately 15 mm x 200 mm x 300 mm (Figure 3).

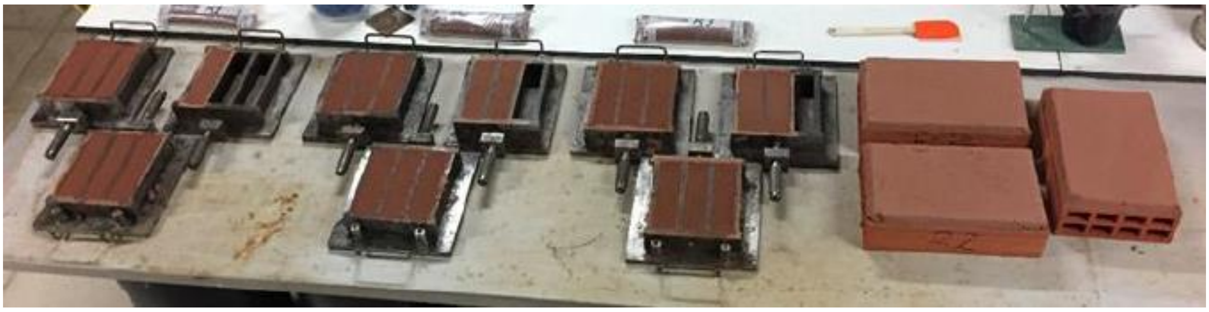

Figure 3. Bioformulated earth mortar samples

The layer of earth mortar was applied to the brick using two molds: one applied around the brick, defining the 15 mm thick layer; and the other positioned on top of the first mold with 70 mm height. Molds were filled with mortar by dropping (from the top of the mold) in order to simulate a constant force of application of the mortar to a brick wall. As in the in-situ application, the brick was previously sprayed with water to avoid excessive absorption from the fresh mortar.

Samples were dried on laboratory conditions at a temperature (T) of 18-21 ºC and a relative humidity (RH) of 46 ± 5% and prismatic samples were demolded after 14 days. Cubic samples with dimensions of 40 mm x 40 mm x 40 mm were cut from half of the prismatic samples resulting from the flexural strength test (Figure 4). The other half prismatic samples were used for the compressive strength test and pieces of about 25 mm x 40 mm x 40 mm were used to assess durability in water. For complementary compressive test of plasters, specimens with 15 mm x 50 mm x 50 mm were prepared from the plaster on hollow bricks samples, eliminating the adjacent plaster. The sampling process is schematically presented in Figure 4.

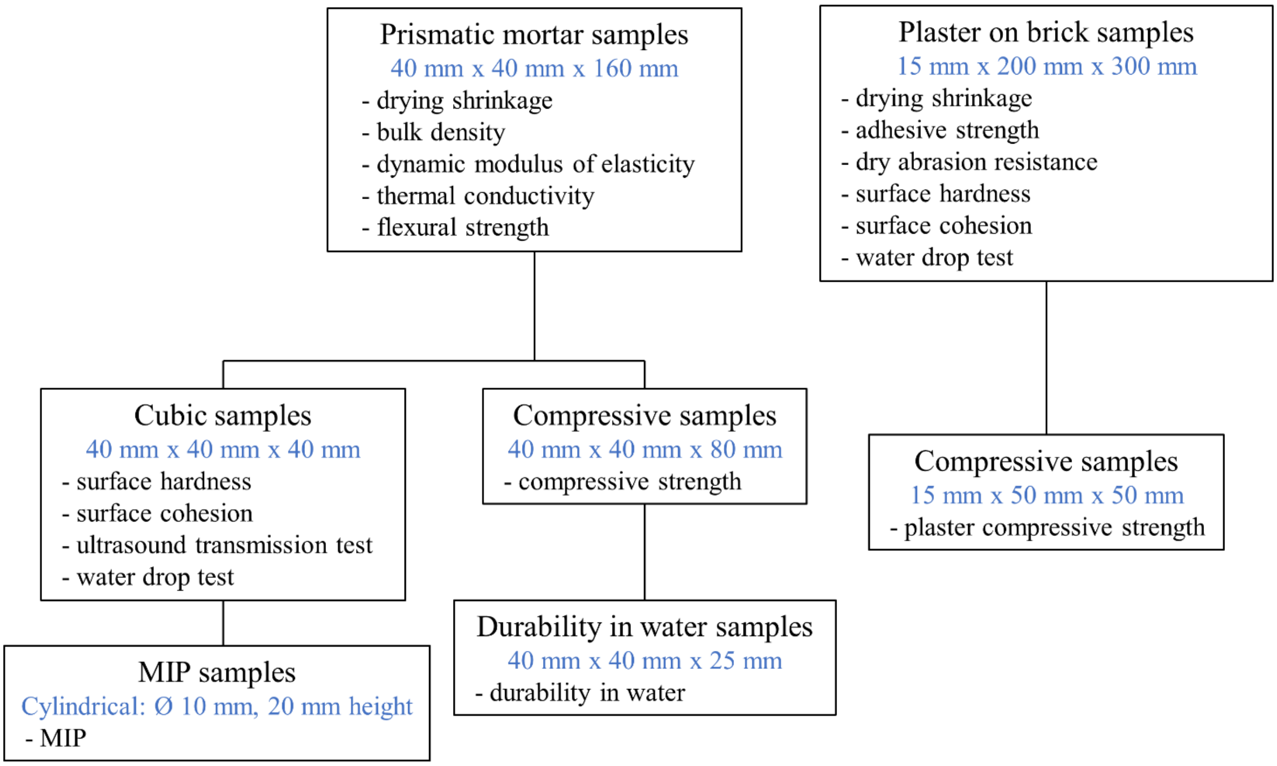

Figure 4. Schematic representation of the samples and testing campaign

3. Methods

3.1. Fresh state mortar tests

Earth mortars were characterized in the fresh state (just after mixing and after 72 h in a sealed plastic bag) for flow table consistency, following EN 1015-3 [47], penetrometer consistency, based on EN 1015-4 [48] and wet bulk density, according to EN 1015-6 [49].

