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**Bioconsolidation of construction
materials – Effect on the durability of an
eco-efficient earthen plaster**

Dissertação para obtenção do Grau de Mestre em
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Abstract

Bioconsolidation is a relatively novel technique used for consolidation, repair, protection and improvement of construction materials. This biotechnology is based on the precipitation of chemical compounds produced by microbial metabolism. It can be used for treatment or formulation.

Although recently applied in several construction materials, such as earth blocks or cement-based materials, bioconsolidation has been unexplored on earth-based plastering mortars. Although the use of earth mortars for earth plasters has been growing on recent years for its eco-efficiency, they are less resistant to damage by contact with water. Therefore, the use of a bioproduct may have a great potential for the improvement of earth mortars' weaknesses.

In the present thesis, two types of bioconsolidation techniques have been studied on earth mortars: as a biotreatment for the improvement of surface properties; and as a component for earth mortars' formulation (bioformulation) for the improvement of the whole material.

The bioproducts used are based on microbial iron mineralization using *Escherichia coli* cells, since iron compounds are present in earth materials, are non-toxic, easy to handle and are not expensive. Several experimental conditions have been studied leading to the proposal that iron concentration is a key parameter.

Tested biotreatments show promising results, producing a slight consolidative effect and significant increasing of water absorption resistance of earth mortars. Bioformulated mortars present a very distinct macrostructure, with a great decrease on mechanical properties. Nevertheless, they achieve a considerable improvement on resistance towards water and a lower thermal conductivity.

The results show the interest on further studies on the use of iron-based bioproducts on earth mortars.

Keywords: Bioconsolidation; Iron-based bioproduct; Biotreatment; Bioformulation; Clayish materials; Earth plaster.

Resumo

A bioconsolidação é uma técnica inovadora que tem despertado interesse para consolidação, reparação, proteção e melhoria das propriedades de materiais de construção. Esta biotecnologia baseia-se na precipitação de compostos químicos através do metabolismo de culturas bacterianas.

Apesar de recentemente aplicada em diferentes materiais, desde blocos de terra a materiais cimentícios, o efeito da bioconsolidação ainda não foi estudado em argamassas de terra para rebocos. A utilização de rebocos com argamassas de terra tem vindo a crescer nos últimos anos devido à sua ecoeficiência. Assim, existe um grande potencial no uso de um bioproduto para atenuar as limitações que estas argamassas apresentam, principalmente face à ação da água.

Na presente dissertação foram estudadas duas abordagens para a aplicação de bioprodutos em argamassas de terra: como biotratamento, para a melhoria das propriedades superficiais; e como um componente na formulação das argamassas (bioformulação), para a sua melhoria em geral.

Utilizaram-se bioprodutos produzidos por culturas de *Escherichia coli* suplementadas com ferro, uma vez que os materiais argilosos contêm compostos de ferro e porque é financeiramente acessível, não tóxico, e fácil de manusear. Foram ensaiadas várias condições experimentais, levando à conclusão de que a concentração de ferro é um parâmetro chave.

Os biotratamentos ensaiados mostram resultados promissores, sendo observado um ligeiro efeito consolidante, mas especialmente uma maior resistência à absorção de água. As argamassas bioformuladas apresentam uma macroestrutura muito distinta, sendo registada uma diminuição significativa das resistências mecânicas. No entanto, verifica-se uma considerável melhoria no comportamento face à água e uma menor condutibilidade térmica.

Os resultados obtidos demonstram o interesse na continuidade de estudos sobre a utilização de um bioproduto à base de ferro em argamassas de terra.

Palavras-chave: Bioconsolidação; Bioproduto à base de ferro; Biotratamento; Bioformulação; Material argiloso; Reboco interior de terra.

Notations

Control – Non-treated or non-bioformulated specimens

“H₂O” – Water treated specimens

“H₂O+Fe” – Specimens treated with an aqueous solution of iron

“LB” – LB medium treated or formulated specimens

“LB+Fe” – LB medium supplemented with iron treated specimens

“E.coli+Fe” – *E.coli* culture supplemented with iron treated specimens

“E.coli+Fe+Dps” – *E.coli* culture expressing Dps supplemented with iron treated specimens

“LB++Fe (1mL)” – 1 mL of LB medium supplemented with five times more concentrated iron treated specimens

“E.coli++Fe (1mL)” – 1 mL of *E. coli* culture supplemented with five times more concentrated iron treated specimens;

“H₂O++Fe (1mL)” – 1 mL of water supplemented with iron treated specimens

“LB++Fe (2mL)” – 2 mL of LB medium supplemented with five times more concentrated iron;

“E.coli++Fe (2mL)” – 2 mL of *E. coli* culture supplemented with five times more concentrated iron treated specimens.

LB – Lysogeny broth

MICP – Microbially induced calcium-carbonate precipitation

MIIP – Microbially induced iron-oxide precipitation

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1. Introduction

1.1. Context and motivations

Bioconsolidation of construction materials is a novel technique based on the use of bacterial cultures supplemented with nutrients for the improvement of construction materials. This biotechnology has been used as a treatment of degraded materials or as component/adjuvant on the preparation of construction materials (bioformulation).

Studies on bioconsolidation of construction materials started with the use of microbial induced calcium-carbonate precipitation (MICP) for the restoration of degraded limestone in ancient monuments. Due to the achievement of very good results, the application of this methodology largely expanded to most of the commonly used construction materials (concrete and cement mortars, ceramic bricks and earth-based blocks).

Sand and soil consolidation has also been studied with resort to microbially induced calcium-carbonate precipitation. More recently, sand and soil consolidation has been performed through microbially induced iron-oxide precipitation (MIIP), achieving as good or better results than the ones obtained with MICP.

MIIP has not yet been tested in construction materials, possibly due to the incompatibility of iron-oxide precipitation with reinforced concrete, the most extensively studied material by MICP. On the other hand, a great compatibility can be obtained with earth-based construction materials, since most commonly used bacterial cultures inhabit earth which contain iron-based minerals.

Earth-based construction has been used for centuries and since the 80s, after falling in disuse for a couple of decades, has been gaining strength mainly because of ecological issues. Considered an eco-efficient construction material, with high capacity for water vapor adsorption and low embodied energy, earth mortars can significantly contribute for the enhancement of living conditions (Lima et al., 2014). On the other hand, earth mortars are considerably fragile, mainly when in contact with water, and there is a crescent need on the achievement of more resistant to weathering and aging earth mortars.

Founded on the above-mentioned concepts, the use of iron-based bioproducts on earth mortars can bring great achievements. As most studies testify (Jroundi et al, 2010a and 2010b; Le Métayer-Levrel, 1999), bioconsolidation can increase cohesion and decrease liquid water absorption without considerably affecting water vapor permeability.

With resort to these iron-based bioproducts, earth mortars' improvement might be achieved either by using the bioproducts on their formulation, or by using the bioproducts as a superficial treatment of previously applied earth plasters.

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1.2. Objectives and methodologies

The present study focused on the development of a bioproduct based on iron biomineralization using *Escherichia (E.) coli* hosting cells grown in a rich culture medium supplemented with iron. The produced iron-based bioproducts are tested on earth plastering mortars. The main aim is to improve plasters behavior when facing water.

Two different application of the bioproducts methods are tested: as a surface consolidation biotreatment; and as a water substitute on the formulation of earth mortars.

Improvements (and drawbacks) on the properties of the resulting earth mortars are assessed using different adequate tests.

1.3. Thesis's organization

After the present Chapter, a bibliographic review on bioconsolidation effects on construction materials is presented on Chapter 2. This review was fundamental for the knowledge of the bioconsolidation processes, their applicability in different construction materials and their improvements. As this study focused on the bioconsolidation of earth mortars, Chapter 2 also gives a general description on earth mortars, their weaknesses and strengths. Besides this, current treatments, additions and admixtures used for the improvement of earth mortars durability are also mentioned.

Used materials and test procedures are described on Chapter 3. Bioproducts and mortars production and methodologies of application of bioproducts are defined; test procedures used to assess possible improvements of each bioproduct application are outlined.

On Chapter 4 results obtained for each bioproduct application are presented, analyzed, discussed and compared with current techniques used for the improvement of earth mortars.

Final conclusions and future project developments are described on Chapter 5.

After References, detailed and individual results of all experimental campaigns can be consulted on the Appendix.

2. Bioconsolidation of construction materials

2.1. Initial remarks

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 and cement mortars, ceramic (fired) clay bricks and (unfired) earth blocks, to limestone and gypsum plasters, it is a sustainable technique that has proven great improvements on the properties of these materials (Achal et al., 2015a). Construction-related biotechnologies have been recently summarized by Ivanov et al. (2015).

Bacterial cells are currently used to precipitate calcium-carbonate on the surface or on the formulation of materials through a biological response mainly denominated as microbially induced calcium carbonate precipitation (MICP). Two major types of applications rely on MICP: as a biotreatment (occurring a biodeposition effect) and as a bioformulation (occurring a biocementation effect). The first one, biodeposition, is a superficial effect in which a bacterial culture is applied at the surface of the material. Biocementation occurs when microbial cells are integrated on the matrix of the material during formulation, acting as a binder, connecting the particles that constitute the material. Bioconsolidation may involve both methods. But consolidation may not be the only improvement that is foreseen by these methods; other improvements may be achieved and included in the general term of bioconsolidation.

Besides MICP, iron oxide producing microorganisms also have potential and have already been tested in soils but 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.

Bioproducts, based on MICP, for biotreatment or bioformulation, have been widely tested in cementitious materials: in concrete (Khaliq et al., 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., 2010a). Both types of materials have shown great improvements.

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

2.2. Microbially induced calcium-carbonate precipitation

Over the years, many different bacteria species have been used in MICP processes, based on their metabolic ability to precipitate calcium-carbonate. For this reason, cells from the *Bacillus* genus have been largely used due to its urease activity and subsequent ability to precipitate CaCO_3 .

In civil engineering, calcium-carbonate precipitation mainly occurs in an induced manner. This means that the type of precipitated mineral depends of the environment conditions (DeMuynck et al., 2010). There are five major factors that control the MICP process: calcium concentration; dissolved inorganic carbon concentration; pH; availability of nucleation sites, that guarantee the conditions for reactions to happen; and presence of urea (Achal et al., 2015b). Besides calcium-carbonate precipitation being a natural phenomenon, the combination of the previous factors with the most adequate bacteria species may lead to a controlled enhanced benefic precipitation of calcium-carbonate in construction materials.

All this process is governed by a series of reactions that occur at the nucleation sites. Hydrolysis of urea, catalyzed by urease, produces ammonium and subsequently carbonate ions (Equation 2.1). The consequent increase of pH stimulates precipitation according with Equation 2.2.



As mentioned before, MICP can be divided in two major forms of applications: biodeposition and biocementation. These two processes tend to overlap in many cases since, in practical terms, they are separated by a thin line.

2.2.1. Biotreatments

Biotreatments are mostly used for building conservation or rehabilitation or for the improvement of pre-casted construction materials. In biodeposition the treatment is superficial, performed on the surface of a porous material, creating a superficial layer with calcite. This layer will consolidate the material, protecting it from different types of intrusion. Some studies have demonstrated that, although being superficial applications, biotreatments can reach depths that may guarantee a long-term bond between the calcite layer and the support (Jimenez-Lopez et al., 2008).

This sustainable technique has been widely used in conservation and repair of limestone, more oriented to monuments' facades with positive results, due to the compatibility between the treatment and the limestone and a decent consolidation effect of the support surface (Le Métayer-Levrel et al., 1999; Jroundi et al., 2010b).

It is applicable to most common construction materials, like bricks and cement-based elements, acting as a waterproofing coating significantly reducing water absorption and improving mechanical resistances, like cohesion and compressive strength.

2.2.2. Bioformulations

Unlike biodeposition from biotreatments, biocementation is mostly an in-depth effect. Bacterial cells are included in the products manufacture as a starting material and, in this case, on MICP calcium-carbonate will work as a binder, a biocement. Precipitated between particles, calcite will bind them forming a cohesive material. This technique might also be used as a substitute (or partial substitute) for common binders, as cement, lime or clay, ensuring adequate mechanical resistances and improving water impermeability. It is noteworthy that in cases where bacteria and binder are used together, bacteria cells should be compatible with the high alkalinity of binders (Achal et al., 2015b).

Since earth construction is a sustainable building technique, the use of biocementation in this type of construction may bring a great innovation, increasing durability by the improvement of cohesion and resistance to water in an eco-efficient way.

The study on the viability of injections of a bioproduct made with fly ashes and *Bacillus cereus* cells for the consolidation of earth walls is an example of a technique that can be combined with the use of a biotreatment, allowing to consolidate a support either in depth and at the surface (Dhami et al., 2013).

2.3. Biotreatments - Literature studies

2.3.1. Limestone

A large part of studies on limestone are related with case studies where biodeposition is an innovative treatment for the conservation and repair of monuments' facades. In France and Spain, biodeposition is a practice that has been growing with very positive results.

The studies on biodeposition for conservation of limestone started in the nineties. In 1993, Le Métayer-Levrel et al. (1999) applied a biotreatment based on bacteria and nutritive medium to the stone of the southeast tower of Saint Médard Church (Paris, France). The researchers found that water absorption had decreased significantly while no changes were observed for water vapor permeability, reducing moisture problems. Furthermore, after four years the appearance remained like the original, what is very important since aesthetics is one of the main points when treating monuments' facades.

More recently, Jroundi et al. (2010a, 2010b) used a biotreatment on the stones in San Jeronimo Monastery and in the Royal Chapel, both in Granada, Spain. In the first case (Jroundi et al., 2010a), researchers have applied two different biotreatment approaches: taking advantage of native bacteria cells that inhabit the stone to be biotreated; and biotreatment with a bacterial *Myxococcus xanthus* culture. In the Royal Chapel (Jroundi et al., 2010b), only the bacteria inhabiting the stone were used. In both cases, bacterial growth was stimulated by spraying of nutritive culture medium. Although the co-cultured bacterial biotreatment demonstrated slightly better results, both approaches resulted in significant improvements.

The superficial consolidation was tested using peeling tapes of 7.0 x 3.5 (cm). The tapes were attached with identical pressure on the surface, detached and weighted before and after treatments. In both San Jeronimo Monastery and Royal Chapel the results showed a weight reduction of the peeling tapes higher than 50%.

Another important point was to verify if changes in aesthetics occurred, because facades must maintain their original aesthetics. Color measurements were performed with a Minolta Chroma Meter spectrophotometer. The results proved that aesthetics was maintained.

Furthermore, Jimenez-Lopez (2008) had already studied the applicability of biotreatment to limestone withdrawal from a quarry in Spain. Specimens of 2 x 5 x 0.5 (cm) were used; half of the specimens were sterilized to inactive bacteria inhabiting the stone. Half of the sterilized and non-sterilized specimens were immersed in nutritive medium while the other half was immersed in nutritive medium inoculated with *Myxococcus xanthus* cells. Superficial consolidation was tested with a sonication test. Presence of bacteria, either the native inhabiting the stone or added bacteria, presented better results with a mass loss

30% lower than the sterilized specimens. Besides the sonication test, back-scattered electron imaging showed that the average thickness of precipitated calcium carbonate is in a range from 10 μm to 50 μm , depending on the growth medium used to cultivate bacterial cells.

All these applications showed excellent results for biotreatment of limestone, either using bacteria inhabiting the stone or adding new bacteria. The common classical techniques use synthetic materials that most of the times are not compatible with the stone and tend to produce damages in the long term. Besides the applicability, eco-efficiency is a matter of extreme importance, and nowadays biotreatment seems to be the more eco-efficient and compatible technique for this type of repair.

2.3.2. Gypsum plaster

Jroundi et al. (2014) tested bioconsolidation in historical pieces of gypsum plaster collected from the Medieval archaeological site of Alcázar de Guadalajara (Guadalajara, Spain). The effect of the bio-based treatment was compared with the ones with conventional consolidants. Three conventional consolidants were applied by brush: tetraethoxysilane (TEOS); a copolymer of ethylmethacrylate and methylacrylate monomers (PEMA/PMA); and polyvinyl butyral (PVB). The biotreatment consisted in enhancing the growth of bacteria inhabiting the gypsum by spraying of nutritive medium M3-P (applied 2 times a day for 6 days). Three different tests were performed to verify the consolidant effect on the different treatments. To evaluate consolidation performance, a Drilling Resistance Measurement System (DRMS) was used. The results obtained before the application of any treatment are in a range of values between 0.24 N/mm and 0.84 N/mm. The treatment with TEOS showed a minimal improvement in consolidation: a 0.84 N/mm drilling resistance was obtained. On the other hand, the use of PEMA/PMA and PVB had an increase of drilling resistance to 2.4 N/mm for depths lower than 3 mm; for higher depths results similar to the ones obtained with specimens before treatment were obtained. However, biotreated specimens achieved a drilling resistance of 1.7 N/mm in the entire drilled depth. These drilling resistance results were confirmed by Scanning Electron Microscopy analysis, which showed that treatment with M3-P reached good depths of bioconsolidation (≈ 14 mm) while treatments with conventional consolidants were limited to a superficial layer.

As in limestone treatments, it is important to maintain original aesthetics. Therefore, color measurements were performed with a Minolta Chroma Meter spectrophotometer. None of the treatments showed a considerable aesthetic change.

Therefore, results obtained for the biotreatment using bacteria inhabiting gypsum are very promising, showing an in-depth consolidation.

2.3.3. Ceramic bricks

The application of biodeposition on ceramic bricks has been investigated to improve bricks properties, specially their resistance towards water absorption. All treatments were performed by immersion of the bricks. Decreases in water absorption of almost 50% have been observed depending on the type of medium used to feed bacteria.

Moreover, since bricks are a porous material, calcite will precipitate easily between the pores and increase mechanical resistances. This technique, apart from improving bricks properties, can be used to repair

deteriorated brick masonry walls, consolidating the wall and the connection between bricks, and improving damp resistance and resistance towards water absorption.

Raut et al. (2014) and Sarda et al. (2009) used *Sporosarcina pasteurii* (formally known as *Bacillus pasteurii*) to precipitate CaCO_3 on the surface of ceramic bricks. The bricks were immersed in nutritive medium inoculated with the bacterial cells. Raut et al. (2014) studied two different types of nutrient medium: OptU, a culture medium optimized in laboratory and Nutrient Broth. Sarda et al. (2009) used Brain Heart Infusion and Nutrient Broth. In this case, the authors performed a screen check for the strain of bacteria most efficient in the production of urea, to easily promote calcite precipitation. Researchers observed that *Sporosarcina pasteurii* had the greater potential.

In both studies, water absorption was tested by immersion of the bricks in water and their weighing before and after immersion. Despite the results being presented in different measurement units, it can be concluded that biotreatment reduces water absorption almost for 50% when OptU medium or Brain Heart Infusion medium were used. It should be noted that the latest are the richest culture medium but also the most expensive. The results obtained with Nutrient Broth have shown less than 20% water absorption reduction when compared with control bricks.