3.2. Hardened state tests on prismatic mortar samples

Prismatic samples were used to assess drying shrinkage and to test mechanical resistance of mortars. Cubic cut samples were used to test surface properties and resistance towards water (Figure 4). All tests were performed in at least three samples, except the mercury intrusion porosimetry (MIP), with only one sample. Apart from the drying shrinkage test, all tests were performed with mortars samples aged for more than 1 month, to assure they were dried and in equilibrium with the laboratory environmental conditions, with a temperature of 18-21 ºC and a RH of 46 ± 5%.

Drying shrinkage, bulk density and porous structure

Linear drying shrinkage was measured according to DIN 18947 [46]. The length of prismatic samples, dried for 14 days, was measured after demolding, and compared with the length of the mold. Linear drying shrinkage is expressed by the percentage of lengths difference.

Bulk density was assessed according to EN 1015-10/A1 [50] and calculated dividing the mass of mortars by the volume obtained by dimensional measuring.

Porosity and porosimetry were measured by MIP using a Micromeritics Autopore II mercury porosimeter. The specimens were cut from the samples and prepared to occupy the maximum volume of a 5 cm^3 penetrometer bulb. Based on the procedure described by Faria et al. [42], the test was performed with low pressures ranging from 0.01 N/mm^2 to 0.21 N/mm^2 and high pressures ranging from 0.28 N/mm^2 to 206.84 N/mm^2.

Thermal conductivity and ultrasound transmission test

Thermal conductivity was evaluated with an ISOMET 2104 Heat Transfer Analyzer with a contact probe API 210412 with 60 mm diameter, at the defined conditions (18-21 ºC and 46 ± 5% RH). Cubic samples, with a surface area of 40 mm x 40 mm, were isolated with polystyrene foam, as shown in Figure 5a. Although the real thermal conductivity of the earth mortar is higher than the measured one, due to the polystyrene foam confinement, results are valid for comparison between the tested mortars.

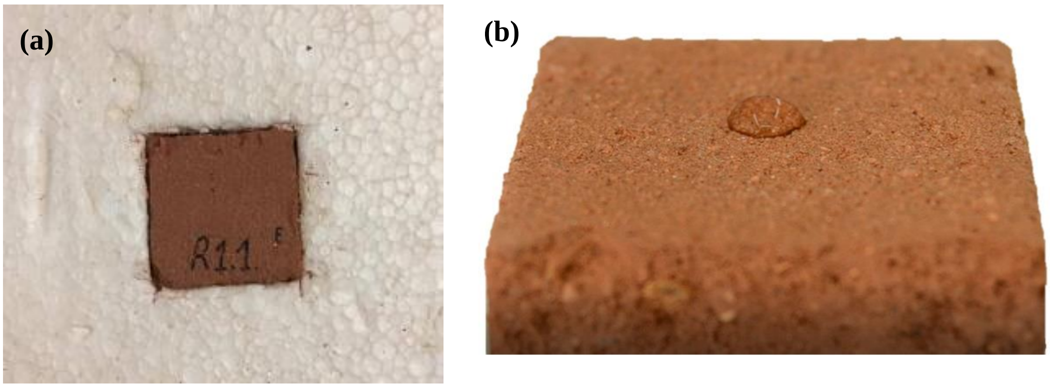

Figure 5. Confinement of earth mortar samples with polystyrene thermal insulation for the thermal conductivity test (a) and drop of water on a sample surface (b)

Ultrasound transmission test was performed according to EN 12504-4 [51] using a Proceq Pundit Lab Ultrasonic equipment by direct measurement. The transducers were applied in opposite sides of the samples and the distance between them was registered for all samples. The results allow to evaluate the compactness of samples.

Dynamic modulus of elasticity, flexural and compressive strengths

Dynamic modulus of elasticity was determined according to EN 14146 [52], using a Zeus Resonance Meter. The test was performed in each sample on four different positions, registering four test results per sample.

Flexural and compressive strengths were determined according to EN 1015-11 [53] using a Zwick Roell Z050 equipment. Flexural strength test was performed at a velocity of 1 mm/min, whereas the compressive strength test was performed at a velocity of 3 mm/min using one half of the samples resulting from the flexural test.

Surface hardness and surface cohesion

All these tests were performed on cubic samples of 40 mm x 40 mm x 40 mm (Figure 4). Surface hardness test was conducted following ASTM D22240 [54] using a digital PCE Shore A durometer, applicable to soft materials. The test was performed in 12 different points along the surface of each sample.

Surface cohesion was assessed using a procedure described by Drdácký et al. [55] and later adapted to earth plastering mortars by Faria et al. [42] and Parracha et al. [18]. Pieces of scotch tape of 50 mm x 50 mm were weighted to register their mass and glued to the sample. A thick neoprene tissue was placed over the sample and a 1.5 kg weigh was placed on top of the tissue, without touching the sample, for 5 minutes. After that period the weight and tissue were removed, the tape carefully removed and weighted. The mass increase in the tape represents the loss of material due to lack of adhesion.

Water drop and durability in water

The water drop test is a simple test that allows to observe the behavior of the tested mortar towards water ingress. In this test, a drop of water was dropped on a surface of a cubic sample and the time until the drop of water was totally absorbed by the sample was registered (Figure 5b).

In order to assess the mortars vulnerability to water, a simple test to assess durability of small mortars' samples (cut from the compressive strength test samples) when submerged in water was established. The test was performed transferring the small samples to glass beakers filled with tap water and registering by video.