Dhami et al. (2012) tested water absorption, compressive strength and durability of two different fired red ceramic ash bricks, rice husk ash and fly ash bricks. Bricks were submersed in a bacterial culture of *Bacillus megaterium* in NBU growth medium (nutrient broth with urea and calcium chloride). After four days, bricks were withdrawn and sprayed with NBU medium and incubated four more days.

Microbial treatment resulted in a significant decrease of water absorption: 7% and 6% decrease, for rice husk ash bricks and fly ash bricks, respectively.

Compressive strength has also shown great improvements when the biotreatment is applied. In rice husk ash bricks, compressive strength was improved from 9.7 MPa to 12.8 MPa, while an improvement from 11.68 MPa to 14.94 MPa was achieved in fly ash bricks.

Durability was tested through a freeze-thaw resistance test. Compressive strength results after freeze-thaw showed no significant decrease in biotreated and untreated bricks. It is possible to conclude that a more superficial bacterial treatment does not contribute significantly to bricks durability in harsh environments.

Raut et al. (2014) also tested the compressive strength of biotreated fired red ceramic bricks. The frogs had to be filled with cement mortar and bricks were tested in a compressive strength testing equipment. When compared with the results obtained for the control bricks (about 4.1 MPa), bricks biotreated with *Sporosarcina pasteurii* and OptU medium had an increase of compressive strength of 83.9% (about 7.54 MPa), while bricks biotreated with *Sporosarcina pasteurii* and Nutrient Broth had an increase of only 24.9%.

The use of biotreatment in ceramic bricks demonstrated very good results, but these treatments have been performed in a small scale. A deeper study, at a larger scale, needs to be performed taking in account the costs related with bacterial cultivation and nutritive medium application.

2.3.4. Cementitious materials

Biotreatment of cementitious materials have been extensively studied. The results of some studies are synthesized.

De Muynck et al. (2008) studied the benefits of a bacterial-based treatment in cement mortar, comparing with conventional techniques. Twenty-seven different types of treatment were tested: 6 surface coatings, 11 penetrating sealants – both considered conventional treatments - and 10 types of bacterial-based treatments.

Conventional treatments were applied by brush. Two different biotreatments were applied: by immersing the cement mortar specimens in a *Bacillus sphaericus* culture and then in a nutrient solution; the other by applying on one of the surfaces of specimens a biopaste of ureolytic mixed bacterial cultures followed by immersing the specimens in nutrient solution.

The authors tested capillary water absorption, according to RILEM 25 PEM (Test II.6) (RILEM, 1980), in cement mortar cubic specimen of 40 mm side. The results have shown that the biotreatment with *Bacillus sphaericus* achieves similar values of resistance to water absorption as conventional treatments. On the other hand, the biotreatment with mixed ureolytic cultures could not reach the same improvement.

Instead of using bacterial cells, pure urease enzyme can be used to precipitate calcium-carbonate. Cardoso et al. (2016) used the enzyme to improve cement mortar cubic specimens. The effect of different treatments was analyzed: i) microbial treatment with *Sporosarcina pasteurii* and nutrient medium; ii) effect of urease and nutrient medium; iii) feeding solution, using only nutrient medium; iv) cure in tap water; and v) air, cured in wet environment.

Water absorption by capillary test showed that only specimens biotreated with enzyme urease had a significant decrease.

As seen in water absorption tests, urease enzyme has shown greater improvements in water vapor permeability, despite being a small increase. If greater results could be achieved, the urease biotreated specimens would have a greater capacity to dry, improving durability and mortar damp resistance.

Ramachandran et al. (2001) tested crack remediation of cement mortar with *Sporosarcina pasteurii*. The authors performed one test where the cement mortar specimen (50.8 x 50.8 x 50.8 (mm)) were cut to simulate a crack. Three different crack depths were made and the cracks were biotreated with a suspension of *Sporosarcina pasteurii*, sand and urea-CaCl₂ broth. The results obtained for compressive strength show that the bacterial treatment is more effective for higher depths, reaching 64% increase when compared with control specimens, which had the crack filled with sand and water.

Bang et al. (2010) have pre-cracked cement mortar specimens and then biotreated the cracks with bacterial cells. Bacteria filled and bond the fissures, resulting in an in-depth biotreatment. To test compressive strength, cubic cement mortar specimens were used. The cracks of the specimens were impregnated with *Sporosarcina pasteurii* immobilized in glass beads, pre-cultured in ATCC 1832 medium. When compared with control specimens, the ones who had the cracks biotreated presented a compressive strength 25% higher, concluding that this treatment works as a crack repair system.

It should be noted that the use of sand on the repair system brought better results than protecting bacterial cells by immobilization because the difference obtained for compressive strength was remarkable.

Stiffness was tested with cement mortar beams of 24.4 x 25.4 x 152 (mm). For this test, the same procedure used to obtain compressive strength was carried out. A 12% higher stiffness in comparison with control specimens was observed.

More recently, Wiktor et al. (2015) applied biotreatment to repair a parking garage with a damaged deck. Researchers used a repair system composed by two solutions, applied concomitantly: Solution A, with sodium-silicate, sodium gluconate and an alkaliphilic bacterium; and Solution B, with calcium-nitrate and an alkaliphilic bacteria. An area of the concrete pavement, 2.0 x 0.5 (m), and three cracks (1-3 mm wide) were biotreated with this bacteria-based repair system.

Resistance to freeze-thaw was tested in laboratory. Six concrete cores were analyzed, 3 from the biotreated area and 3 from an untreated area. The biotreated cores had a mass loss of $1.9 \pm 0.3 \text{ kg/m}^2$; the untreated area had a mass loss of $3.6 \pm 1.3 \text{ kg/m}^2$, expressing the positive effect of the microbial treatment.

To verify cracks sealing, an *in situ* water permeability test was performed. A wooden frame was placed on top of the cracks and sealed with silicon glue, prior to pouring 5 liters of tap water. The untreated cracks were heavily leaking, while two of the biotreated cracks exhibited a few dripping spots and the third biotreated crack was not leaking at all.

These results are very encouraging to re-enforce the benefits of a bacteria-based repair system for concrete.

2.4. Bioformulation - Literature studies

2.4.1. Earth-based blocks

Bioformulation or biocementation can have an important role in earth construction, limiting water absorption and increasing consolidation.

The use of bioformulation in earth blocks has been studied by Dhimi et al. (2015) to help stabilizing the blocks, decreasing their water absorption. The blocks were prepared with 50% soil and 50% sand, with the addition of Nutrient Broth medium supplemented with CaCl_2 and urea, inoculated with *Bacillus megaterium*. 40% reduction in water absorption and a decrease in linear expansion were observed resulting in a more stable earth block.

A similar study was described by Mukherjee et al. (2013), using soil-cement blocks of 230 x 110 x 60-75 (mm) made with an undefined percentage of cement. *Bacillus megaterium* culture was added while making the blocks. Blocks were cured by spraying nutritive medium for 28 days and kept for drying for more than 30 days.

Water absorption was tested by immersing the blocks in water for 24 hours; the blocks were weighted before and after immersion. Untreated blocks had a 9.9% water content, while bioformulated blocks had 6.6%, a 33.6% reduction.

A wet compressive strength test was performed by Mukherjee et al. (2013) by immersing the earth-cement blocks in water for 48 hours. The bioformulated blocks had a 10% improvement in wet compressive strength when compared with untreated blocks.

To evaluate how water affected the dimensional stability of blocks, linear expansion on saturation was tested by soaking the blocks in water for 48 hours, measuring it before and after soaking. While untreated blocks had a linear expansion of 0.09%, the bioformulated blocks presented a linear expansion of only 0.05%. The previous results can be supported with the ones obtained by Mercury Intrusion Porosimetry (MIP). Untreated blocks had a total porosity of 25.4%, while bioformulated blocks only had 17.4%, a 31% reduction. It is possible to conclude that calcite crystals act as a biosealant, improving the behavior of blocks.

Bernardi et al. (2014) tested different types of sandy earth blocks. Blocks with dimensions of 91 x 58 x 200 (mm) were made with silica rich sandy earth and for stabilized ones, a binder was added: natural hydraulic lime (EN 459-1 NHL5) (CEN, 2015) or type II/V cement (according to ASTM C150 (ASTM, 2016)). NHL5 was added at 20, 25, 30, 40 and 50% (v/v). Cement was added at 5, 10, 15, 20 and 25% (v/v). After mixing the sandy earth – and one of the binders for stabilized blocks - with water, blocks were tamped for 50 times with a steel tamper from a 2.54 cm high and left to cure for 7, 14 and 28 days.

Blocks without binder stabilization were biocemented with *Sporosarcina pasteurii* ATCC 11859 culture. *Sporosarcina pasteurii* was grown in Ammonium-Yeast Extract (ATCC 1376) for 24 hours. Blocks were set vertically to allow the percolation of bacterial culture from top to bottom, for 4 hours. Blocks were then fed with urea-calcium medium, in an average of 3 times a day, and compressed with a low confining stress of approximately 10 kPa. Blocks were treated 21, 42 and 84 times during the 7, 14 and 28 days curing.

Compressive strength tests were performed. In bio unstabilized blocks, high compressive strengths were achieved, up to 2.2 MPa with longer biocementation. Similar results were obtained with sand-lime blocks and sand-cement blocks, with lime blocks reaching values of about 1 MPa and cement blocks 2.5 MPa. When compared with stabilized blocks, bacteria-treated blocks can reach as good or higher strengths as the stabilized ones.

P-wave velocity tests and calcite concentration measurements were performed by Bernardi et al. (2014) to verify how calcite precipitation was dispersed along the bacteria biocemented blocks. Contradictory results were obtained; while p-wave velocity measurements show that, in general, velocity decreased from top to bottom, calcite concentration tends to be higher on the bottom of the block. From these contradictory results, it was possible to conclude that precipitation of calcite on the blocks was not homogeneous.

Despite showing good results, these biocemented sand-based blocks need further investigation in order to achieve a more homogeneous calcium-carbonate precipitation. It should be noted that even if a treatment-like application method is being used, the principal objective is the occurrence of a biocementation process.

2.4.2. Sand and soil consolidation

Sand bioconsolidation results have been one of the main boosters of using MICP in soil consolidation in Geotechnical Engineering (Umar et al., 2016). Most of the above-mentioned studies have been performed to understand the binding capacity of calcium biomineralization in sand-based construction materials.

As mentioned above for the case of Bernardi et al. (2014), despite sand and soil consolidation present a treatment-like application, the main objective is reaching biocementation.

Achal et al. (2011) performed a simple test preparing sand columns with *Sporosarcina pasteurii*, bioconsolidating either with Corn Steep Liquor or Nutrient Broth medium.

Upon the bioconsolidation of specimens, nutritive medium was shed through the sand columns and flow rate was measured. After 10 days of biocementation the control specimen presented a flow rate of 2.6 mL/min; specimens treated with Corn Steep Liquor were totally obstructed after 8 days, while total obstruction was observed after 10 days-treatment with Nutrient Broth.

As Achal et al. (2011), Dhami et al. (2012) studied the effect of *Bacillus megaterium* cells on sand columns. NBU culture medium (nutrient broth with urea and calcium chloride) was used to feed the columns and to measure flow rates. Control and bacteria treated specimens presented an average initial flow rate of 15 mL/min. After 7 days of feeding, biotreated specimens were totally obstructed, while after 10 days the control specimens were still exhibiting a flow rate of 12.3 mL/min.

Using the EDTA (ethylenediaminetetraacetic acid) titration method, the authors concluded that a feeding by gravity method leads to a heterogeneous precipitation of calcite through the sand columns, with 31% calcite on the top, 16% on the center and 9% on the bottom. Consolidation occurs mainly on the top of the sand column, limiting the nutritive medium that reaches the bottom of the column.

Recently, Cardoso et al. (2016) tested the durability of sand columns biocemented with *Sporosarcina pasteurii*. The top 5 cm of the specimens were cut and submersed in water for more than one month. About 3 cm of the bottom of the sample crumbled, while the upper 2 cm was intact reinforcing the idea of a higher calcite precipitation on the upper part of the sand columns.

Consequently, new types of sand biotreatments were tested to improve bacterial treatment efficiency.

Different types of application of nutritive medium and bacteria in sand columns have been studied by Tobler et al. (2012): i) co-injection of bacteria culture and nutritive medium at the same time; and ii) injection of bacteria followed by addition of nutritive medium, testing different concentrations and injection rates. Researchers concluded that a parallel injection immobilized bacteria cells on top of specimens, which lead to an irregular consolidation. On the other hand, a phased injection showed a more uniform consolidation of sand columns.

Dhami et al. (2013) tested bacterial viability of a prepared bioproduct with different types of bacteria immobilized in fly ash. During 12 months bacterial' population was monitored concluding that *Bacillus cereus* had the higher viability. Based on these results, researchers tested the applicability of the fly ashes and *Bacillus cereus* grout in a sand column. When water absorption was tested, control specimens absorbed 12.15% water, while treated specimens only absorbed 8.84% water. Researchers also tested porosity with MIP, verifying the sealing of the pores: control specimens presented a total porosity of 25.3% compared with 19.2% of the biotreated specimens.

These results show that it may be possible to produce a bacteria-based repair product ready for commercialization for sand consolidation.

2.4.3. Cementitious materials

Bioformulation has been applied in cement mortars in different ways, always trying to improve microbial methodologies and mortars' characteristics. As for biotreatments, many studies have focus on cementitious materials bioformulation and results of some are synthetized.

Sierra-Beltran et al. (2014) tested the bond between bio-based cement grouts (without sand) and mortars (with sand) and a concrete support. Bio-based grouts and mortars were prepared with CEM I 42.5N, fly ash, blast furnace slag and *Bacillus cohnii* cells previously impregnated in lightweight aggregates. Different mixtures have been prepared with or without sand (mortars or grouts, respectively) and *Bacillus cohnii* cells.

An adhesion test has been performed by applying a 12 mm layer of bio-based cement paste on a concrete support. A strength of 2.89 MPa was obtained, fulfilling the requirements defined on EN 1504-3 (CEN, 2005) for concrete repair mortars.

Results from drying shrinkage test and from restrained shrinkage test are contradictory. Despite bio-based cement mortars present higher drying shrinkage, when applied to concrete they tend to bond better than pure cement mortars, presenting lower delamination in time.

This study confirmed the possibility of application of a bio-based mortar as a concrete repair system.

Besides bio-based grouts, Achal et al. (2011) created a bioconcrete made with cement, sand, coarse aggregate and *Sporosarcina pasteurii* cells. Specimens were cured for 28 days in two different nutritive media: Corn Steep Liquor and Nutrient Broth.

Capillary water absorption and water permeability have been tested. Results show that water absorption tends to decrease 5 times more in bioformulated specimens in comparison with control specimens, independently of the nutritive medium used. On the other hand, specimens bioformulated with Corn Steep Liquor are more efficient than the ones bioformulated with Nutrient Broth in reducing penetration depth for water.

Chloride penetration was also tested showing a resistance towards chloride penetration about 3 times higher for bioformulated specimens.

Recently, Bravo da Silva et al. (2015a) tested self-healing in cement mortars with addition of Cyclic EnRiched Ureolytic Powder (CERUP). The researchers reinforced the specimens with a steel bar and cracks were performed by applying forces to the bar. Results showed that bacteria bioformulated specimens presented 80% more crack closure than the control specimens.

Khaliq et al. (2016) tested the self-healing capacity of a bio-based concrete. Four mixtures were prepared: Mix 1 – control specimens; Mix 2 – concrete with *Bacillus subtilis* cells; Mix 3 – concrete with *Bacillus subtilis* cells impregnated in light weight aggregates; and Mix 4 – concrete with *Bacillus subtilis* cells impregnated in graphite nanoplatelets.

Compressive strength test results show an increase in compressive strength by ascending order - Mix 1 (26.28 MPa), Mix 2 (with a slight increase compared to Mix 1), Mix 3 (28.86 MPa) and Mix 4 (29.43 MPa) - similar to the ones obtained by other researchers, showing that using a carrier will not necessarily affect compressive strength but improve bacteria viability.

To test self-healing ability, specimens with 3, 7, 14 and 28 days of curing were positioned in the compressive strength testing equipment and compressed until cracks with 1 mm wide appeared. Dimension of cracks was monitored using a crack measuring microscope at 3, 7, 14 and 28 days of self-healing.

After 28 days of self-healing, Mix 4 had almost filled the crack, 0.81 mm; Mix 3 was able to fill more than half of the crack, 0.61 mm; Mix 2 had a maximum repair of 0.37 mm, which could be explained by the lack of protection of bacterial cells. Mix 4 presented better results for specimens cracked at 3 and 7 days of curing while Mix 3 had better results for specimens cracked at 14 and 28 days of curing.

Luo et al. (2015) did a similar test to Khaliq et al. (2016). In this case, the researchers tested different crack widths, concluding that the wider the crack, more difficult it was to repair. In accordance with the results of Khaliq et al. (2016), Luo et al. (2015) defined 0.8 mm as the limit of width reparable with a bioproduct.

Khaliq et al. (2016) obtained better results for compressive strength with *Bacillus subtilis* cells than other researchers obtained with *Sporosarcina pasteurii*. It is further noted that the use of a bacterial protection like lightweight aggregates increase strengths, increasing the viability of bacterial cells.

Besides superficial cracks, Liu et al. (2016) reported changes on the microstructure of bio-based cement mortars. With resort to coda wave interferometry, the researchers obtained results that evidence that bacteria-based cement mortars may repair internal microcracks.

Erşan et al. (2015) studied the applicability of 8 different types of bacterial protection in cement mortars: diatomaceous earth, zeolite, expanded clay, granular activated carbon, metakaolin, air entrainment, CERUP and Activated Compact Denitrifying Core (ACDC). *Bacillus sphaericus* and *Diaphorobacter nitroreducens* strains were used in this study. The best results for compressive strength were obtained for specimens treated with *Diaphorobacter nitroreducens*, achieving 10% improvement when compared with controls.

On the other hand, Wang et al. (2012) also tested cement mortar specimens with *Bacillus sphaericus* bacterial cells immobilized on diatomaceous earth. In this study, the authors observed the sealing of cracks due to drying. Light microscopy analysis allowed to observe a complete closure of these cracks, leading to a reduction of water absorption on the cement mortars.

Tziviloglou et al. (2016) underlines the importance of a proper protection for bacteria when applied as self-healing agent in concrete. In fact, the authors obtained excellent crack sealing results, but the use of LWA (Light Weight Aggregates) as a protection for bacterial cells in concrete resulted in a significant decrease in compressive strength, which may limit the use of this technique.

Unlike the results obtained by Khaliq et al. (2016), the protected ureolytic bacteria used by Erşan et al. (2015) and Tziviloglou et al. (2016) decreased compressive strength values. In this case, the decrease in compressive strength must be accounted, even when assessing concrete self-healing ability. The authors mentioned a field application of self-healing concrete where bacteria were protected with LWA. This bio-based material was applied on a canal in Ecuador, where the concrete walls exhibited significant cracking. Since July 2014, when the bio-based self-healing concrete was applied, up to the date of submission of the mentioned manuscript, no cracks were observed on the section where self-healing concrete was applied.

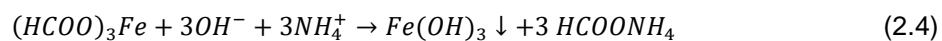
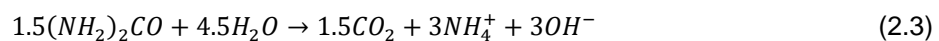
These results demonstrate the need of further studies using bacterial cells protection techniques.

Besides bio-concrete ability of crack sealing, reducing the possibility of reinforcements corrosion in concrete, Erşan et al. (2015) concluded that nitrate reducing bacteria may improve reinforcements resistance towards corrosion. Both observations guarantee an active and a passive technique for reinforcements' protection towards corrosive agents.

2.5. Microbially induced iron-oxide precipitation

Parallel to the utilization of calcium-carbonate precipitation for repair or improvement of construction materials, iron mineralization through iron-oxide precipitation can also be used for these purposes.

Iron-based biogrouts for soil improvement have been investigated by Ivanov et al. (2010) who tested two different biogrouts: an iron-based biogrout and a calcium-carbonate precipitating biogrout as control. The control biogrout was constituted by calcium-chloride, urea and urease-producing bacteria, while iron-based biogrout consisted in iron-reducing bacteria with iron ore and organic waste. The researchers described the iron precipitation process using two main reactions (Equations 2.3 and 2.4):



The researchers tested the compressive strength and water permeability of a soil samples. Despite the iron-based biogrout could not achieve compressive strengths as high as the calcium-based biogrout, water permeability was significantly reduced. The results showed that an iron-based biogrout could be a competitive solution for most common grouts, at lower cost.

Ivanov et al. (2014) performed a similar study. Besides reaching similar conclusions, the researchers highlighted two observations: the precipitate from the iron-based biogrout had a gel-like appearance instead of the crystal appearance of the calcium-based biogrout, which could mean an easier clogging of the treated material; and the fact that use of iron reducing bacterial cells is one of the most inexpensive ways to produce an iron-based biogrout.

As for calcium-carbonate, great results can be expected from an iron-based bio-product for the application on construction materials. But this technique has two features that must be considered: the color of iron-oxide, which is not appropriated, for example, for limestone consolidation and repair, and the reaction with steel. The reinforcements on concrete can be damaged by this technique.

Therefore, it is expected that an iron-based product for application on earth construction, earth blocks or ceramic bricks bioconsolidation can reach as good or higher results than the ones obtained when MICP is used, along with lower costs.

2.6. Advantages and disadvantages of bioconsolidation

The use of microbially induced calcium-carbonate precipitation in construction materials has a great potential. This technique may surpass the ones used nowadays for the obtainable compatibility between the bioproduct and material to be treated, despite being a sustainable technique.

Biotreatment on limestone has been deeply studied and implemented. Monuments' facades have already been repaired using microbial treatments with very good results.

Biotreatment and bioformulation in cementitious materials also has a great potential. Many different types of bacterial strains and application techniques have already been studied and described. Calcite deposition increases compressive strength, protects from chloride penetration and decreases water absorption, leading to a greater durability of concrete structures. Bacteria-based bioproducts have already been used in concrete structures repair, also leading to significant achievements.

The most promising bacteria application in concrete is the production of a self-healing concrete: with more studies on the viability of this technique, service life of the treated concrete can be more precisely estimated and an easier embracement by the construction community can be achieved (De Belie et al., 2016).

Like in cementitious materials, the precipitation of calcite in ceramic bricks will act as a biosealant. It is expected that reductions on water absorption of bricks can reach 50%.

Earth blocks and probably other earth construction components have a lot to gain from bacterial treatments, decreasing water absorption and consolidating the material. More studies are needed to better understand the potential of MICP use in earth construction, for instance applying MICP on a damaged earth wall. Most of the studies tested sand columns, with promising results.

Besides all the advantages, some considerations must be taken. When put into practice, it is necessary that favorable conditions are gathered so that a microbial treatment can achieve the expected results.

This is not yet a simple technique so qualified workers are required. In addition, the use of bacteria in construction materials may not be acceptable by everyone, what takes one to a problem of changing mentalities. Besides all the proven benefits using bacteria, it is necessary for people to understand that the type of bacteria used are harmless.

When performed in large scale, the costs associated to biotreatments must be deeply analyzed and taken in consideration; almost all studies have been performed in small scale.

De Muynck et al. (2010) performed a cost evaluation for biotreatment and bioformulation. The costs associated with the biotreatment of limestone with bacteria are considerably high and more competitive costs must be obtained. On the other hand, bacteria bring a lot more advantages, namely the compatibility with the preexistent materials and reversibility, both fundamental when conservation of architectural built heritage is under taken.

When it comes to the use of bacteria for bioformulation of cement based materials, the costs diverge on the use of bacteria precipitate as a binder because a regular binder is considerably less expensive than a bacteria-based bioproduct. Considering the advantages for biobased repair mortars, the price put to the product may not compensate. On the other hand, bioconcrete – a bacteria based concrete – has a lot more advantages, like self-healing capacity and, consequently, no need of regular inspections and repair, what can overlap the extra price of the material.

More recently, Achal et al. (2015b) refers that studies that used more affordable medium, reusing industrial byproducts, can reach as good or higher results but at a competitive price. The importance of reaching

less expensive techniques for a large scale and more real application is remarkable (Bravo da Silva et al., 2015b).

A more inexpensive solution that can reach similar results is an iron-based bioproduct. This technique of construction materials improvement is still growing, with studies been made only for soils consolidation, but a lot more can be done. Similar studies than those which have been made for calcium-carbonate precipitation in earth-based materials (blocks, plasters) and ceramic bricks and other ceramic products can be performed with microbially induced iron-oxide precipitation.

Ivanov et al. (2016) have summarized disadvantages of microbial treatment of construction materials, with more emphasis in MICP that has been extensively explored. Some of them are: i) high cost of bacteria culture media or enzyme preparations; ii) toxicity of compounds from the metabolism of bacteria, namely products resulting from the hydrolysis of urea in MICP processes; iii) increase of pH in MICP, that may induce materials degradation; or iv) life-time of calcite crystals. Some of these drawbacks can be circumvented using iron-based biotreatments. In fact, MIIP produces a less brittle biomineral, without formation of cells toxic compounds beside being less expensive.

2.7. Consolidation of earth mortars

It is known that earth-based mortars were one of the primarily used construction materials, with its' utilization being dated at least from the Neolithic (Bruno et al., 2008) where earth mortars were used to fill branch structures used for sheltering. Construction with earth fell in disuse with the arrival of more resistant construction materials but has been regaining strength with the crescent concern on sustainable and eco-friendly construction techniques.

Earth mortars are mainly constituted by water, sand and a clayish earth. All these components are easily accessible, no heavy industrial process is necessary for their preparation and, if used without chemical binder stabilization, can be reused for the same purpose (Lima et al., 2016b).

A high adsorption and desorption capacity is one of the main advantages of earth mortars use as plasters. This ability leads to the control of relative humidity in leaving places, improving the comfort and even contributing for health problems like asthma and allergies control (Lima et al., 2016b).

On the other hand, earth mortars are less resistant and more water degraded than the most commonly used mortars, like cement mortars. Even though earth mortars can achieve the required mechanical and adhesive strengths to be applied as plasters, the same can not be achieved regarding their resistance towards water. When in contact with water, erosion rapidly occurs and, if saturation is reached, earth mortars regain plasticity (Lima, 2013).

In order to achieve higher resistance, natural stabilizers, as oils and fats, and chemical stabilizers, as lime, cement and gypsum, have been used in earth mortars (Eires et al., 2017). Despite improvements might be obtained with the use of stabilizers, some lead to the dissolution of earth mortars main strengths: the capacity for reuse and the contribution to control indoor humidity.

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

Lima et al. (2016a) studied the effect of the addition of gypsum on the formulation of earth mortars based on an ilithic clay. The study mainly focused on resistances and, when compared with earth mortars with no stabilization, improvements were achieved. The authors obtained a decrease on linear shrinkage, meaning a lower probably for the occurrence of superficial fissures. Mechanical strengths (flexural and compressive) were increased, along with dry abrasion resistance and surface cohesion. Adhesive strength was roughly the same. Both mechanical strengths increased with the increase in volume ratio of gypsum on the earth mortar. The same behavior was observed for dry abrasion resistance and surface cohesion with higher improvements being obtained for higher gypsum concentrations

Similarly, Lima et al. (2016c) compared the properties of an air lime mortar and a natural hydraulic lime mortar with an ilithic earth mortar. The same test procedures as Lima et al. (2016a) were conducted. Linear shrinkage was similar on all mortars. Different results were obtained for mechanical strengths: while the natural hydraulic lime mortar had similar strengths to earth mortars, on air lime mortar a decrease was observed. Adhesive strength on air lime mortar was the same observed on earth mortars, but the natural hydraulic lime mortar had a significant increase. Dry abrasion resistance was substantially lower on lime mortars. The same results were not observed for surface cohesion: while the natural hydraulic lime mortar showed a higher surface cohesion, the air lime mortar had considerably lower results.

Stabilization of earth mortars with low percentages of Portland cement was studied by Gomes et al. (2016). Flexural and compressive strengths were tested and water absorption coefficient assessed. In general, obtained results were worse than for non-stabilized mortars. Lower flexural and compressive strengths were obtained on Portland cement stabilized earth mortars. In addition, a higher water absorption coefficient was obtained.

Stazi et al. (2016) used commercially available products, 8 different types of earth mortar stabilization have been tested: 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). Among others, the researchers assessed compressive strength in earth mortars with additions, measured contact angle and performed the drip erosion test. Earth mortars with additives reached slight lower compressive strengths than control mortars. Contact angle test showed that even if some improvements might be obtained from the use of additions on formulation, a good impermeability can 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 additions and mortars with surface treated with silicon nano-particles or silane-siloxane had 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. The use of natural fibers for the improvement of earth mortars has been widely studied. Lima et al. (2015) studied the addition of oat straw fibers and typha fiber-wool on the formulation of earth mortars. Linear shrinkage, mechanical and adhesive strengths were assessed. Earth mortars formulated with oat straw fibers showed considerably lower linear shrinkage than control earth mortars; the ones formulated with typha fiber-wool also showed low linear shrinkage. The addition of typha fiber-wool on earth mortars lead to increase of flexural, compressive and adhesive

strengths. On the other hand, mortars with oat straw fibers had low flexural and compressive strengths and adhesive strength only slightly increased.

Besides organic fibers, oils have also been studied to ameliorate earth mortars properties. The addition of linseed oil to earth mortars has been tested by Lima et al. (2016c). Linear shrinkage, mechanical and adhesive strengths, dry abrasion resistance and surface cohesion have been evaluated. Linear shrinkage was not affected by the addition of linseed oil. Results from all other tests showed improvements: mortars where 5% linseed oil was added reached higher strengths than air lime and natural hydraulic lime mortars also tested by Lima et al. (2016c).

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. The objective of that study was to evaluate resistance towards water degradation and mechanical strengths. Flexural and compressive strength were assessed, contact angle was measured and a drip erosion test was performed. Flexural and compressive strength tests were only conducted in earth mortars formulated with and without chitosan. Earth mortars formulated with chitosan showed an increase of almost 100% on both compressive and flexural strengths, when compared with reference mortars. Contact angle and drip erosion were assessed in earth mortars without treatment, mortars formulated with chitosan and mortars with a surface treated with chitosan (tests were performed on the treated surface). Contact angle in mortars formulated with chitosan reached an average value of about 70°; this value was only obtained for mortars with the highest concentration of chitosan. Mortars' surface treated with chitosan presented a higher contact angle, reaching an average value of about 90°. Contact angle in reference earth mortars was nonexistent. On the drip erosion test, earth mortars without treatment had the degradation process due to erosion rapidly occurring. In mortars formulated with chitosan, erosion was lower, but mortars with a higher concentration of chitosan were not affected. The best improvements were obtained for chitosan treated surface earth mortars, with practically no erosion being observed.

Despite already been tested in earth-based materials, the bioconsolidation effect, both by biotreatment or bioformulation, has not yet been studied in earth plastering mortars. This eco-efficient construction material may have a lot to gain from the use of an eco-friendly biotechnology for its enhancement. Furthermore, earthen-based materials have only been bioconsolidated by MICP and not with bio-products based on iron precipitation. This fact justifies the experimental campaign of the present thesis.

3. Materials and methods

3.1. Initial remarks

In the present thesis, iron-based bioproducts have been used in two distinct areas: as a biotreatment, acting on the surface of construction materials; or as component, being added on the production of construction materials. Different tests were performed in different specimens in order to assess improvements obtained by the use of these iron-based bioproducts.

Figure 3.1 summarizes the tests performed on each specimen for the different approaches of the bioproducts. Different cubic specimens (40 x 40 x 40 mm) of the earth mortar were used for biotreatments and for bioformulations. Specimens composed by a plaster of the earth mortar on hollow brick were only used for bioformulations.

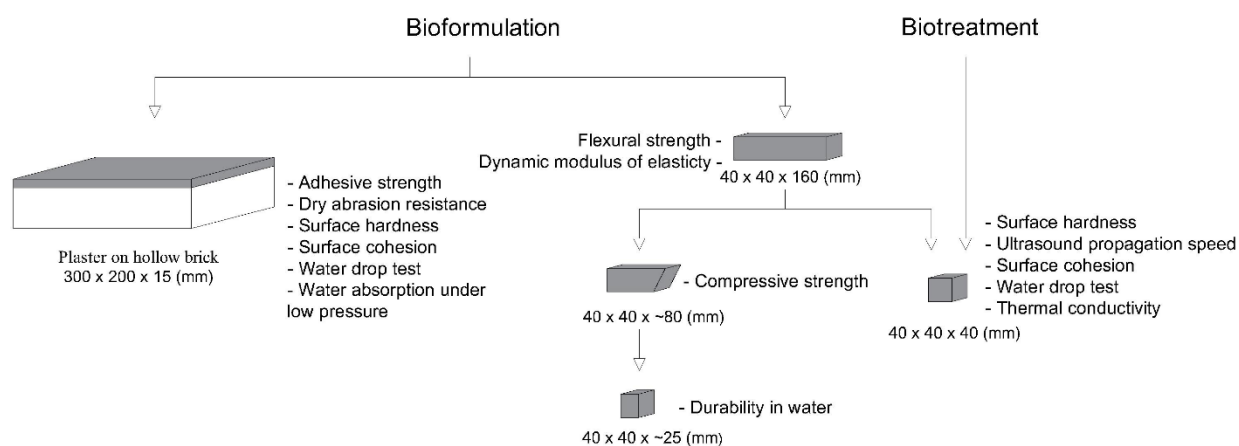


Figure 3.1 – Schematic representation of specimens and tests made with them

The specimens for biotreatment were produced previously to the thesis by one of the supervisors in the Building Materials Laboratory of Civil Engineering Department of FCT NOVA. The specimens for the study of bioformulation were produced with the same ready-mixed plaster product within this thesis. The bioproducts were produced within this thesis in a UCIBIO Laboratory of Chemistry Department of FCT NOVA. All the experimental campaign took place on both these facilities.

3.2. Production of iron-based bioproducts

As iron-based bioconsolidation has already been studied in soils, with good results (Ivanov et al., 2014), earth/soil based construction materials may have a lot to gain from this novel biotechnology. One of the advantages is that most earths have iron on its composition and, therefore, have affinity with the iron-based bioproducts. Another is the fact of being one of the less expensive and more eco-friendly ways of inducing biomineralization (Ivanov et al., 2014).

In this study, earth plastering mortars were either surface biotreated or bioformulated with this iron-based bioproduct and compared with a non bioconsolidated reference mortar.

Escherichia (E.) coli BL21(DE3) was used to produce all biotreatments. It is a well-characterized microbiological organism not known to consistently cause disease in immunocompetent adult humans, that presents minimal potential hazard to laboratory personnel and the environment. *E. coli* is a facultative gram-negative, non-sporulating, rod shaped bacterium, with an optimal growth temperature of 37°C. Bacteria cells were cultured in LB (Lysogeny broth) medium, a nutritionally rich medium that contains 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl. To evaluate the effect of iron mineralization (formation of ferric oxides minerals) on the biotreatment, iron ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution) was added to some bacterial cultures. The effect of the presence of a protein from the ferritin family, Dps, that catalyzes iron biomineralization was also assessed.

The bioproducts were produced and applied as liquids, with different viscosities.

3.3. Surface biotreatments for earth mortar

3.3.1. Materials

The earth mortar used in the present study was produced with a ready-mixed plastering product composed by clayish earth, siliceous sand and cut oat fibers. This product was prepared by the Embarro company (<https://www.embarro.com/en/products/>) with the main constituents being extracted from the South region of Portugal, Algarve. This ready-mixed product has been used and extensively studied by Faria et al. (2016). The researchers have obtained interesting results from XRD analysis about its composition. The presence of calcite (CaCO_3) and hematite (Fe_2O_3) was observed, both constituents that may be used by bacterial cells inhabiting the earth mortar or bacteria added with the biotreatment, supporting the bioconsolidation process.

To test the biotreatments, cubic earth mortar specimens (40 x 40 x 40 mm) were used (Figure 3.2). These specimens were cut from prismatic samples (40 x 40 x 160 mm) that were prepared by Faria within a Workshop on Earth Plasters that took place at FCT NOVA in 2013, with the ready-mixed product with resort to a Putzmeister MP25 mixing and pumping equipment. For each biotreatment, three cubic earth mortar specimens' replicates were tested. The upper surface of the samples (that has not been in contact with the metallic mold) were tested.



Figure 3.2 - Example of an earth mortar cubic specimen used in this work

This study was performed in two phases. The first consisted in the screening (here designated 1st screening) of experimental conditions to establish optimal conditions, that were then used in the second phase (2nd screening) to biotreat mortar specimens.

3.3.2. 1st Screening

Before the 1st screening, a simple test was performed to gauge the appropriate volume of liquid biotreatment to be applied on the surface of each mortar (40 x 40 mm). Water was applied to scraps from the cutting of prismatic specimens in different volumes, leading to a volume to be applied of 1 mL, that was applied using a micropipette.