3.3. Hardened state tests on plaster on ceramic brick samples

Firstly, three distinct tests were performed on these samples: visual analysis to observe eventual formation of cracks due to shrinkage; adhesive strength to characterize the bond between the mortar and the brick; and dry abrasion resistance to assess the surface cohesion of the mortar layer. After performing these tests, the same samples were used to test surface hardness and cohesion, resistance towards water absorption and compressive strength and results were compared with the ones obtained for the prismatic samples (without the influence of plastering a substrate).

Adhesive strength

Adhesive strength was determined following EN 1015-12 [56]. Circular specimens were cut on the mortar layer and metallic pins with a base with the same 50 mm diameter were fixed with epoxy glue to the mortar. When the glue dried, the pins were pulled out with a Zwick Roell Z050 equipment, at a velocity of 1 mm/min. Using the load strength registered by the equipment software and the glued area, the adhesive strength of the plaster to the brick was calculated and the type of mechanically induced fracture was identified. These can be cohesive, when the fracture occurs in the mortar layer, meaning that the adhesion plaster/support is higher, or adhesive, when it emerged at the interface of the mortar and the brick.

Dry abrasion

Dry abrasion test was performed based on DIN 18947 [46]. In this test a medium hard rotating brush [42] was pressed against the surface of the earth plaster, with a constant applied pressure of 2 kg, to simulate wear conditions (Figure 6a). After 20 rotations, the loose particles were cleaned from the surface and the plastered brick sample was weighed on a scale with a precision of 0.1 g, before and after each test. The mass change and the relief left by the brush are indicative of the surface durability to abrasion.

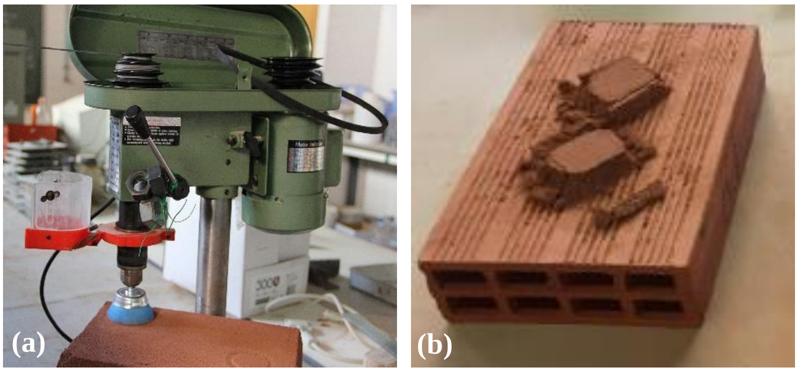

Figure 6. Dry abrasion test being performed on a plaster sample (a) and plaster samples on brick after the compressive strength test (b)

Compressive strength

Compressive strength of plaster samples was assessed using a Zwick Roell Z050 equipment. The test was performed on samples with approximate dimensions of 50 mm x 50 mm, still attached to the ceramic brick, after removing the surrounding areas of plaster (Figure 6b).

Surface hardness, surface cohesion and water drop test

Surface hardness, surface cohesion and water drop tests were also performed on earth plasters on ceramic brick samples as described above (see Section 3.2). However, these samples represent a real plaster in comparison with the ones of Section 3.2.

4. Results and discussion

4.1. Characterization of fresh state mortars

As previously stated, the control earth mortar (Control) was produced by mixing the solid components with water, adjusting its volume to allow a good workability. Although the same ratio (25% of the dry pre-mixed product) was used for the E.coli+Fe and LB mortars, different workability was obtained. The E.coli+Fe and LB mortars presented a “foamy” workability. The foamy consistency of these mortars may be explained by the foam generated by the mechanical mixing of components, and may be associated to the LB culture medium, since it was observed in both mortars bioformulated with the *E. coli* bioproduct and just with the LB.

LB is a nutritionally rich medium, widely used for laboratory cultivation of *E. coli* species. The observed foam could be explained by the presence of biological molecules such as proteins, peptides and vitamins in the LB. Their mechanical denaturation/degradation could also contribute to the formation of foam. In fact, the role of proteins and peptides on formation and stabilization of foam has been the focus of many studies and has been recently reviewed by several authors [57-59].

No significative odor was noticed on fresh bioformulated mortars in comparison to Control, when mixing and after removal from zipper-bags.

The earth mortars removed from the zipper-bags 72 h after mixing, to assess mortar's pre-batch viability, were less workable when compared with the ones tested just after being mixed, though still acceptable to be applied as a plaster. Once the bags were hermetically closed (and there is no water evaporation on clayish mortars), the observed loss of workability can be justified by the water absorption of the clay particles or by the degradation of biological molecules. One should mention that the alkaline pH (pH~9, according with Santos et al. [59]) of earth mortars could, in fact, contribute to the denaturation of peptides, proteins and vitamins, as well as to lyse bacterial cells, altering the chemical and biological activity of the bioproducts. Moreover, water formulated (Control) and bioformulated mortars presented a similar workability after 72 h, in comparison to those tested immediately after mixing.

It is noteworthy that in both Control and bioformulated mortars no liquid halo around the mortars was observed on the flow table at the end of the tests. Higher flow table diameters and lower slump heights were obtained for bioformulated mortars in comparison to Control mortars (Figure 7). These results are in accordance with the observed different workability.