Two triplicates were prepared as controls and four biotreatments were tested:

- Control - Non-treated earth mortar specimens;
- H₂O - Treatment of earth mortars with H₂O
- LB – biotreatment with LB medium;
- LB+Fe - LB medium supplemented with iron;
- E.coli+Fe - *E. coli* culture supplemented with iron;
- E.coli+Fe+Dps - *E. coli* culture expressing Dps supplemented with iron.

All specimens were labelled before applying the treatments (Figure 3.3).

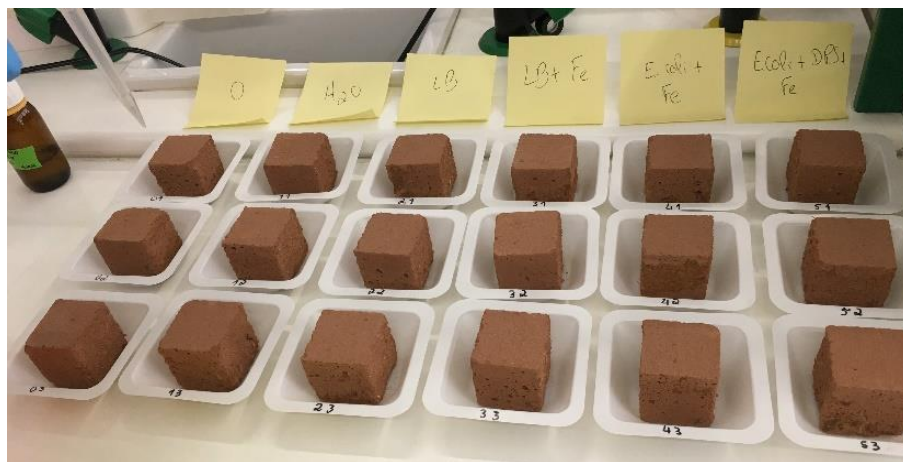


Figure 3.3 - Cubic earth mortar specimens used on the 1st screening

Besides testing the effect of each biotreatment, percolation and dissipation at the surface and in depth were also assessed. Biotreatments and water were applied on a central point of the 40 x 40 (mm) surface to be treated, concentrating the biotreatment to the point of application and to the surrounding area.

After application of each treatment, specimens were left to dry for 72 hours, and fed with 1 mL of LB nutritive medium, or water in the case of the “H₂O” control. Refeeding, in the same conditions, was performed daily for four more days, according with the calendar presented on Table 3.1.

A first experimental campaign was conducted. The same treatments were re-applied on treated mortar specimens; in this case, only one day feeding was performed (with 72 hours’ rest between treatment and

feeding). Two more experimental campaigns were conducted after the application of this second treatment. The calendarization of the 1st screening is presented on Table 3.1.

Table 3.1 - Calendarization of 1st screening

1 Application of 1st treatment	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 experimental campaign	12	13	14
15 Application of 2nd Treatment	16	17	18 Single day feeding	19	20	21 2nd experimental campaign
22	23	24	25 3rd experimental campaign			

Beside the experimental campaigns mentioned above, another experimental campaign was conducted 115 days after the last application of treatment (130 days after the 1st biotreatment).

3.3.3. 2nd Screening

Based on the results obtained on the first screening, the biotreatments were ameliorated and some of the testing parameters were altered: application method, volume applied and iron concentration.

As it was noticed in the first screening that the application method damaged the central part of the specimens surface, in the second screening all biotreatments were applied throughout the entire surface area, instead of being applied on a single central point. Biotreatments were also applied with resort to a micropipette.

The treatments that obtained better results were tested in higher volume (application of 2 mL) and with higher concentrations of iron (five times more concentrated).

For the second screening, 10 different triplicates of cubic earth mortar specimens were tested (Figure 3.4):

- Control – specimens with no treatment;
- H₂O (1mL) – treatment with 1 mL of water;
- LB+Fe (1mL) – 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;
- H₂O++Fe (1mL) – treatment with 1 mL of water supplemented with iron;
- H₂O (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.

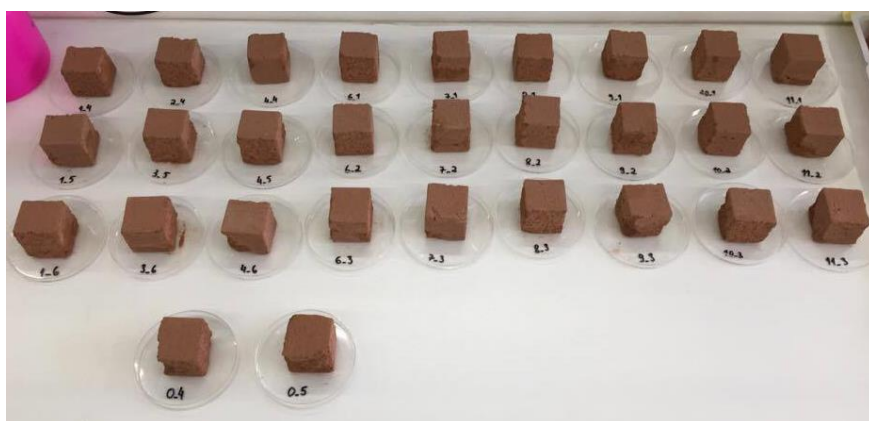


Figure 3.4 - Cubic earth mortar specimens used on the 2nd screening

The treatment procedure used on the 2nd screening was similar to the second treatment applied on the 1st screening. The treatments were applied and left to rest (and dry) for 72 hours and then one feeding was applied. Feeding was performed using the same volumes of the treatments, respectively. “H₂O (1 mL)”, “H₂O++Fe (1 mL)” and “H₂O (2 mL)” were fed with water while the biotreatments were fed with LB medium.

One experimental campaign was conducted 4 days after the feeding was performed, and another 65 days after treatment application to assess treatment durability.

3.4. Bioformulation of earth mortars

3.4.1. Materials

For the bioformulation of earth mortars, the same ready-mixed product used by Faria et al. (2016) was used. As mentioned before, the ready-mixed product is composed by clayish earth, siliceous sand and cut oat fibers.

By the time Faria et al. (2016) studied the used ready-mixed product, it presented an average loose bulk density of 1.17 kg/dm³. For the preparation of this work, the loose bulk density of the ready-mixed product was necessary and it was re-measured. The average loose bulk density had now changed to 1.47 kg/dm³. That may be due to the loss of some oat fibers during the storage of the ready-mixed product, considering that the product was tested in similar relative humidity conditions.

The number of earth mortar formulations were limited to three, due to the availability of ready-mixed product. For control specimens, the earth mortar was formulated with tap water (“Control”). For the bioformulated specimens, two different bioproducts were tested: one with Luria Broth medium (“LB”); and one with *E. coli* culture supplemented with iron (“*E.coli*++Fe”). In both cases the bioproducts totally replaced the kneading water.

This part of the work was performed after the 1st screening. The bioproduct based on *E. coli* culture supplemented with iron had shown the best achievements on the first screening. Therefore, it was chosen to be tested for bioformulation. The treatment with LB medium was used as a bio-control to understand

what was more beneficial, the bacteria inhabiting the ready-mixed product or the addition of exogenous *E. coli* cells.

3.4.2. Production of earth mortars

Earth mortars are mainly constituted by clayish earth and sand; these two constituents should be mixed in specific mass ratios depending on their characteristics. In this case, as a ready-mixed product was used, there was no need for a granulometric study.

According to Santos et al. (2015), who also used the same ready-mixed product, water should be added in a volumetric ratio of 0.2. During the production of the earth mortars used in this study, 20% water, in volume, was added but it was noticeable that the mortars were still dry. 22.5% water volume was also tested, but a decent workability was only obtained for 25%. Therefore, all mortars were prepared using a volumetric ratio of kneading liquid of 0.25. The control mortars were formulated with 25% water in volume, while the bioformulated mortars were prepared with 25% in volume of each bioproduct.

Mortars were mechanically produced in laboratory with resort to a mixer (Figure 3.5). The water or bioproduct is firstly placed on the mixer, followed by pouring the appropriate volume of ready-mixed product. The mixing process, following standard procedures of DIN 19847 (DIN, 2013) involves three steps: 1 minute mixing, 5 minutes resting, and 30 seconds mixing. For a more efficient process, the mixing blade and the vat should be slightly moistened.

The leftovers mortars' formulation were saved on different zipper bags and re-used 72 hours after the mixing.



Figure 3.5 – Laboratory mortar mixer used for earth mortars preparation

3.4.3. Earth mortar specimens

Two different types of specimens were produced: prismatic earth mortar specimens, with dimensions of 40 x 40 x 160 mm; and a layer of earth mortar applied on a ceramic hollow brick, simulating a plaster with dimensions of approximately 15 x 200 x 300 mm. Six cubic specimens and one plaster on a brick were

performed for each mortar formulation. One prismatic specimen was performed for “H₂O” mortar after 72 hours and two for each of the bioformulated mortars after 72 hours. (Figure 3.6).



Figure 3.6 - Bioformulated earth mortar specimens

The prismatic earth mortar specimens were produced based on EN 1015-11 (CEN, 1999) with resort to metallic molds and a tamping machine (Figure 3.7). The molds were filled in two layers, being each layer tamped for 20 times. The excess mortar was scrapped from the top of mold. Specimens dried on laboratory conditions during January 2017 and were demolded after 14 days.



Figure 3.7 - Equipment used on the compaction of prismatic bioformulated earth mortars

The layer of earth mortar was applied to the brick with resort to two molds: one applied around the brick, defining the 15 mm thick layer; and one positioned on top of the first mold with 7 cm high, the mortar is dropped from the top of this mold in order to simulate a constant force of application of the mortar to a brick wall. The brick is previously moistened to avoid excessive absorption of water from the mortar still fresh.

3.5. Test procedures

3.5.1. Surface tests

The same experimental procedures were conducted throughout all campaigns directed to biotreatments. Surface tests were also conducted in bioformulated mortar specimens.

All the tests were performed on laboratory conditions with 18/21°C and relative humidity of 46±5%. Earth mortars are highly hygroscopic (Lima et al., 2014). Therefore, specimens mass was controlled to discard or understand abnormal results due to environment conditions variation of RH.

3.5.1.1. Surface hardness

Surface hardness evaluation was performed according to ASTM D2240 (ASTM, 2000) using a PCE Shore A durometer, applicable to soft and rubber-like materials.

The durometer is constituted by a spike that is pressed against the surface of the specimen until the base of the durometer is parallel and in contact with the surface to be tested. Through a system of springs, the relative hardness of the surface is presented on a display. The scale of the durometer goes from 0 to 100 (Figure 3.8).



Figure 3.8 - Shore A durometer (left) and test procedure (right)

Surface hardness of the treated surface of the cubic earth mortar specimens (40 x 40 mm) was evaluated in 12 different points along the surface (Figure 3.9). Initially, the surface was tested in 9 points (surface divided in 9 equal regions), but due to the 1st screening application method of the biotreatment, the central region of the surface had a concavity that did not allowed a correct measure of the surface hardness.

1	2		3
4	5	6	9
	7	8	
10	11		12

Figure 3.9 - Scheme of measurement areas of the surface hardness test

3.5.1.2. Ultrasound propagation speed

Ultrasound test was performed according to EN 12504-4 (CEN, 2004) with resort to a Proceq Pundit Lab equipment.

The ultrasound equipment is constituted by two transducers, a transmitter and a receiver, that through a vibration impulse measure the time between the two transducers. The results from the ultrasound test allows to evaluate the compactness from the tested specimens and, indirectly, the thickness of the biotreatment.

As the biotreatment was applied on one of the surfaces of the cubic specimens, the ultrasound test was performed on the perpendicular direction to the treated surface (Figure 3.10).



Figure 3.10 - Ultrasound propagation speed test procedure

Before the test was performed, the height of all specimen was registered. The equipment outputs the time (t), in μs , between the transducers and knowing the distance (d) between them, it is possible to calculate the ultrasound propagation speed (v_{us}) using equation 3.1.

$$v_{us}(m/s) = \frac{d(mm) \times 10^{-3}}{t(\mu s) \times 10^{-6}} \quad (3.1)$$

3.5.1.3. Surface cohesion

The test procedure used to assess the surface cohesion of the treated specimens was based on the method described by Drdácý et al. (2014) and applied to earth plasters by Faria et al (2016).

An adhesive tape was cut in pieces of 50 x 50 mm and placed on the treated surface of each specimen. A weight of 1.5 kg was positioned on top of the adhesive tape for 5 minutes (Figure 3.11). This technique allows to perform the test with an equal pressure applied to all specimen.

The adhesive tape is peeled from the surface and weighted. The mass value obtained expresses the surface cohesion of the surface of the treated and untreated specimens. All weightings were performed on a scale with precision of 0.0001 g.

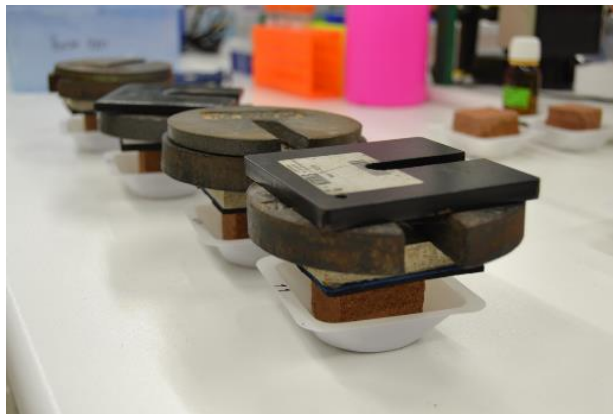


Figure 3.11 - Surface cohesion test procedure

3.5.1.4. Water drop test

The water drop test is a simple test that allows to observe the behavior of the tested mortars towards water ingress. In this test, a drop of water is spilled on the surface of the specimen (Figure 3.12). The whole procedure is video recorded and the time until the drop of water is totally absorbed by the specimen is measured.



Figure 3.12 - Observation of the water drop during the water drop test

3.5.2. Mortars fresh state tests

The following test procedures were only performed on bioformulated mortars by the time they were performed and on bioformulated mortars saved on zipper bags for 72 hours.

Due to the maximum volume of the mixer, two mixtures of each formulation were prepared. The total volume needed for each formulation was divided in two in order to guarantee the same mixing conditions. Fresh state tests were performed in all mixtures.

3.5.2.1. Flow table consistency

Flow table consistency was measured according to EN 1015-3 (CEN, 1999). Furthermore, the slump height of the mortar was measured after measuring the flow diameter. The flow table equipment is presented on Figure 3.13.

Before starting the test, the table and the conic mold were moistened. The mortar was placed on the mold in two layers, being each layer tamped at least ten times with a pounder. The excess of mortar was scraped by the top of the mold and the mold removed. 15 strokes were made in 15 seconds using a crank of the flow table.

After the test is performed, the diameter was measured in three different directions and the slump was measured on the center of the table. The higher the diameter and the lower the height, the less consistent is the mortar.



Figure 3.13 - Flow table (left) and test procedure (right)

3.5.2.2. Penetrometer consistency

Penetrometer consistency was measured according to EN 1015-4 (CEN, 1998). The consistency of the mortar is defined by the penetration depth of the penetrometer.

The mortar was placed on a cup in two layers, being each layer tamped two times in each quadrant of the cup by lifting the top of the cup. The excess of mortar was scraped by the top of the cup. The cup was then positioned on the base of the equipment and the penetrometer dropped from a height of 10 cm, measuring the penetration depth with the scale of the penetrometer (Figure 3.14). The higher the penetration depth, the less consistent is the mortar.

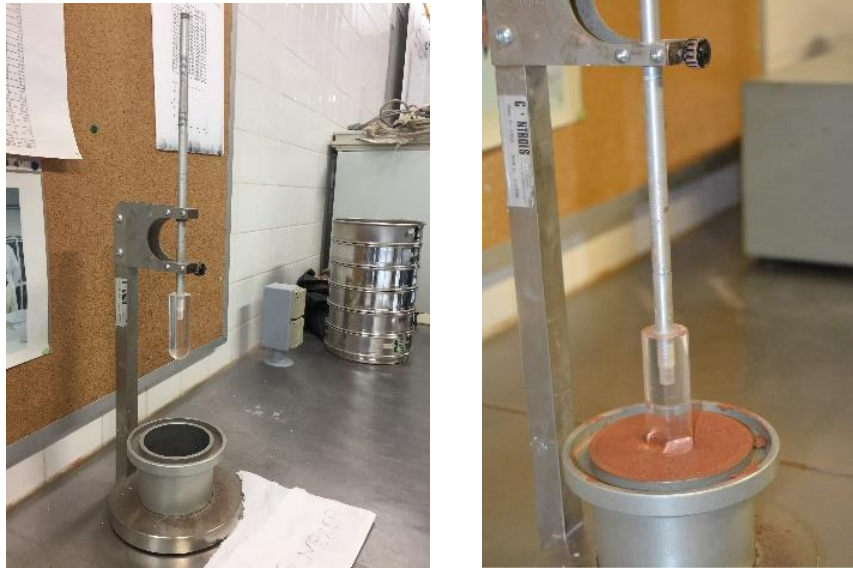


Figure 3.14 - Penetrometer (left) and test procedure (right)

3.5.2.3. Wet bulk density

Wet bulk density was measured according to EN 1015-6 (CEN, 1998), with resort to a cup of 1 dm³ and a scale with precision of 0.1 g (Figure 3.15).

The cup was firstly placed on the scale and the scale tared. The mortar was then placed on the cup in two layers, being each layer tamped two times in each quadrant of the cup by lifting the top of the cup. The excess of mortar was scraped by the top of the cup and the cup cleaned. The weight of 1dm³ of mortar was then measured.



Figure 3.15 - Scale and cup used to assess the wet bulk density

3.5.3. Hardened state – prismatic earth mortar specimens

Prismatic specimens were used to control shrinkage and to test mechanical resistances of bioformulated mortars. From the prismatic specimens, different specimens were cut and used to test surface properties and resistance towards water.

3.5.3.1. Drying shrinkage

Linear drying shrinkage was measured according to DIN 18947 (DIN, 2013). Before the prismatic specimens were prepared, the length of each mold was measured. After 14 days, the specimens were demolded and their length measured. Linear drying shrinkage is given by the lengths difference.

3.5.3.2. Bulk density and dynamic modulus of elasticity

Bulk density was assessed according to EN 1015-10/A1 (CEN, 1999). Prismatic specimens were measured and weighed before the dynamic modulus of elasticity test.

Dynamic modulus of elasticity was measured according to NP EN 14146 (IPQ, 2007), with a Zeus Resonance Meter (Figure 3.16). As it was necessary to perform the test, all dimensions and weight of the specimens were registered. The test was performed on four different faces, being registered four dynamic modulus of elasticity values for each specimen.



Figure 3.16 - Dynamic modulus of elasticity test procedure

3.5.3.3. Flexural and compressive strengths

Flexural and compressive strengths were determined according to EN 1015-11 (CEN, 1999) with resort to a Zwick Rowell Z050 equipment (Figure 3.17). Both tests were performed 48 days after the specimens were produced.