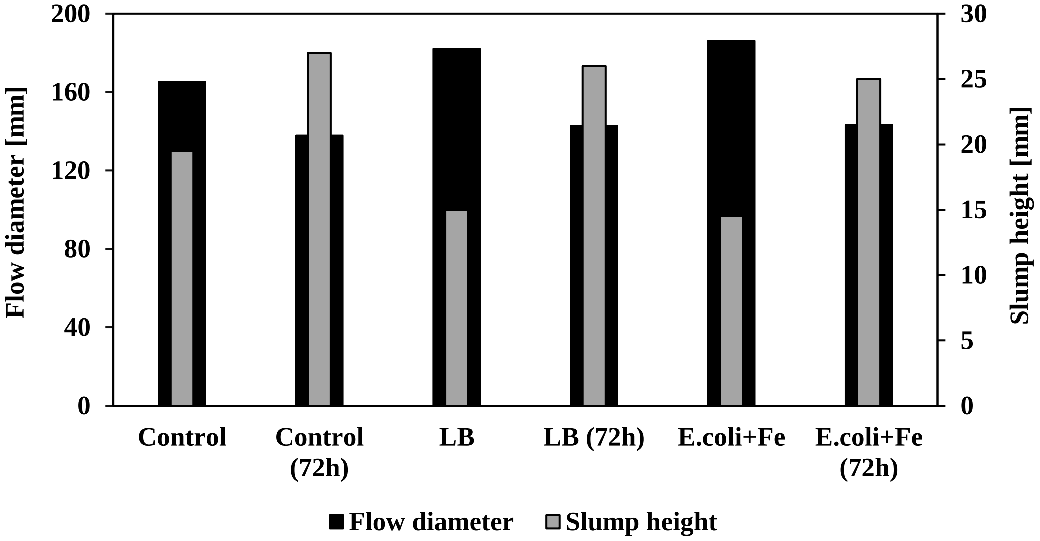

Figure 7. Flow diameter and slump height of mortars

All mortars tested immediately after production presented a flow table between 162 mm for the Control and 180-182 mm for the bioformulated, close to the range of 175 ± 5 mm defined by the standard DIN 18947 [46] what is justified by the use of a flow table in accordance to EN 1015-3 [47] and not the subsequent amendment of that standard. The slump height was inversely proportional: higher for the Control and lower for the bioformulated mortars. Since all the mortars were formulated with the same volumetric ratio of kneading liquid/dry product of 0.25, results show that the bioproducts increased the consistency of mortars, decreasing their flow. Nevertheless, bioformulated mortars were still quite workable, most probably due to their foamy consistency.

The foamy consistency of the bioformulated mortars disappeared after 72 h inside zipper-bags, and all mortars presented lower flow table diameters, around 140 mm. In this case, the slump height of bioformulated mortars increased, also with values closer to the ones obtained for the Control mortars.

Penetrometer consistency results (Figure 8) are in accordance with the results obtained for flow table consistency. Bioformulated mortars present a higher penetration depth than Control mortars and all mortars tested after 72 h are in a closer range of values for penetration depth, showing that consistency is quite different for bioformulated mortars when compared with Control mortar, immediately after mixing. Nevertheless, this distinct behavior disappears after 72 h in a close bag. This fact shows that the air entraining effect caused by the use of bioproducts on mortars formulation can only be maintained if the mortars are not pre-batched.

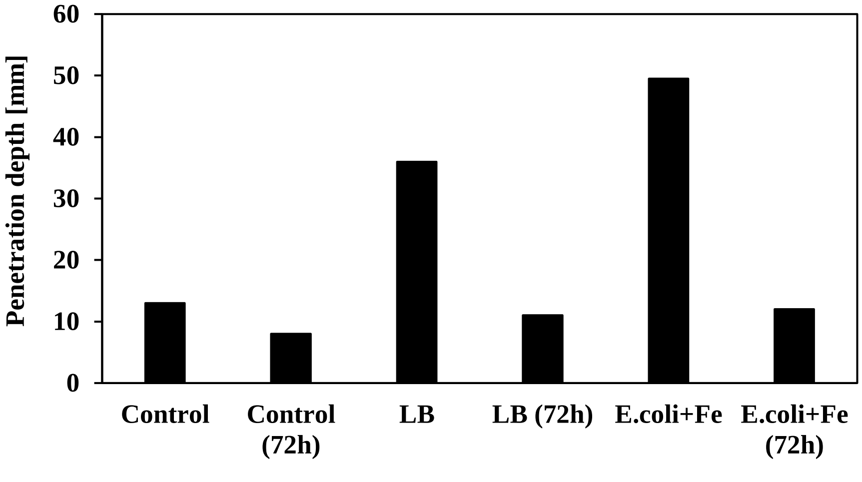

Figure 8. Penetrometer consistency of mortars

The results of the consistency of bioformulated mortars inspire more in-depth studies on the fresh state characteristics to understand the influence of components on consistency and workability of mortars. The proposal that the foamy consistency observed after production was associated to the LB, acting as an air entraining or foaming agent, could be further explored/optimized, for example, to produce lightweight earth mortars.

It was not possible to measure the wet bulk density of Control mortars after 72 h. Nevertheless, no significant change is expected since the consistency of the Control mortar did not change significantly with time (Figures 7 and 8). Results of wet bulk density are presented in Figure 9.

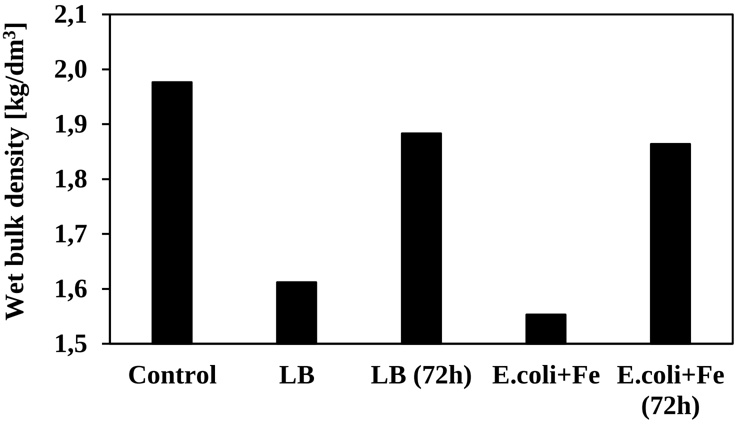

Figure 9. Wet bulk density of mortars

As expected through visual observation and workability assessment of bioformulated mortars, their wet bulk density was lower than that obtained for the Control mortar. However, all mortars have the minimum wet bulk density of 1.2 kg/dm^3, defined by DIN 18947 [46]. 72 h after production, the wet bulk density of bioformulated mortars significantly increased, approaching the value obtained for the Control mortar immediately after preparation.