Flexural strength test was performed at a velocity of 1 mm/min, while the compressive strength test was performed at a velocity of 3 mm/min with the half samples resulting from the flexural test.

Flexural strength was calculated with resort to equation 3.2.

$$Flx = \frac{3}{2} \times \frac{F \times 100}{b \times h^2} \quad (3.2)$$

In equation 3.2 F (N) is the obtained load, b (mm) is the perpendicular dimension to the load and h (mm) is the parallel dimension to the load. Compressive strength was calculated by dividing the obtained load by the area of the compression equipment (40 x 40 mm).



Figure 3.17 - Test procedure for flexural (left) and compressive (right) strengths analysis

3.5.3.4. Thermal conductivity

Thermal conductivity was evaluated with an ISOMET 2104 Heat Transfer Analyzer with a 60mm contact probe API 210412 (Figure 3.18). In order to avoid measurement errors due to RH differences, the specimens were kept for 72 hours on the same room at defined temperature and RH.

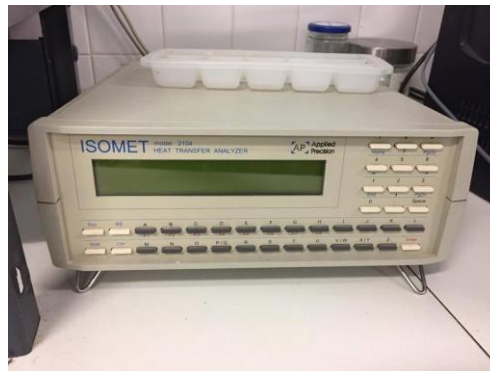


Figure 3.18 - ISOMET 2104 Heat Transfer Analyzer

As the available earth mortar specimens had a surface of 40 x 40 mm, the specimens were confined with polystyrene thermal insulation (Figure 3.19) in order to perform the necessary 60 mm diameter of the probe.



Figure 3.19 - Confinement of the mortar specimens with thermal insulation

Due to the lack of availability of the thermal conductivity testing equipment, only two measurements were performed for each of the three formulated mortars.

3.5.3.5. Durability in water

To assess the mortars resistance towards liquid water, a simple test was performed where samples of mortars that resulted from the compressive strength test are submersed in water.

Glass beakers were used and filled with tap water and the samples were submersed (Figure 3.20). The behavior was registered and video-recorded.

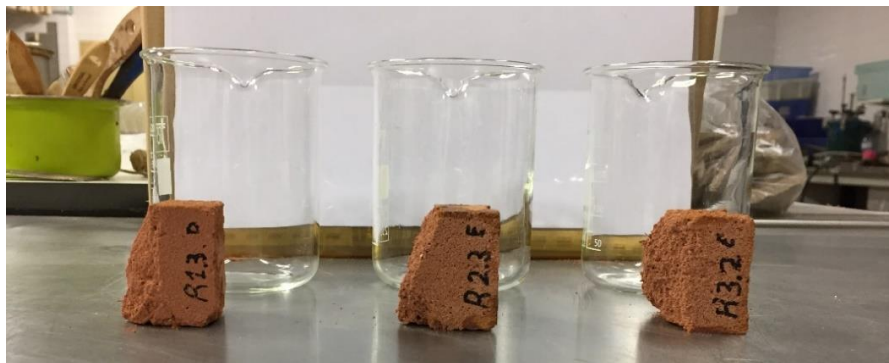


Figure 3.20 - Water resistance test procedure

3.5.3.6. Surface tests

The surface tests were performed on cubic specimens of 40 x 40 x 40 mm that were cut from one of the half prismatic specimens resulting from the flexural strength test.

Surface hardness, ultrasound propagation speed, surface cohesion and water drop test were performed according to the procedures described on 3.5.1.

3.5.4. Hardened state – earth mortar plaster on ceramic brick

Firstly, three distinct tests were performed on these specimens: visual analysis, in order to observe possible cracks due to shrinkage; adhesive strength, to characterize the bond between the mortar and the brick; and dry abrasion resistance, in order to assess the surface cohesion of the mortar layer. These tests were only performed on bioformulated mortars. After the above-mentioned tests were performed, the specimens were used to test surface hardness, surface cohesion, resistance towards water absorption and compressive strength for comparison with similar mortars but without the influence of a plastering application on a support.

3.5.4.1. Adhesive strength

Adhesive strength was performed according to EN 1015-12 (CEN, 2000). Three assays were performed in the specimen of each of the three formulations. With resort to a drill, circular holes were made on the mortars layers and metallic pins, with a base of 50 mm diameter, were glued to the mortar on the center of the hole with an epoxy glue (Figure 3.21). After the glue dried, the pins were pulled with a the same Zwick Rowell Z050 equipment used for flexural and compressive strength at a velocity of 1 mm/min. The load is registered by the equipment software, the type of rupture was observed and the diameter of the base of the mortar sample is measured, allowing to calculate de adhesive strength and if the rupture was cohesive (in the thickness of the mortar) or adhesive (in the interface of the mortar and the brick).



Figure 3.21 - Adhesive strength test procedure

3.5.4.2. Dry abrasion resistance

For dry abrasion resistance analysis, three tests were performed for each mortar formulation. A medium hardness brush was attached to a rotating device with a constant pressure of 2 kg (Figure 3.22). Plastered bricks were positioned under the brush, the brush rotates for 20 times and the loose particles were cleaned from the surface. Bricks were weighed on a scale with a precision of 0.1 g before and after each test, being the mass difference and the relief left by the brush an indicative of the surface cohesion.



Figure 3.22 - Dry abrasion resistance test procedure on the opposite side of specimen previously submitted to adhesion test

3.5.4.3. Water absorption under low pressure by Karsten pipe

Water absorption under low pressure was performed with resort to Karsten pipes, according to EN 16302 (CEN, 2013). The pipes were set perpendicular to the surface and the interface between the pipe and the surface sealed with resort to plasticine. Pipes were filled with 4 mL of water and the time until the water was absorbed was registered (Figure 3.23).

This test was performed after adhesive strength and dry abrasion resistance tests. Since the diameter of the brush used for dry abrasion resistance was higher than the diameter of the base of the Karsten pipes and the central part of that area was not eroded, the test was performed on the same place of the dry abrasion resistance test.



Figure 3.23 - Karsten pipes test procedure

3.5.4.4. Surface tests

Surface hardness, surface cohesion and water drop test were also performed on earth plasters on ceramic brick specimens, in accordance with the procedures described on 3.5.1, as these specimens more closely represent an *in situ* plaster.

Surface hardness, in accordance with the performed on 40 x 40 x 40 (mm) mortar specimens, was performed in 12 different points distributed along the specimens' surface. Due to the lack of space on the specimens (adhesive strength and dry abrasion resistance had already been performed), only two surface cohesion tests were performed for each specimen/mortar. Schematic representations of both tests are presented in Figure 3.24

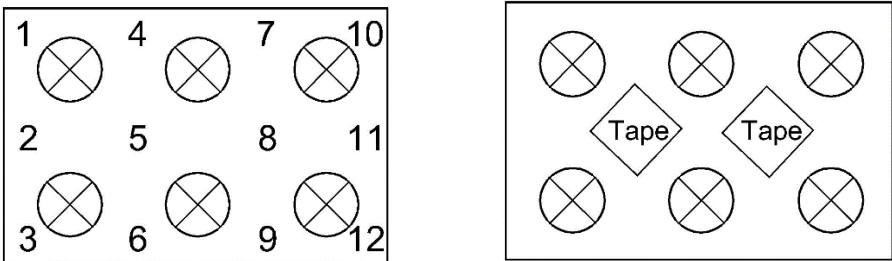


Figure 3.24 – Schematic representation of adhesion samples, measures performed on the surface hardness test (left) and from surface cohesion test (right) on earth mortar layer specimens

Water drop test was performed on three distinct points distributed along the surface. Ultrasound propagation speed test was not performed on these specimens.

3.5.4.5. Compressive strength

Compressive strength of samples from the plasters was assessed with resort to the same Zwick Rowell Z050 equipment used previously for mechanical tests at a velocity of 3 mm/min. The test was performed with samples (approximately 50 x 50 mm) of the earth plastering mortar still attached to the ceramic brick, after the removal of all the other areas of plaster. Figure 3.25 shows the mortar samples after the test was performed.

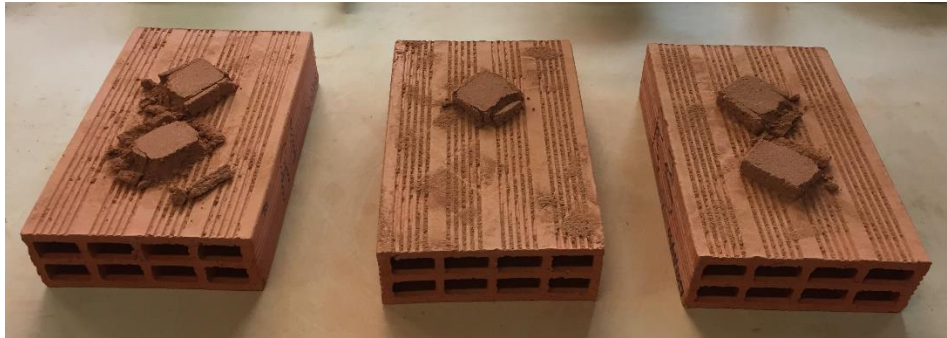


Figure 3.25 - Samples after compressive strength test

4. Results and discussion

4.1. Initial remarks

Average results of tests are presented in this section graphically with standard deviation (when possible). Individual detailed results are presented on the Appendix.

4.2. Surface biotreated earth mortars

4.2.1. 1st Screening

“1st Treatment” and “2nd Treatment” designations refer to the experimental campaigns performed 72 hours after application of the biotreatments (treatment plus feeding). Other labels are identified with the respective time after treatment.

4.2.1.1. Surface hardness

On a first approach, surface hardness was measured on 9 distinct point of the treated surface. As the application method used on the first screening damaged the surface on the central application point of the biotreatment (Figure 4.1), the measured procedure was changed to the 12 points described on Figure 3.9 (section 3.5.1.1).



Figure 4.1 – Surface damage created by the application of biotreatment on the 1st screening

Surface hardness measurements of the specimens of 1st Treatment were performed with the 9 points grid and results of the central point (treatment application point) were ignored. Surface hardness was re-measured 6 days after application by the 12 points grid method. Average results are graphically presented in Figure 4.2.

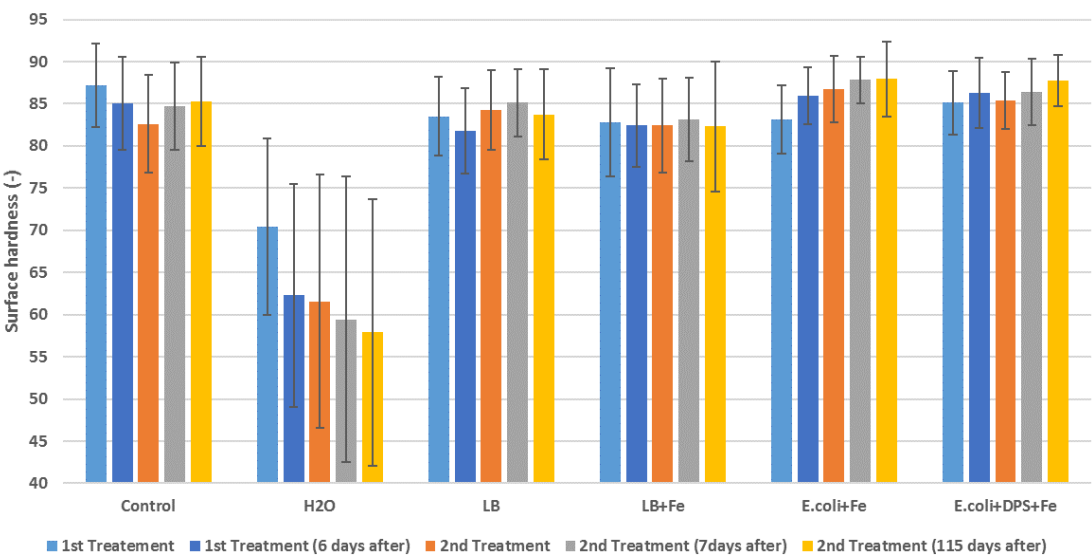


Figure 4.2 - 1st screening surface hardness results

When compared with control specimens, biotreated earth mortars had no significant improvements. Despite the “E.coli+Fe” treated had an increase in surface hardness, the results are not substantial and it is not correct to assume that there really was an improvement because measurement errors should be taken into account.

On the other hand, results obtained for the “H₂O” specimens, although with a very high standard deviation, demonstrate that the application of a liquid on the surface of these earth mortars led to a significant decrease of the surface hardness. Since all the bioproducts are aqueous suspensions and the surface hardness of biotreated specimens was similar to the control specimens, in comparison with the “H₂O” it can be seen that a consolidation effect is being created on the biotreated surfaces.

4.2.1.2. Ultrasound propagation velocity

Despite being only a surface treatment, if consolidation from the biotreatment happens in-depth, an increase in the ultrasound propagation velocity is expected. Results are presented on Figure 4.3.

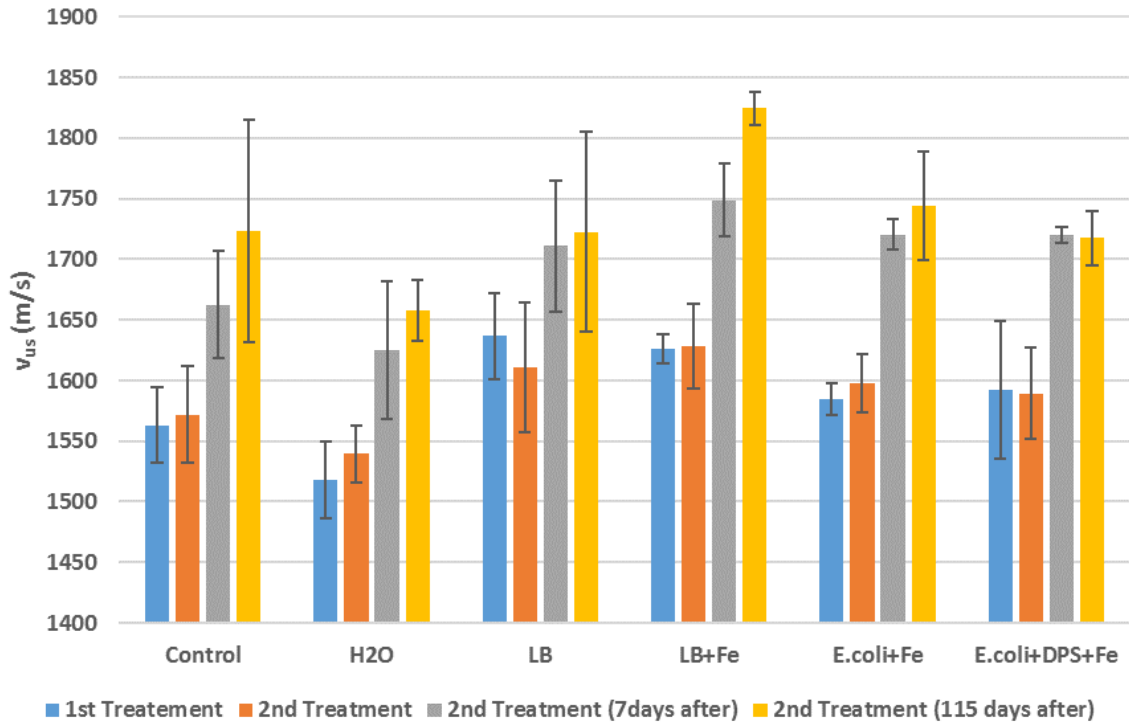


Figure 4.3 - 1st screening ultrasound propagation speed results

All the biotreated specimens present higher ultrasound propagation velocity than the “H₂O” and control specimens. Therefore, and as happened with the results from surface hardness, a slight improvement in compactness was noticed with all the bioproducts.

As Figure 4.3 shows, a crescent ultrasound propagation velocity was obtained for all tested specimens with time. This behavior may be due to different laboratory conditions and a crescent adsorption of water vapor by the earth mortar specimens and it is probably the reason of the jump between “2nd Treatment” and “2nd Treatment (7 days after)” results.

4.2.1.3. Surface cohesion

All adhesive tapes from the surface cohesion tests were re-attached to a white sheet. A pattern on the adhesive tapes was noticed on the treated specimens: a halo of loose particles around the application point had been formed, derived from the application method (Figure 4.4).



Figure 4.4 - 1st screening surface cohesion adhesive tapes from “Control” (right), “H₂O” (center) and “E.coli+Fe” (left) specimens

As observed in Figure 4.4, the specimens treated with the bioproduct have a very distinct pattern from the control specimens. Even if the results show improvements, this phenomenon needs to be avoided. Obtained results are presented in Figure 4.5.

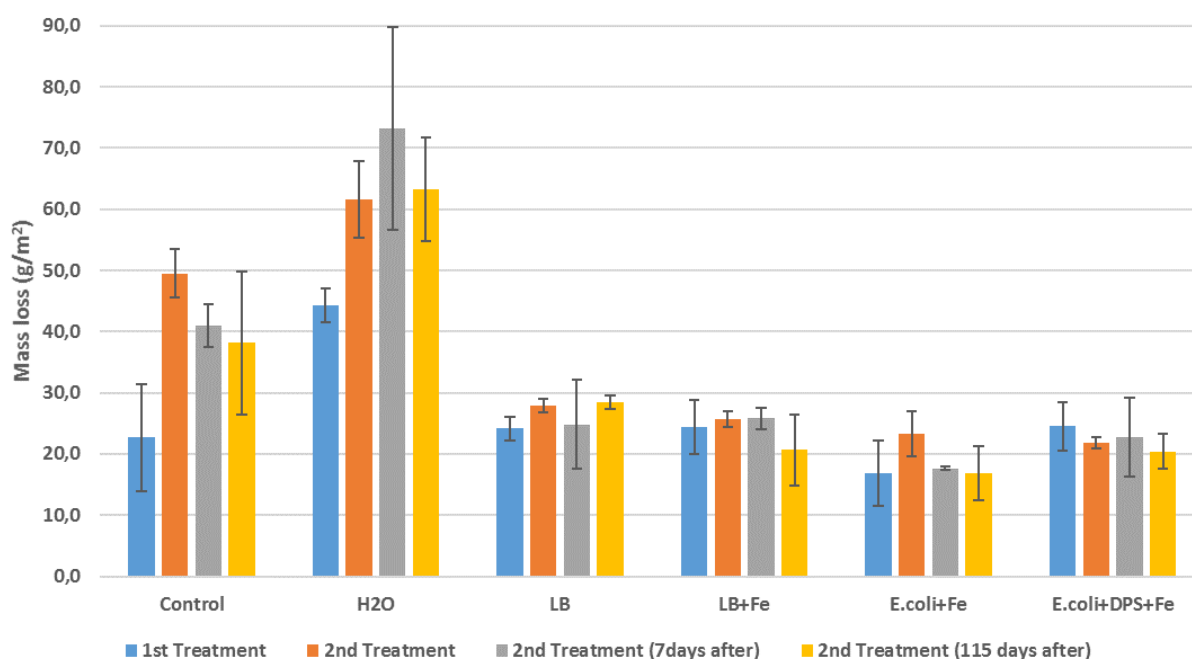


Figure 4.5 - 1st screening surface cohesion test results

The results obtained for surface cohesion (Figure 4.5) support the idea presented in section 4.1.1.1 that despite a liquid treatment may damage the surface of the specimen, the surface cohesion of biotreated specimens is maintained or even improved when compared with control ones. “H₂O” specimens have almost the double mass loss than other specimens.

“E.coli+Fe” treated specimens continue to show improvements. “E.coli+Fe+Dps” treated specimens have similar results to the previous tests.

The experimental campaigns conducted after the 2nd treatment was applied demonstrated that the control specimens were more degraded, maybe due to the action of the previously performed tests. “H₂O” specimens had a similar behavior.

Biotreated specimens showed no great improvements after the 2nd treatment. Mass loss did not increase, supporting a possible consolidation effect.

4.2.1.4. Water drop test

The water drop test exhibited the most evident results, especially after application of the second treatment. Despite being difficult to evaluate with accuracy the time between the moment the drop touches the surface until its total absorption, significantly different behaviors are observed (Figure 4.6).

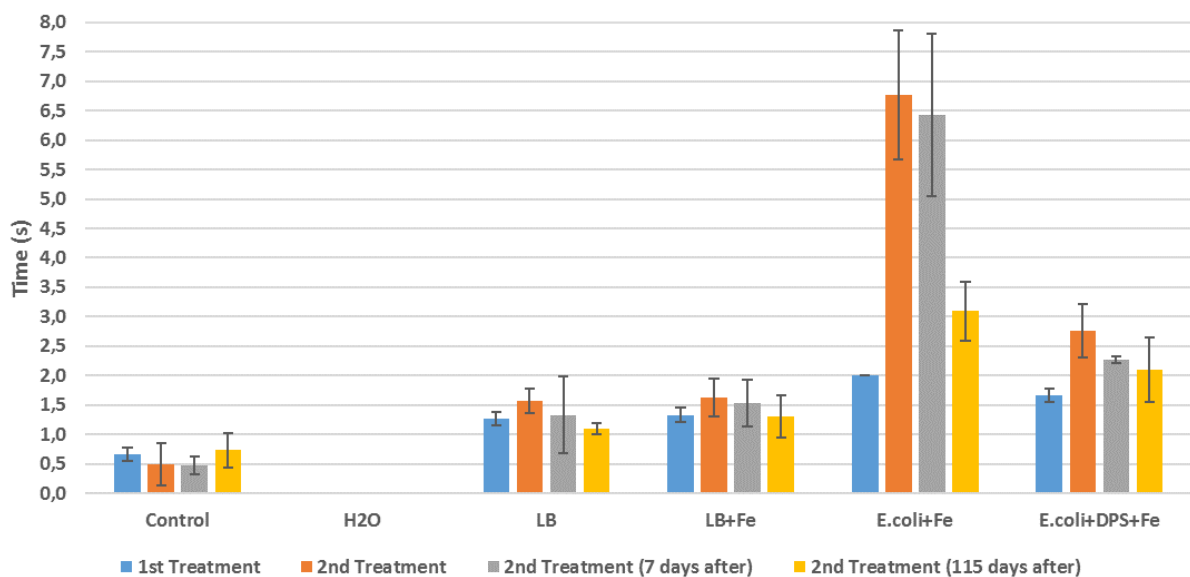


Figure 4.6 - 1st screening water drop test results

As it was expected, “H₂O” specimens had lower values than the control specimens. While control specimens showed an absorption time of approximately 0.5 seconds, on the “H₂O” specimens the water drop was instantly absorbed.

After application of the 1st treatment all biotreated specimens had an increase on water absorption drop time.

Water drop test performed after the 2nd treatment was applied showed that “E.coli+Fe” treated specimens had an increase of more than 850% (about 9 times higher) when compared to control specimens. 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 the “E.coli+Fe” treated specimens, where time to water absorption considerably decrease but was still the highest. This behavior might be due to the consecutive tests that damage the treated surface, that might be more explicit on the treatment that showed higher improvements.

4.2.2. 2nd Screening

As mentioned before, after analysis of the results obtained on the 1st Screening, some parameters were re-adjusted for the 2nd Screening. It is noteworthy that the 2nd Screening started before the experimental campaign at 115 days of the 1st Screening and, therefore, observations from this stage were not taken into account.

The application method was changed due to the degradation of the central part of the surface of specimens observed visually and on the previously obtained results on surface hardness and surface cohesion tests.

As no great differences were observed on the results from the “LB” and “LB+Fe” specimens and since the increase on iron concentration was one of the parameters that needed to be tested, “LB” biotreatment was not used on the 2nd screening.

The use of Dps did not led to significant results, in fact, lower results than the “E.coli+Fe” biotreatment were obtained. As an iron scavenger protein, Dps stores the iron, decreasing iron availability, which probably led to the decrease of the consolidation effect of the biotreatment. Thus, “E.coli+Fe+Dps” biotreatment was not used for the 2nd screening.

As it will be observed, the application of 2 mL volume led to interesting results, but while the treatments were applied, difficulties were felt related to the application of a larger volume on a small surface of specimens.

As only two “Control” specimens were used on the 2nd screening, standard deviation values are not graphically presented for ultrasound propagation speed, surface cohesion and water drop tests.

4.2.2.1. Surface hardness

The same 12 points grid used on the 1st Screening was used for the 2nd Screening. No degradation due to the application method was observed this time, but the same test procedure was conducted. Average results are graphically presented on Figure 4.7.

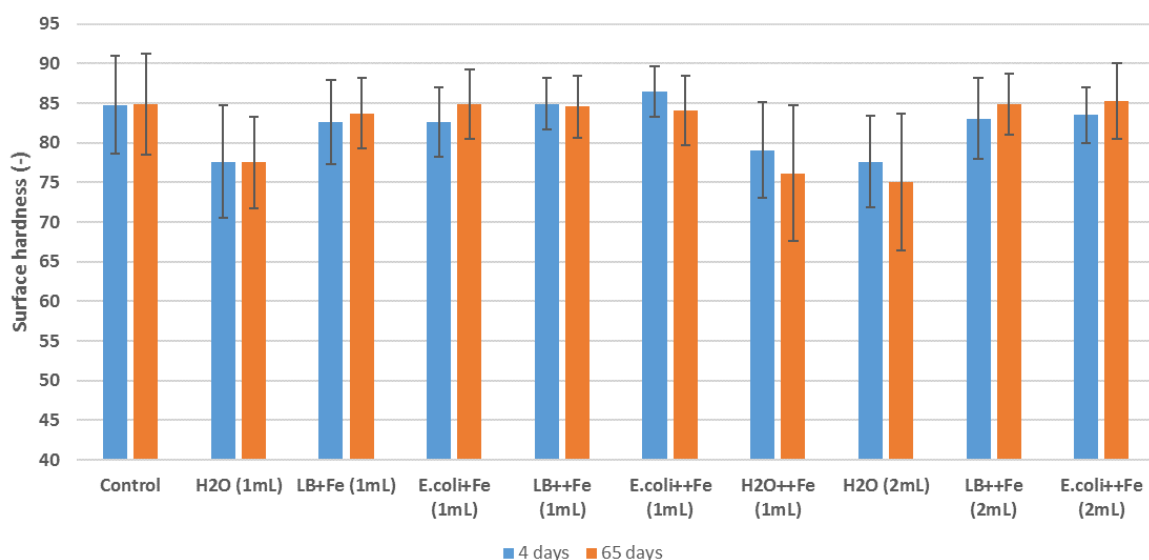


Figure 4.7 - 2nd screening surface hardness test results

As it was expected from the 1st screening, specimens treated with water had a significant decrease in surface hardness (Figure 4.7).

All biotreated specimens showed surface hardness values in the same range as the control specimens (non treated at all, therefore not damaged by any liquid), reaffirming the idea that even with a possible degradation of the surface due to the application of a liquid, the surface hardness is maintained and a consolidating effect is occurring.

In treatments where iron concentration was increased and 1 mL volume was applied, a slight increase in surface hardness can be noticed. Even the “H₂O++Fe (1mL)” biotreatment had higher surface hardness results than “H₂O (1mL)” and “H₂O (2mL)” treatments.

“LB++Fe (1mL)” and “E.coli++Fe (1mL)” biotreatments reached higher surface hardness than the ones obtained for the control specimens, while “LB+Fe (1mL)” and “E.coli+Fe (1mL)” had a slight decrease. This suggests that iron concentration may be a key factor for the increase of surface hardness and the consolidation of the surface.

Re-tested biotreatments, prepared as volume controls – “LB+Fe (1mL)” and “E.coli+Fe (1mL)” - did not show significant differences when compared with the results obtained on the 1st screening.

Surface cohesion results obtained 65 days after the treatment application did not show significant differences from the ones performed 4 days after the treatment. Some biotreated specimens had their surface hardness decreased while others showed a slight increase, with no pattern being defined.

4.2.2.2. Ultrasound propagation velocity

Results of ultrasound propagation velocity from the 1st Screening did not lead to significant conclusions, maybe due to low penetration depth of the treatments. For the 2nd screening, as 2 mL treatments were applied, a more in-depth consolidation might have been obtained (Figure 4.8).

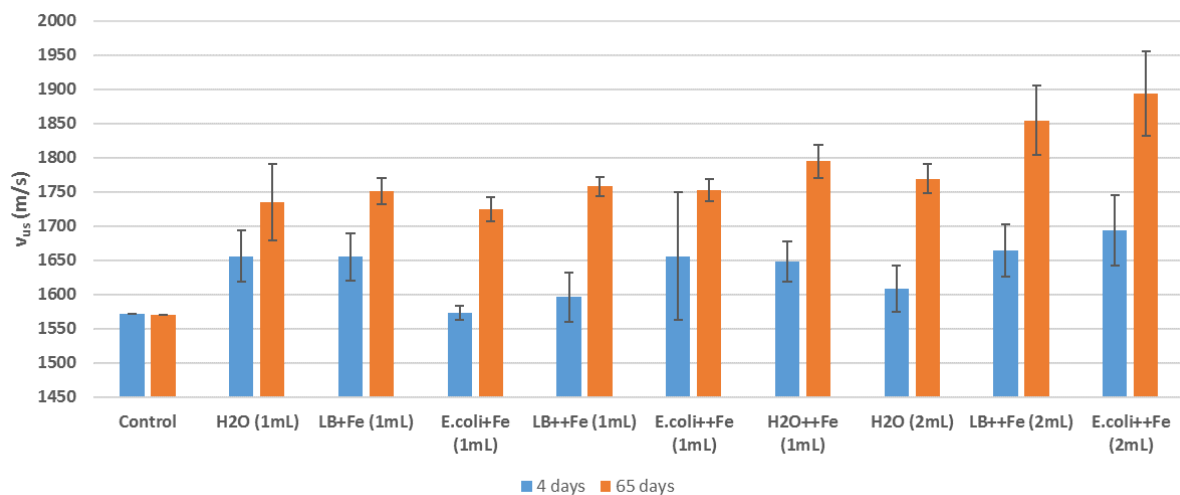


Figure 4.8 - 2nd screening ultrasound propagation velocity test results

Even if better results were obtained for the “LB++Fe (2mL)” and “E.coli++Fe (2mL)” biotreatments, almost all specimens had higher ultrasound propagation velocity than the control specimens.

Control specimens are in the same range of ultrasound propagation velocity as the ones obtained on the 1st screening, but all other specimens registered higher values than the control, leading to some

contradictory results when compared to the 1st screening. Probably, the hole created by the application method on the 1st screening led to misleading results and comparison is not possible.

Results for 65 days show a considerable increase in ultrasound propagation speed in all specimens with exception of the control specimens, that remained similar to the 4 days results. If control specimens also showed an increase in ultrasound propagation speed, the results could be justified by a slight increase in relative humidity (47% to 51%). No further conclusions could be withdrawn.

4.2.2.3. Surface cohesion

On the 1st screening, difficulties were encountered when cutting and weighing the adhesive tape. Therefore, adhesive paper was used on the surface cohesion test of the 2nd screening. Nevertheless the adhesive paper had lower adhesive strength than the adhesive tape. Mass loss results obtained from the surface cohesion test are presented on Figure 4.9.

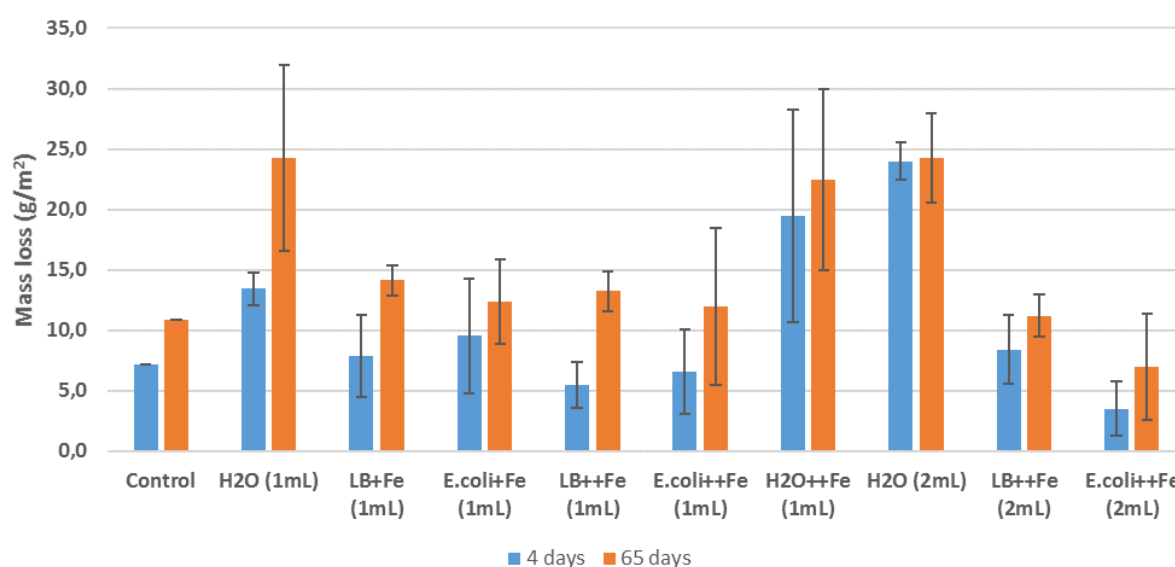


Figure 4.9 - 2nd screening surface cohesion test results

As no degradation effect was visually observed, there were considerably less free particles on the surface of the specimens. A pattern of free particles distributed along the surface was common to all specimens.

Similar results to the 1st screening were observed: water treated specimens presented lower surface cohesion; biotreated specimens were in the same range of values for surface cohesion as control specimens.

It should be noted that the “E.coli++Fe (2mL)” treatment showed in both ages of test lower mass loss than the control mortar. The consolidation effect obtained for this treatment is close to the ones of the treatments used by Jroundi et al. (2010a, 2010b) to repair degraded limestone (both treatment methods are described on 2.3.1).

When compared with the results obtained for surface hardness, an analogous effect occurs for surface cohesion: biotreatments with higher iron concentration reach higher surface cohesion. As surface hardness and surface cohesion are related, the obtained results support the idea that iron has a main role on the consolidating effect.

Higher mass loss was obtained in all specimens in the test performed 65 days after the treatment application. Even if these results tend to lead to the conclusion that the treatment has a low durability, the decrease in surface cohesion can be due to the successive testings that tend to degrade the surface, specially the surface hardness test. In order to discard this hypothesis, in future experimental campaigns different specimens should be used to assess the treatment durability.

4.2.2.4. Water drop test

Before the test was conducted, when the feeding was performed, a resistance towards water absorption was evident on both “E.coli++Fe (1mL)” and “E.coli++Fe (2mL)” biotreatments. This behavior can be observed on Figure 4.10.

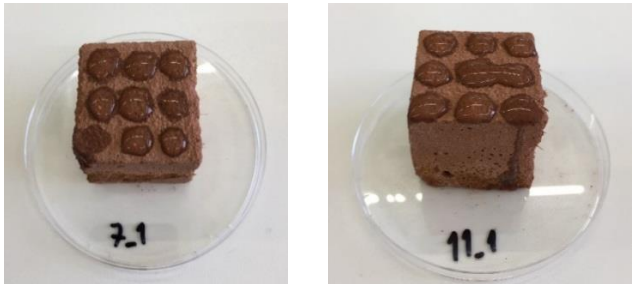


Figure 4.10 - Observation of “E.coli++Fe (1mL)” (left) and “E.coli++Fe (2mL)” (right) specimens absorption resistance during feeding

As the feeding was being applied, the LB medium was hardly absorbed by the earth mortar. This observation led to the conclusion that the biotreatment itself was creating a waterproofing effect. So, may be feeding was unnecessary.

The results from the water drop test confirm the waterproofing effect observed during the feeding of the “E.coli++Fe (1mL)” and “E.coli++Fe (2mL)” specimens (Figure 4.11).