4.2. Hardened state – prismatic earth mortars

As previously mentioned, tests on mortars samples prepared immediately after preparation were performed in a minimum of three replicates, except for MIP.

4.2.1. Visual observation and drying shrinkage

When comparing by visual observation the surface of the three mortars, it stands out the big pores (diameter up to 2 mm) presented by the LB and E.coli+Fe samples. No visual shrinkage was observed in any of the mortars. Control samples presented an average linear drying shrinkage of 0.4%, whereas LB mortar samples had 0.0%, and E.coli+Fe mortar samples 0.2%. Although not significantly different, bioformulated mortars present lower shrinkage than the Control mortar, which is a positive result, since some studies indicate that the durability of earth buildings may be reduced due to shrinkage cracks [60]. All tested mortars are way below the maximum drying shrinkage of 2.0% defined by DIN 18947 [46]. Although the LB and E.coli+Fe mortars are characterized by high wet bulk density variation in the first 72 h when inside a sealed plastic bag, the absence of drying shrinkage of the mortars inside the molds show that the same effect does not seem to occur when mortars are in contact with air. Moreover, the drying process of these mortars is very slow, not depending on a chemical reaction, as when using common mineral binders such as limes or cements are used.

4.2.2. Bulk density and pore structure

Bulk density, determined geometrically, and bulk density and porosity, accessed by MIP, are presented in Table 2.

Table 2. Bulk density, porosity, median pore diameter, most frequent pore diameter, maximum differential incremental mercury intrusion, thermal conductivity and dynamic modulus of elasticity of mortars

Mortar	Bulk density [kg/dm³]		Porosity [%]	Pore diameter [µm]		M.Intr. [ml/g]	Thermal conducti. [W/(m.K)]	Ultrasound velocity [m/s]
	Geometric	MIP		Median	Frequent			
Control	1.69 ± 0.03	1.74	33	29.8	33	0.05	0.77	1138 ± 24
LB	1.38 ± 0.07	1.44	44	92.5	218	0.12	0.54	1021 ± 45
E.coli+Fe	1.38 ± 0.06	1.50	38	83.6	217	0.16	0.41	1050 ± 13

As it was expected from what was observed during the formulation of mortars, bioformulated mortars have lower bulk density than the Control mortars. These results are in accordance with the ones obtained for wet bulk density, supporting the idea that bioformulated mortars are substantially more porous than Control mortars. Although the water loss between the wet and dry bulk densities is up to 300 kg/m^3, the absence of visual shrinkage on the prismatic samples may be justified by the high-water absorption of the earth clay particles.

Lima and Faria [61] studied the addition of oat straw fibers and typha fiber wool on the formulation of earth mortars with similar water content, obtaining values of bulk density ranging between 1.66 and 1.91 kg/dm^3. The results obtained in the present study for Control mortars are in that range. However, bioformulated mortars present lower values of bulk density. Gomes et al. [62] determined a bulk density of 1.7 kg/m^3 for earth-based mortars formulated with a kaolinitic earth without any addition. However, the bulk density decreased when these researchers added hemp fibers to earth mortars formulation or a combination of fibers and binders. Bulk densities of 1.5 kg/m^3 and 1.5-1.6 kg/m^3, respectively, were described. The results of the present bioformulated mortars are lower than those reported by Gomes et al. [62], even when compared with the results obtained for earth mortars formulated with the addition of hemp fibres and binders.

Bulk density determined by geometric and MIP methods are similar [42], but the MIP method allowed to differentiate LB and E.coli+Fe mortars. Therefore, whenever a more precise method is not available, the geometric method seems a good possibility to preliminary assess earth plasters bulk density and, indirectly, their porosity.

Faria et al. [42] obtained a porosity of 30-31 % for a pre-mixed earth plastering mortar with earth from the same quarry. Table 2 shows that the Control mortar presented a slightly higher porosity of 33 % while the E.coli+Fe mortar and particularly the LB mortar presented a higher porosity, 38 % and 44 % respectively. This was already expected due to the air entraining effect detected when the mortars were mixed and the fact that no significant shrinkage occurred in the prismatic samples, indicating the preservation of the porous structure. Figure 10 plots the incremental mercury porosimetry curves of the tested mortars.

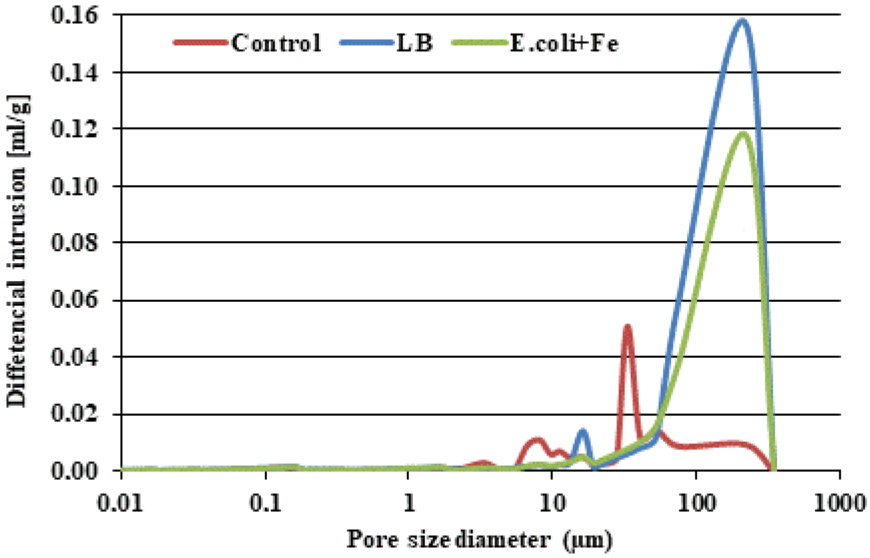

Figure 10. Differential incremental mercury porosimetry curves of mortars

It can be observed that all the mortars exhibited a bi-modal distribution (more significant for Control). Median pore diameter, most frequent pore diameter and maximum differential incremental mercury intrusion are presented in Table 2. All results confirmed the much higher porosity of bioformulated mortars in comparison to the Control and demonstrate the possibility of using LB-based bioproducts as air entraining agents for earth mortars or, eventually, for other types.