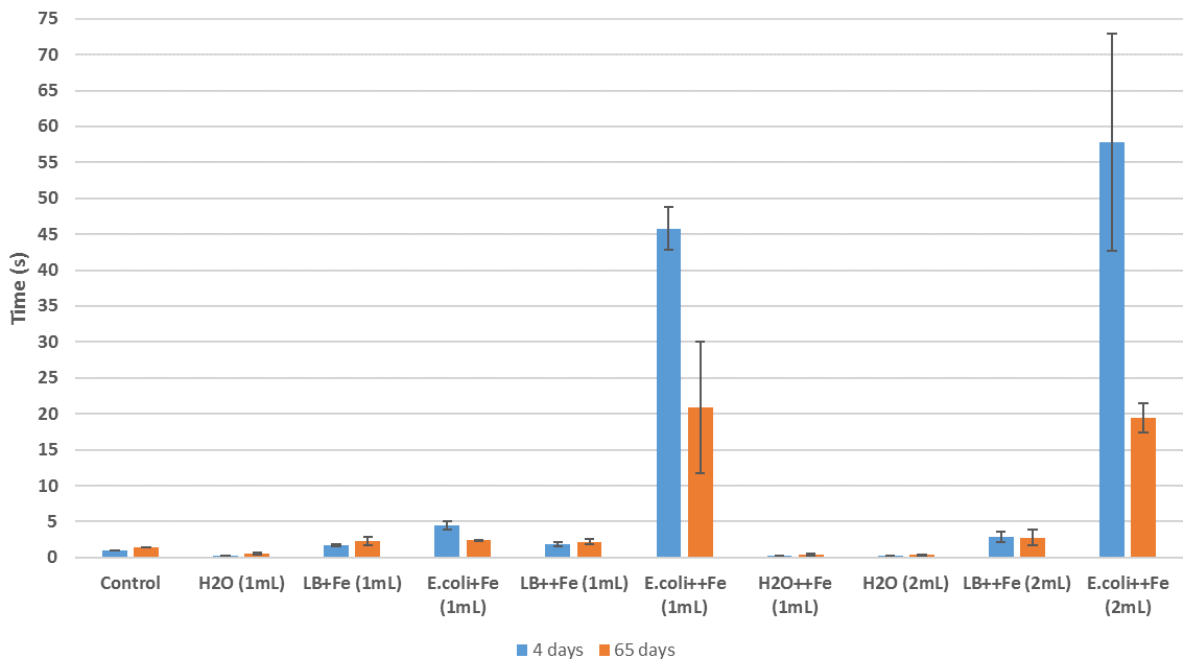


Figure 4.11 - 2nd screening water drop test results

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 has previously observed on the 1st Screening.

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 great results were achieved. Like the results obtained for surface cohesion, a tendency to affirm that the treatment is not durable rises with the results obtained for this test. But again, damage on the surface of the specimens produced by previous tests repeated in each test phase may justify this decrease.

These are very promising results, since degradation by water is one of the major weaknesses of earth mortars. Even if compared with the results of the commercially available treatments studied by Stazi et al. (2016) and described in 2.7, that can reach a complete waterproof, these iron-based biotreatments reach inspiring improvements without affecting the re-use of earth mortars. On the other hand, as hygrometric conditions control is one the greatest strengths of earth plasters, a hygroscopic test should be performed to assess the sorption-desorption behavior of these biotreated mortars.

4.3. Bioformulated earth mortars

As mentioned before, bioformulation of earth mortars were performed after the 1st screening. As the “E.coli+Fe” specimens were the ones who presented the best results, this bioproduct was used to bioformulate the mortars. “LB” bioproduct was tested has a biocontrol.

4.3.1. Fresh state

Water formulated mortars (control specimens) had the volume of water adjusted for a good workability. For bioformulated mortars, the same volume percentage (25%) was adopted for the bioproducts, but a similar workability was not obtained. Bioformulated mortars presented a “fluffy” aspect, visually with a high air content.

After fresh state tests and the production of all specimens, a portion of fresh mortars were kept on zipper-bags. As the above-mentioned behavior of bioformulated mortars was observed, the remains were re-used after 72 hours to infer if the same behavior would occur.

When earth mortars were removed from the zipper-bags, it was clear that they were drier but water formulated mortars and bioformulated mortars had now a similar workability.

As only two mixes of each mortar were performed, standard deviation was not obtained for fresh state tests, except for flow table consistency.

4.3.1.1. Flow table and penetrometer consistencies

Flow table consistency results confirm the “fluffy” aspect observed during the mixing of bioformulated mortars. It seemed that the mortars had air entrained. Higher flow diameter and lower slump height (Figure 4.12) than control mortars were registered for both bioformulated mortars.

It is noteworthy that in both water formulated and bioformulated mortars no excess volume of water or of the bioproduct was added and no liquid halo around the mortar was observed on the flow table at the end

of the test. This behavior may be due to foam produced by shaking while handling the LB medium (*E. coli* culture has LB medium as a constituent).

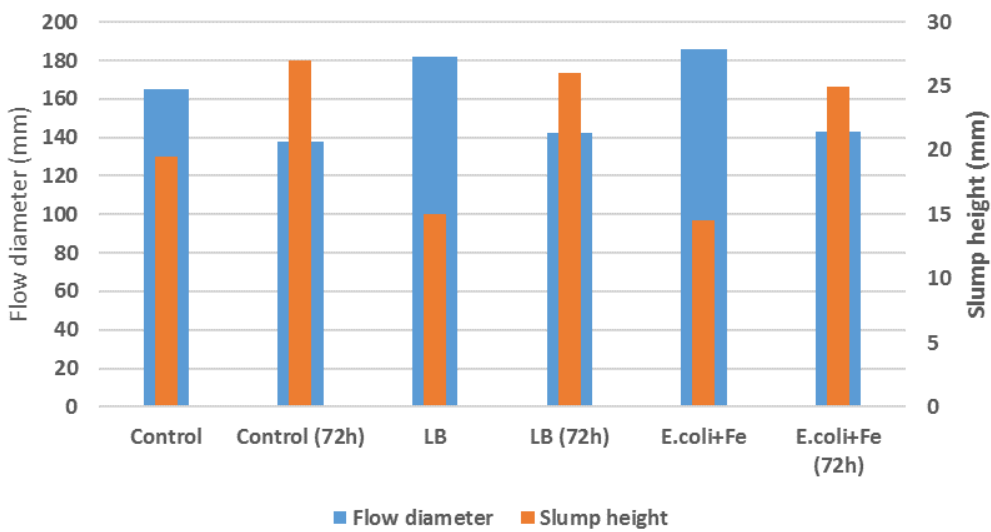


Figure 4.12 - Bioformulated earth mortars flow diameter and slump height

All mortars tested immediately after production presented a flow table within a range of 20 mm, between 162 mm for the control and 180-182 mm for the bioformulated, close to the range of 175±5 mm defined by DIN 18947 (DIN, 2013). The slump height was proportional: higher for the control and lower for the bioformulated mortars. When tested after 72 hours all the mortars presented the same but lower flow table diameter, about 140 mm. The slump height of all the mortars increased and became closer for the control and the bioformulated mortars. Therefore, it seems that the fluffy consistency of bioformulated mortars disappears after some time of mixing.

Penetrometer consistency results, presented on Figure 4.13, are in accordance with the obtained for flow table consistency. Bioformulated mortars present a higher penetration depth than control mortars and all mortars tested after 72 hours are in a much closer range of values for penetration depth, showing that consistency is quite different for bioformulated mortars in comparison with control mortar immediately after mixing but the different effect disappears after some hours.

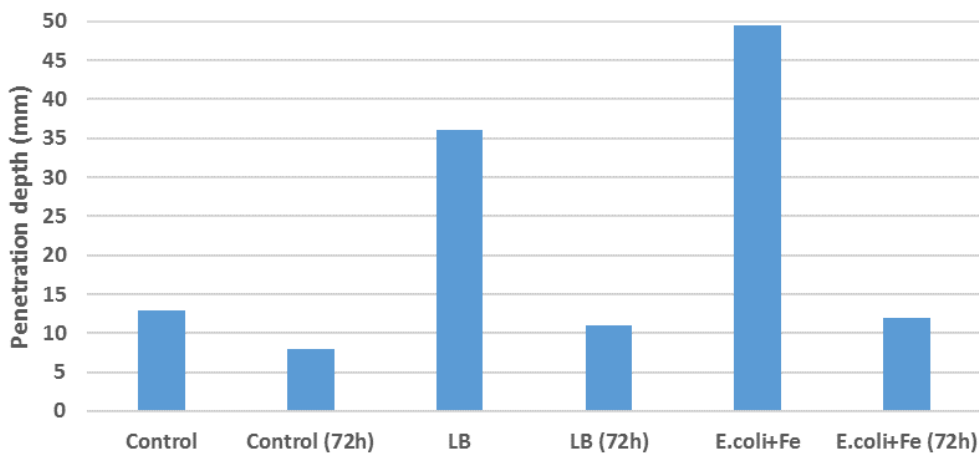


Figure 4.13 - Bioformulated earth mortars penetrometer consistency results

Bioformulated mortars justifies a further study in order to understand the influence of components on consistency and particularly on workability. May be this is justified by the LB medium, which could be deconstructed and the gas producing component identified.

4.3.1.2. Wet bulk density

It was not possible to measure wet bulk density of control mortars after 72 hours due to the lack of necessary mortar volume. Nevertheless, it is expected that it does not change as much as the bioformulated mortars because workability did not change with time as happened with the other mortars. Results are graphically presented on Figure 4.14.

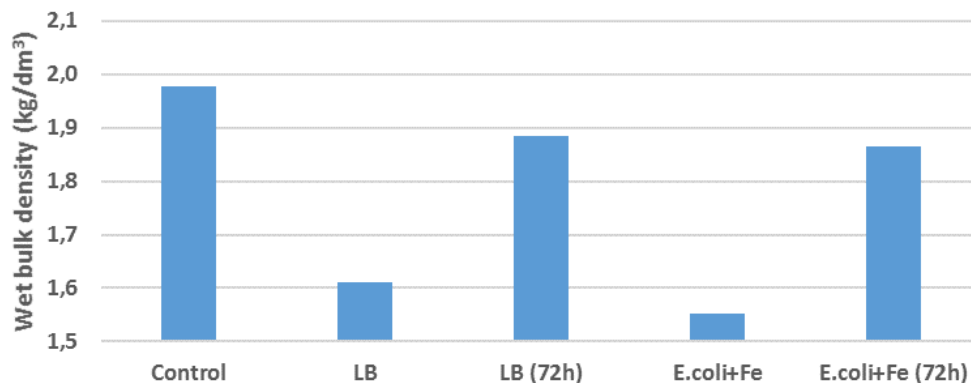


Figure 4.14 - Bioformulated earth mortars wet bulk density results

As it was expected after the visual observation and workability of bioformulated mortars, wet bulk density was lower than the control mortar. Nevertheless, all mortars are in accordance with a minimum wet bulk density of 1.2 kg/dm³ defined by DIN 18947 (DIN, 2013).

72 hours after production, wet bulk density of bioformulated mortars increased quite significantly and turn to be more similar to the initially obtained in the control mortar, but still lower.

4.3.2. Hardened state – prismatic earth mortar specimens

Difficulties were encountered when demolding the bioformulated mortar specimens. Bioformulated mortar specimens were strongly attached to the molds and only specimens on molds where demolding oil was used in excess kept intact. One of the prismatic specimens of “E.coli+Fe” mortar performed 72 hours after the mixing broke when demolding.

4.3.2.1. Drying shrinkage

No visual shrinkage was observed in any of the formulated mortars. Control specimens presented an average linear drying shrinkage of 0.4%, “LB” mortar specimens 0.0% and “E.coli+Fe” mortar specimens 0.2%. Although it is not a significant difference, bioformulated mortars present lower shrinkage than the control mortar.

All mortars are in accordance with the maximum drying shrinkage of 2.0 % defined in DIN 18947 (DIN, 2013).

4.3.2.2. Bulk density and dynamic modulus of elasticity

While demolding the prismatic specimens, specially the bioformulated mortars were considerably attached to the mold and some material was lost. Even though some specimens were not complete, bulk density was assessed and average results taken (Figure 4.15).

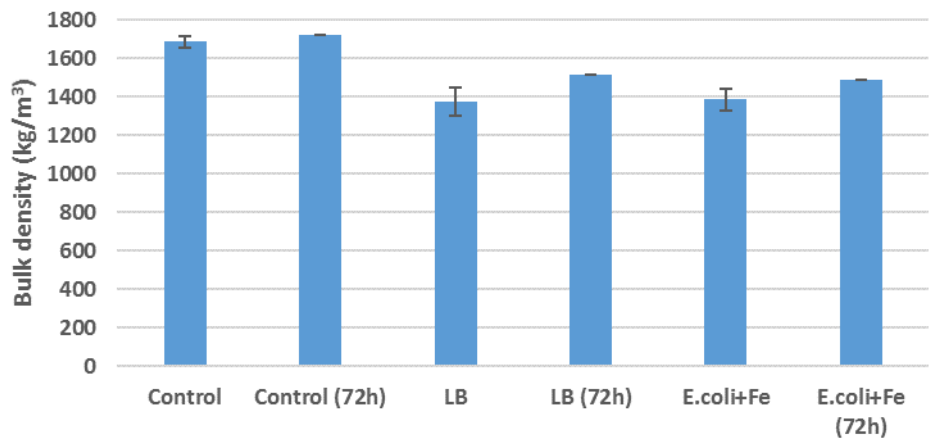


Figure 4.15 - Formulated mortars bulk density

As it was expected from what was observed during mortars’ formulation, bioformulated mortars have lower bulk density than control mortars. Mortar specimens molded 72 hours after the formulation show an increase in bulk density, more pronounced on bioformulated mortars. These results are in accordance with the ones obtained for wet bulk density, supporting the idea that bioformulated mortars are considerably more porous than control mortars.

A few specimens did not retrieve conclusive results for dynamic modulus of elasticity, which lead to the annulation of these results. This may happen due to fissures on the specimens. No results could be obtained for “E.coli+Fe (72h)” mortar. All results are graphically presented on Figure 4.16.

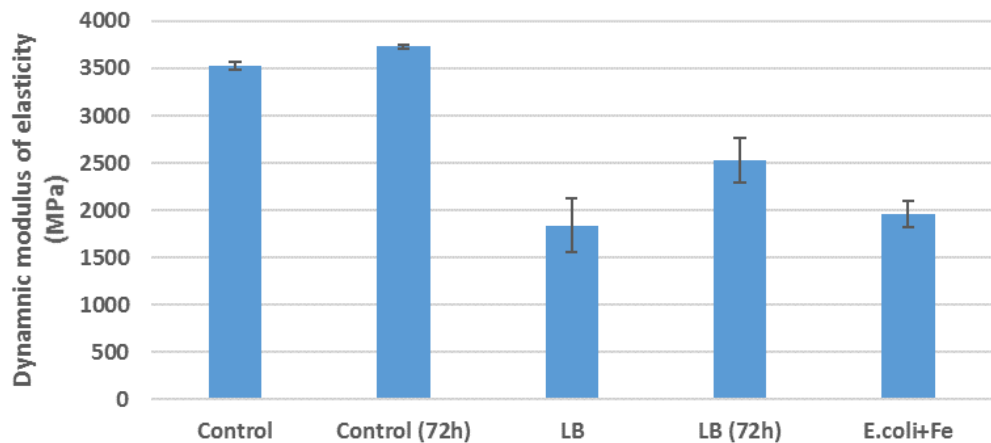


Figure 4.16 - Bioformulated mortars dynamic modulus of elasticity results

When compared with control mortar, a considerably lower dynamic modulus of elasticity was obtained for bioformulated mortars, meaning they are less rigid and may have a higher capacity to absorb deformations. Despite not having results for “E.coli+Fe (72h)”, the “LB (72h)” mortar had a significant increase in the

dynamic modulus of elasticity when compared with “LB”, what can probably suggest an increase in both bioformulated mortars. This should be justified by the increase on bulk density.

4.3.2.3. Flexural and compressive strengths

Flexural strength and especially compressive strength results for bioformulated mortars are negatively affected by the degraded and irregular form of the specimens due to demolding.

Average results for flexural and compressive strengths are presented in Figure 4.17. Standard deviation was not obtained for mortars used 72 hours after mixing because of short number of specimens.

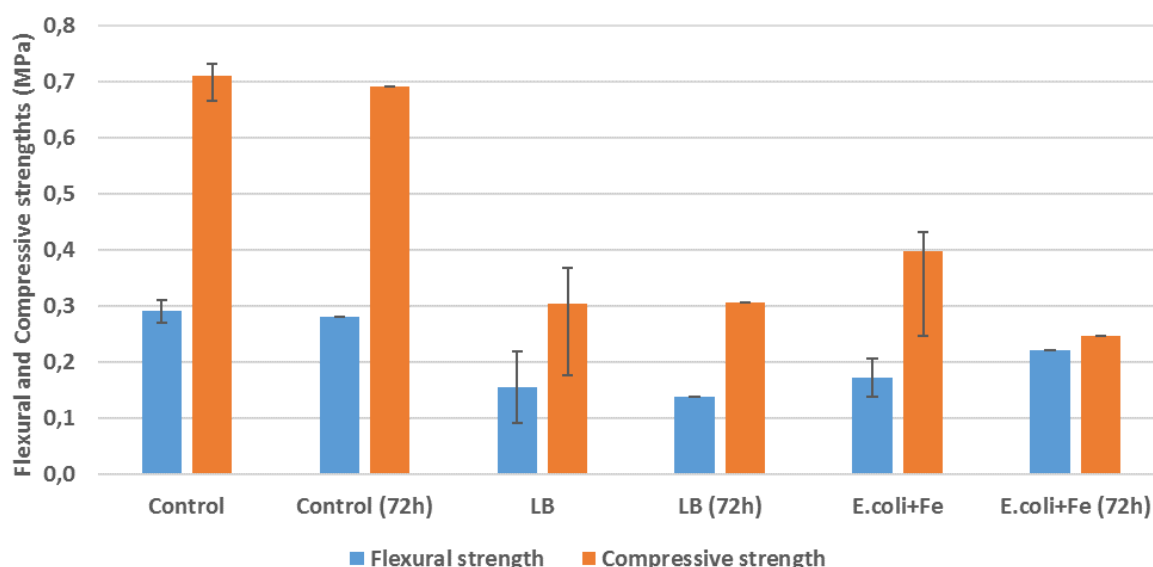


Figure 4.17 - Bioformulated mortars flexural and compressive strengths 48 days after production

With the results previously obtained for the dynamic modulus of elasticity, it was already expected that bioformulated mortars had lower flexural and compressive strengths. As Ivanov et al. (2010) and Ivanov et al. (2014) have demonstrated, it is possible to have similar iron-based bioproducts acting as binders in sand. So, it is imperative to perform a more in-depth study of the used iron-based bioproduct in order to avoid the observed behavior.

No significant conclusions can be taken for the mortars used 72 hours after mixing. Despite only one test was performed for each mortar, the results for flexural and compressive strength are irregular and no pattern can be noticed. Nevertheless, considering that the fresh state properties significantly changed when specimens were produced 72h after mixing and the wet bulk density increased, it could be expected that an increase on mechanical strength also occurred, what was not a pattern.

Flexural and compressive strengths of mortars freshly used are presented in more detail on Table 4.1.

Table 4.1 - Formulated mortars flexural and compressive strengths results

Specimen	Control	LB	E.coli+Fe
Flexural strength (MPa)	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Compressive strength (MPa)	0.7 ± 0.0	0.3 ± 0.1	0.4 ± 0.2

In both flexural and compressive strength, all mortars present lower strengths than the minimum of 0.3 MPa for flexural strength and 1.0 MPa for compressive strength defined by DIN 18947 (DIN, 2013).

4.3.2.4. Surface hardness

Obtained results for surface hardness of bioformulated mortars are graphically presented on Figure 4.18.