4.2.3. Thermal conductivity and ultrasound pulse velocity

As bioformulated mortars showed visible porosity, a lower thermal conductivity is expected. Results are shown in Table 2. As predicted, a significant decrease on thermal conductivity was observed on bioformulated mortars, when compared with the Control, more expressive for the E.coli+Fe mortars. It seems that the use of bacterial culture for the bioformulation of earth plastering mortars can represent an alternative to an increased addition of natural fibers for thermal conductivity enhancement. In fact, earth-based mortars and blocks prepared with natural fibers showed a reduction on the dry bulk density and a greater porosity [61,63]. Kaolinitic earth mortars with 1% or 2% of natural fibers (corn pith, barley straw and barley wool) presented thermal conductivity ranging between 0.3-1.4 W/(m.K), increasing with the decrease on the fibers content [64]. Although measured by a different method, the thermal conductivity data determined for the bioformulated mortars characterized in the present work are within the range of the ones reported by Palumbo et al. [64].

These mortars, however, cannot be classified as thermal mortars [65], since they exhibited a thermal conductivity higher than 0.2 W/(m.K). Nevertheless, the E.coli+Fe mortar presented a very positive result, especially when comparing with the behavior of plasters for thermal bridges corrections, such as EPS-cement and cork-cement mortars [66].

After observing the foamy mortar texture and visual pores of bioformulated mortars, a higher percentage of porous was expected for these mortars and, consequently, lower compactness. Averaged ultrasound propagation velocity of mortars is presented in Table 2. These data showed that bioformulated mortars seem to be less compact than the Control, results that are in agreement with thermal conductivity.

4.2.4. Dynamic modulus of elasticity, flexural and compressive strengths

When compared with the Control mortar, a considerably lower dynamic modulus of elasticity was obtained for bioformulated mortars (Table 3), indicating that these mortars are less rigid and may have a higher capacity to absorb deformations. Results of dynamic modulus of elasticity are in accordance to bulk density (Table 2). Santos et al. [59] characterized a ready-mixed earth plastering mortar and obtained 4331 ± 25 N/mm^2 of dynamic modulus of elasticity. The values obtained in the present study (Table 2) were always lower than that presented by Santos et al. [59]. Averaged results of flexural and compressive strengths are presented in Table 3. The results are consistent with the ones obtained for bulk density and dynamic modulus of elasticity (Tables 2 and 3). Flexural and compressive strength of bioformulated mortars are affected by the low bulk density obtained for bioformulated mortars due to their very porous structure. Therefore, based on the results of dynamic modulus of elasticity, lower flexural and compressive strengths were expected.

In both flexural and compressive strength parameters, all mortars present lower strengths than the minimum of 0.3 N/mm^2 for flexural strength and 1.0 N/mm^2 for compressive strength defined by DIN 18947 [46]. Therefore, the ready-mixed mortar used in the present study is a low strength earth mortar. However, when compared to EN 998-1 [65], the E-coli+Fe mortar can be classified as a general-purpose mortar, with a minimum compressive strength of 0.4 N/mm^2.

Mukherjee et al. [10] achieved an improvement of 10% for compressive strength of bioformulated earth-based blocks (Table 1). Bernardi et al. [24] attained similar compressive strength for bioformulated sandy earth blocks and analogous earth blocks but stabilized with cement or lime (Table 1). While these two last studies report a slight improvement on compressive strength of bioformulated earth blocks, probably due to the biomineralization of calcium carbonate and the binder used to stabilize the earth, the same tendency of compressive strength increase was not verified in the present study. One possibility is the effect that the binder used for stabilization may have on bioproducts induced mineralization. Nevertheless, even the addition of a low binder content to an earth mortar can decrease its strength, as observed by Santos et al. [59] when 5% of air lime was added to an illitic earth mortar with oat fibers, similar to the one tested here, resulting in about 50% reduction of the flexural strength (from 0.2 N/mm^2 to 0.1 N/mm^2) and of compressive strength (from 0.43 N/mm^2 to 0.22 N/mm^2). In that case the decrease on mechanical strength was more significant than in the present study. Further studies are needed to deeply understand the phenomena.

4.2.5. Surface hardness and surface cohesion

Table 3 presents the results obtained for surface hardness and surface cohesion of bioformulated mortars.

Table 3. Surface hardness, surface cohesion and ultrasound velocity of mortars molded after mixing

Mortar	Surface hardness [Shore A]	Mass loss by lack of cohesion [g/m^2]	Dynamic modulus of elasticity [N/mm^2]	Flexural strength [N/mm^2]	Compressive strength [N/mm^2]
Control	86 ± 5	12.1 ± 4.7	3527 ± 45	0.3 ± 0.0	0.7 ± 0.0
LB	78 ± 9	12.5 ± 4.0	1838 ± 287	0.2 ± 0.1	0.3 ± 0.1
E.coli+Fe	57 ± 11	40.6 ± 11.1	1958 ± 142	0.2 ± 0.0	0.4 ± 0.2

Surface cohesion results are in accordance with the results obtained for surface hardness, with the E.coli+Fe bioformulated mortar being the one exhibiting lower surface cohesion (Table 3). The LB formulated mortar presented a mass loss similar to the Control one, while E.coli+Fe mortar had around the triple mass loss. These results are in accordance with surface hardness, although less distinct.

4.2.7. Water drop absorption

The results obtained for the non-standardized water drop test are shown in Table 4, in terms of time to water drop absorption and percentual increase of that period. In Control samples the absorption of water was much faster than in LB or E.coli+Fe samples.

Table 4. Water drop test results of mortars

Mortar	Absorption time [s]	Increase [%]
Control	1.4 ± 0.4	-
LB	2.8 ± 0.9	100
E.coli+Fe	1.9 ± 0.6	35.7

Parracha et al. [18] tested the same bioproducts used in the present study for surface biotreatment of a similar ready-mixed earth-based plastering mortar and achieve significant positive results, increasing its resistance to water. The researchers obtained an increase of 2150 % on the absorption time of a drop of water 4 days after the biotreatment with E.coli+Fe bioproduct. The results obtained in the present study are less expressive, although bioformulated mortars showed some waterproofing effect.

4.2.8. Durability in water

Figure 11 presents a photograph of this simple test taken about 1 minute after samples of mortars were submersed in water. As it can be observed, the bioformulated mortars have considerably higher durability than the Control mortar, especially the E.coli+Fe mortar. Control mortar sample was placed in water and instantly started to disrupt. LB mortar was slowly crumbling and after about 30 seconds started to collapse. More than 1 minute after the mortars were placed in water, E.coli+Fe mortar was still intact and considerably less loose particles than the other mortars could be observed on the bottom of the container.

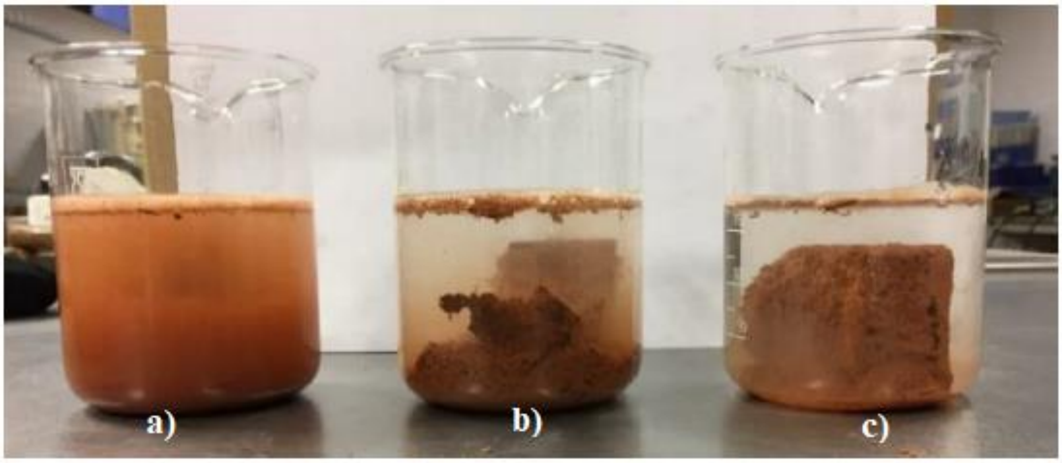

Figure 11. Mortar samples degradation when submersed in water: (a) Control; (b) LB and (c) E.coli+Fe

The results showed that bioformulated earth mortars do have significantly higher resistance towards liquid water than Control mortars. This behavior may assure that bioformulated earth plasters are less vulnerable than Control mortar when applied in more harsh environments, such as close to a window left open when it rains or during indoor cleaning maintenance actions with water. The same is verified if mortars are applied as sacrificial renders on conservation, namely on archaeological sites.

4.3. Hardened state – earth mortar plaster on ceramic brick

4.3.1. Visual observation and adhesive strength

No cracks were visually observed on the surface of any of the plaster on brick samples, which is in agreement with the fact that no shrinkage was recorded on the prismatic samples.

Three different types of fractures were observed on the adhesive test: total disruption of the mortar from the brick (adhesive rupture); partial disruption of the mortar from the brick; and disruption by the mortar (cohesive rupture). Control samples presented, in all three tests, a total disruption of the mortar from the brick, indicating that the cohesion of the mortar is higher than the adhesion to the brick. Tests in all LB and E.coli+Fe samples resulted in a partial disruption of the mortar from the brick, suggesting that the adhesion to the brick was close to the cohesion of the bioformulated mortar.

Quantitative results are shown in Table 5. Although the EN 998-1 [65] does not have a minimum value for general purpose plasters, the Control and E.coli+Fe plasters were close to the minimum value of 0.05 N/mm^2 of adhesive strength defined by DIN 18947 [46]. Furthermore, the value of adhesive strength obtained for E.coli+Fe mortar was not lower than that obtained for the Control.

Table 5. Adhesive strength, compressive strength, dry abrasion resistance, surface hardness and surface cohesion of earth mortar plaster on ceramic brick

Mortar	Adhesive strength [N/mm^2]	Compressive strength [N/mm^2]	Mass loss by dry abrasion [g]	Surface hardness [Shore A]	Surface cohesion [g/m^2]
Control	0.04	3.2	3.1 ± 1.7	82 ± 5	1.4
LB	0.02	1.3	2.4 ± 0.6	77 ± 6	2.3
E.coli+Fe	0.04	1.1	3.3 ± 0.5	78 ± 7	5.2

Lima and Faria [61] determined an adhesive strength of 0.09 N/mm^2 for earth plastering mortars formulated with oat fibers, 0.11 N/mm^2 for a similar plaster but using typha fibers instead of oat fibers, and 0.07 N/mm^2 without any addition in the formulation. In the present study, all earth plasters presented lower adhesive strength in comparison to the results obtained by Lima and Faria [61]. Nevertheless, Faria et al. [67] showed that for earth plasters, the cut of the specimens for adhesive test (before or after the mortar is dried) and the application of a clay grout or just water previous to the plaster have significative influence on test results, which is important to low strength plasters such as earthen or air lime based. The authors obtained an adhesive strength of 0.04 N/mm^2 for similar earth plaster but with oat fibers addition. This result is in accordance with the one obtained in the present study for the Control and E.coli+Fe mortars.

4.3.2. Compressive strength

Table 5 presents the results obtained for compressive strength. These results are in accordance with the minimum of 1.0 N/mm^2 defined by DIN 18947 [46] for earth plasters but were obtained by a distinct method, although more representative of on-site requirements. Nevertheless, bioformulated mortars presented considerably lower compressive strength than Control mortars.

Despite higher compressive strengths were obtained for samples applied on the brick for all three formulations in comparison with the prismatic ones, the difference between Control and bioformulated mortars was similar to the strengths obtained on earth mortar prismatic samples (up to 2-3 times higher).

4.3.3. Dry abrasion resistance, surface hardness and surface cohesion

Due to the lack of cohesion observed during the demolding of bioformulated mortars prismatic samples, a medium hardness brush was used to measure dry abrasion resistance. The results are shown in Table 5.

Despite what was observed in the prismatic samples, weight loss from abrasion in bioformulated mortars was similar to the one obtained for Control mortar. In fact, even LB bioformulated mortar showed lower mass loss.

The difference in the finishing surface between the samples on brick and the prismatic samples was that in the first ones a plastic float trowel was used. This finishing method, close to the one performed *in situ*, probably caused deaeration at the surface of the bioformulated mortars layer, increasing its cohesion.

All mortars present higher mass loss than the maximum of 1.5 g defined by DIN 18947 [46].

Faria et al. [42] assessed dry abrasion resistance of an earth plaster using a similar medium hardness brush, following the experimental procedure described on section 3.3. A weight loss of 4.5 g was obtained, which is higher than any of the results obtained in the present research study.

When handling the bioformulated prismatic samples, they were considerably more friable than the Control mortar samples. The same friability was not observed on the earth plastering mortars applied on brick because the mortars were thrown on top of the brick (in the laboratory, falling from a defined height) and pressed against the brick to align and compact the surface of the plaster, simulating an *in situ* plaster application. Also, one must consider the influence of the brick itself and the drying surface. For these reasons, surface hardness was also tested on these samples (Table 5). These results showed, within the experimental error, that bioformulated and control mortars have similar surface hardness.

When compared with the results obtained on the cubic samples, E.coli+Fe plaster samples presented a surface hardness almost 40% higher. This different behavior may be associated to the method used to apply the mortar on brick samples - a more similar method to the currently used on-site.

For surface cohesion, bioformulated mortars showed higher mass loss and, consequently, lower surface cohesion, than Control mortars (Table 5). All formulated mortars cubic samples presented considerably higher mass loss than the plaster samples.

4.3.4. Water drop

The results obtained for water drop test are shown in Table 6. The values obtained for bioformulated mortars (LB and E.coli+Fe) presented a significant increase on the time to absorb a drop of water relative to those obtained for Control mortars, showing a considerably lower water absorption rate.

Table 6. Water drop test results for earth mortar plasters on ceramic brick.

Mortar	Absorption time [s]	Increase [%]
Control	1.1 ± 0.5	-
LB	6.0 ± 2.6	445
E.coli+Fe	8.7 ± 0.6	691

On the cubic samples, bioformulated mortars had already shown positive results, although less evident than the ones obtained for the earth plasters on brick.

5. Conclusions

In this research study, the effect of an innovative iron-based bioproduct applied in the formulation of earth mortars was assessed. The main conclusions are the following:

- The properties of the plaster simulated on brick samples were different from samples of the same mortar molded and tested in prismatic samples. This distinct behavior was probably due to the method used to apply the mortar layer over the brick, simulating in-situ application conditions – the mortar was left to fall from a defined height to simulate the energy of in-situ applications and a trowel was employed to compact the surface of the mortar on the brick – and the water suction of the brick from the fresh mortar. This effect was particularly noticed due to the porous structure of the bioformulated mortars.
- The bioformulated mortars presented lower mechanical characteristics than Control mortar what was justified by a lower density of those mortars, due to their highly porous structure.
- Since the highly porous structure was observed in both LB and E.coli+Fe bioformulated mortars, one can infer that was associated to the presence of LB medium in the two bioproducts. When higher strength plasters are intended, most probably the LB medium can be removed or decomposed in its constituents (yeast extract, peptone and sodium chloride) to identify the "foamy" component. In parallel, the effect of LB can be optimized so that LB-based bioproducts can be studied as innovative air entraining agents for other mortars and concrete.
- In comparison with Control, bioformulated mortars showed longer durability in water, especially the E.coli+Fe bioformulated mortar, suggesting a positive activity of bacterial cells. Further studies on the microstructure of these bioformulated mortars are fundamental to explain their greater durability.
- Thermal conductivity of bioformulated mortars was considerably lower, suggesting a potential application of these mortars to contribute to thermal insulation. This enhancement was justified by the high porosity of bioformulated mortars.

The reversibility and compatibility of earthen mortars with old substrates and their increased resistance towards water make them more durable for conservation interventions, namely when applied to protect archaeological structures as sacrificial renders. It is considered that a more in-depth study on the bioproducts may surpass the weakening of mechanical strength of bioformulated tested mortars. In parallel, the same effect can be further optimized for lightweight water-resistant earthen plasters to be applied on new construction with contribution to thermal insulation. Therefore, further studies using eco-friendly nontoxic iron-based bioproducts for earthen mortars optimization are justified, so that this type of ecological plasters can be even more efficient and widely used.

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