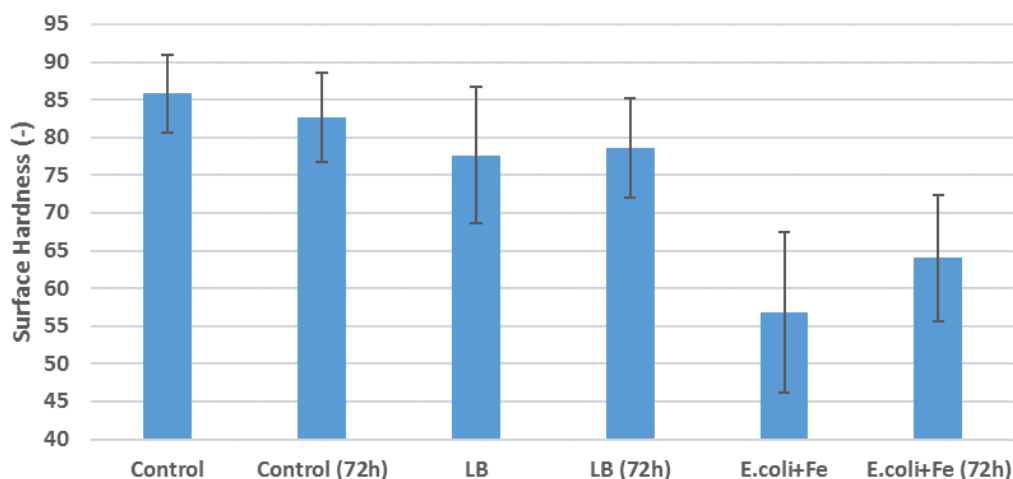


Figure 4.18 - Bioformulated mortars surface hardness test results

Unlike the results obtained on the 1st and 2nd screenings, bioformulated mortars presented lower surface hardness than biotreated mortars. Specially on “E.coli+Fe” biotreated mortars, the ones obtaining better results, when the “E.coli+Fe” bioproduct was used on the formulation of mortars the surface hardness considerably decreased.

These results are not in total accordance with the ones obtained for dry abrasion resistance. When handling the bioformulated prismatic specimens, they were considerably more friable than the control mortar specimens. The same friability was not observed on the earth plastering mortars applied on brick because the mortars were thrown to the brick (in the laboratory, they fall from a defined height) and pressed against the brick to regularize the surface of the plaster – simulating an *in situ* plaster application. Also, there was the influence of the brick itself and the drying surface. For this reason, surface hardness was also tested on these specimens.

In all mortars, no significant differences (considering the standard deviation) were observed for mortars used 72 hours after mixing. Nevertheless, bioformulated mortars with E.coli+Fe presented the lowest hardness.

4.3.2.5. Surface cohesion

While handling the bioformulated mortar specimens they were considerably friable than the control specimens, specially the “E.coli+Fe” specimens. Surface cohesion test results are graphically presented on Figure 4.19.

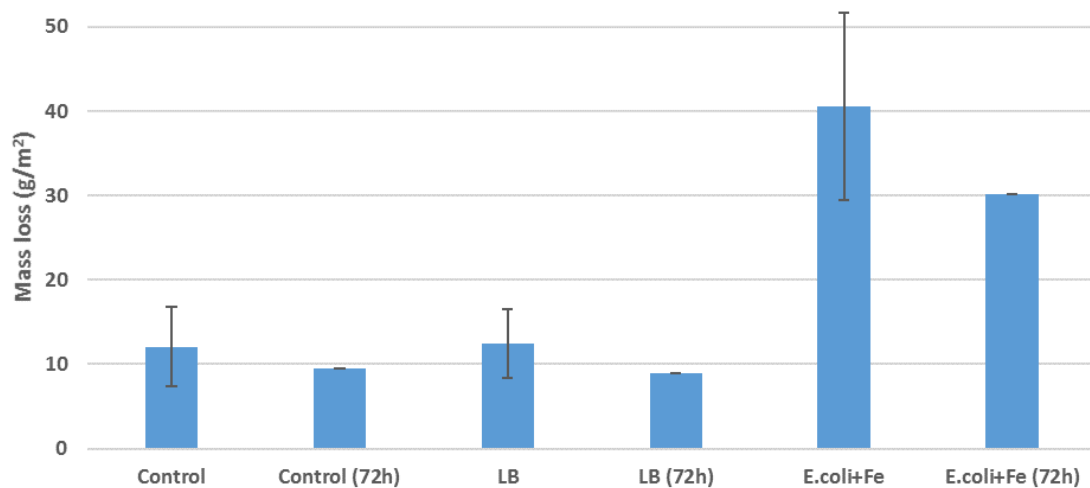


Figure 4.19 - Bioformulated mortars surface cohesion test results

Surface cohesion tests are in accordance with the results obtained for surface hardness, being the “E.coli+Fe” bioformulated mortar the one with lower surface cohesion.

The water formulated mortar and the LB medium formulated mortar had similar mass loss results, while “E.coli+Fe” mortar had around the triple mass loss. “E.coli+Fe” mortar has been showing a considerable higher brittleness than the other mortars, what needs to be avoided.

Despite no decrease, no great difference was observed for mortars used 72 hours after the mixing, except for the “E.coli+Fe” mortar but mass loss was still considerably high.

4.3.2.6. Ultrasound propagation velocity

After observing the “fluffy” aspect and visual pores of the bioformulated mortars, higher porous percentage was expected for these mortars and, consequently, lower compactness.

Average ultrasound propagation velocity of mortars is presented on Figure 4.20. Standard deviation values were not obtained for mortars used 72 hours after mixing.

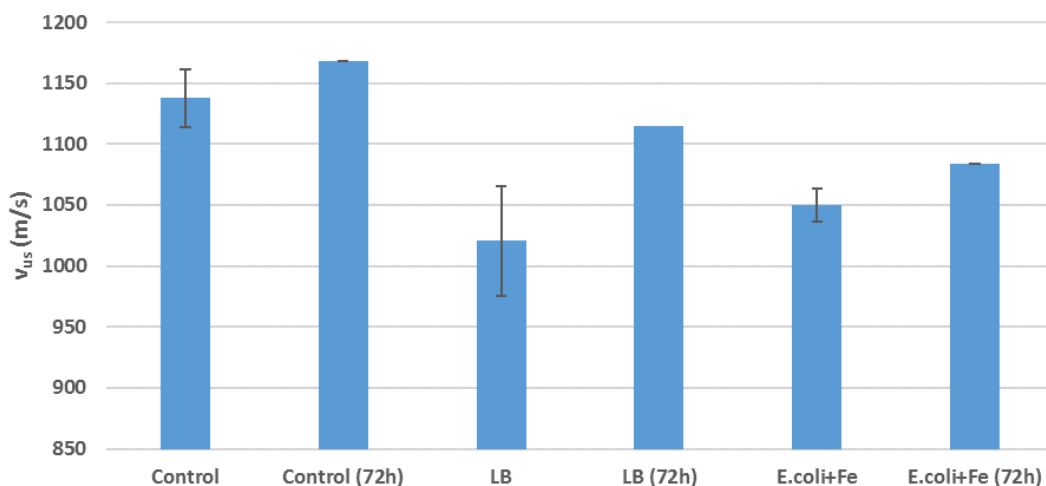


Figure 4.20 - Bioformulated mortars ultrasound propagation velocity test results

Ultrasound propagation velocity results show that bioformulated mortars are in fact less compact than control mortar. “E.coli+Fe” mortar may be slightly more compact than “LB” mortar due to the production of iron-oxide on the porous structure.

Mortars molded 72 hours after mixing show higher compactness than mortars used immediately after mixing.

4.3.2.7. Thermal conductivity

As the bioformulated mortars showed visible porosity, it was expected a decrease of thermal conductivity. Average thermal conductivity results are presented on Table 4.2.

Table 4.2 - Thermal conductivity test results

Specimen	Control	LB	E.coli+Fe
Thermal conductivity (W/m.K)	0.773	0.545	0.416

A significant decrease was observed on bioformulated mortars, when compared with control mortars. These results might be due to the above-mentioned visual observation of pores in bioformulated mortars.

Although there has been a decrease of flexural and compressive strengths and a loss of cohesion, these results may lead to the application of these bioformulated mortars for thermal insulation purposes.

4.3.2.8. Water drop test

Similar results to the 1st screening were obtained in the water drop test, with bioformulated mortars tending to resist more to water penetration. Obtained results are presented on Figure 4.21. Standard deviation values were not obtained for mortars used 72 hours after mixing.

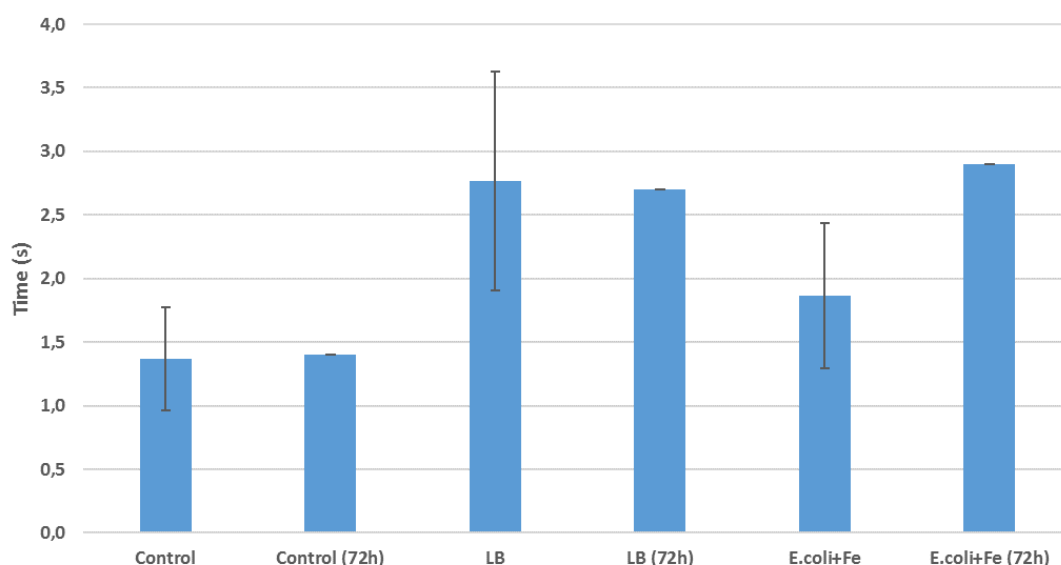


Figure 4.21 - Formulated mortars water drop test results

When compared with the results obtained by Aguilar et al. (2016), a similar trend is observed, with bioformulated mortars showing a slight waterproofing effect but not as evident as the results obtained for the biotreatments.

4.3.2.9. Durability in water

Although not regulatory, this test showed promising results. This test helped to understand the behavior of bioformulated mortars when submerged in water, since resistance to water absorption was one of the best improvements observed on the 1st screening.

Figure 4.22 is a photograph taken about 1 minute after samples of mortars were submersed in water. As it can be observed, the bioformulated mortars have considerably higher durability when submerged in tap water than the control mortar, specially the “E.coli+Fe” mortar.

The moment the control mortar sample was placed in water it immediately started to dissolve. “LB” 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 with considerably less loose particles than the other mortars could be observed on the bottom of the container.

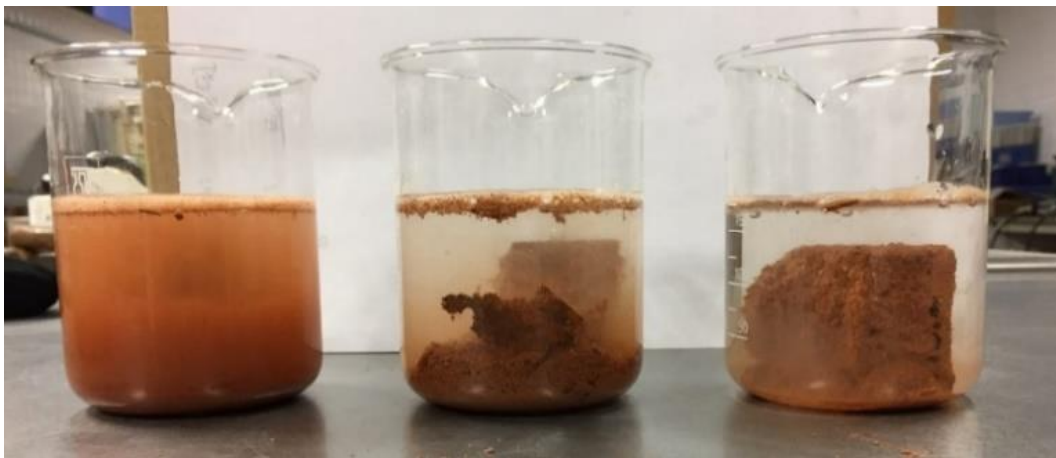


Figure 4.22 – “Control” (left), “LB” (center) and “E.coli+Fe” (right) mortars degradation when submersed in water

These results suggest that bioformulated earth mortars may have considerably higher resistance towards liquid water than control mortars. This behavior may guide earth mortars to application as renders or in more harsh environments.

4.3.3. Hardened state – earth plaster on brick

4.3.3.1. Visual observation

Disconnection from the brick was noticed on the sides of bioformulated mortars (Figure 4.23). On the other hand, no cracks were observed on the surface of these mortars, neither on the control mortars (Figure 4.24)



Figure 4.23 - Disconnection from the bricks observed on “LB” (right) and “E.coli+Fe” (right) mortars

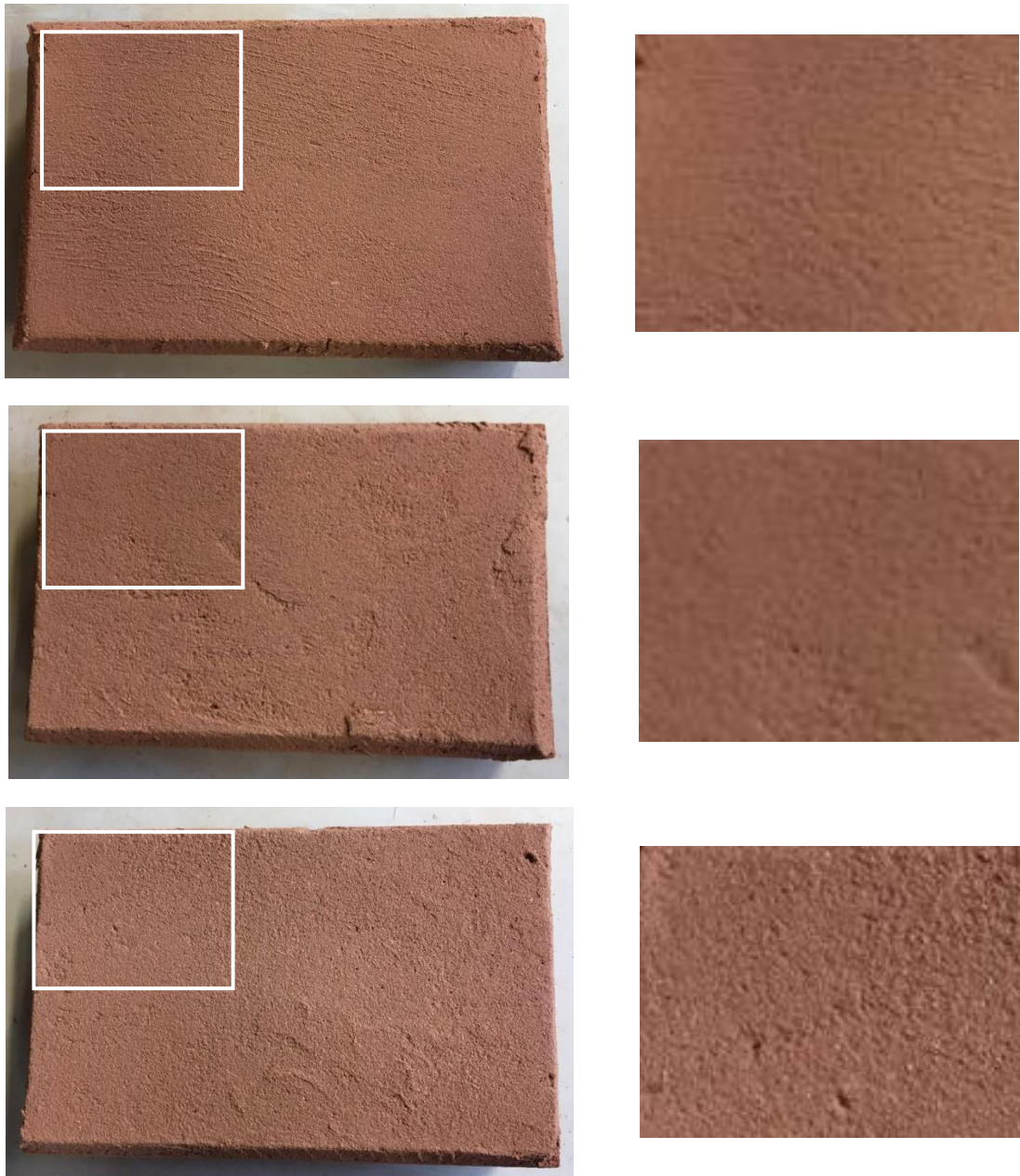


Figure 4.24 - Observation of surface on "Control" (above), "LB" (center) and "E.coli+Fe" (below) specimens; zoomed image on the right

4.3.3.2. Adhesive strength

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) (Figure 4.25).

Control specimens had in all three tests a total disruption of the mortar from the brick, meaning that the cohesion of the mortar is higher than the adhesion to the brick. One of tests in "LB" specimens could not be performed because the mortar fracture had already happened. All other tests in "LB" specimens and "E.coli+Fe" specimens had a partial disruption of the mortar from the brick, meaning that the adhesion to the brick is close to the cohesion of the bioformulated mortar.



Figure 4.25 - Obtained disconnection fractures on "Control" (left), "LB" (center) and "E.coli+Fe" (right) specimens

The first test of control mortars was performed on a hydraulic press that did not had the precision to register the adhesive strengths of these earth mortars, leading to the annulment of the result. On the "LB" mortars, in addition to the hole where fracture had already occurred, one of the other two tests returned an abnormally low adhesion strength, also leading to the annulment of this result. Due to these events, standard deviation was not obtained and only average results are presented on Table 4.3.

Table 4.3 - Formulated mortars adhesive strength results

Specimen	Control	LB	E.coli+Fe
Adhesive strength (MPa)	0.04	0.02	0.04

"E.coli+Fe" bioformulated mortar presented as good results as the control mortar. "LB" mortar had half of the adhesive strength of the control one, but no great conclusions can be taken from this mortar because of the number of tested samples.

The control and E.coli+Fe mortars are close to the minimum value of 0.05 MPa of adhesive strength defined in DIN 18947 (DIN, 2013).

4.3.3.3. Dry abrasion resistance

Due to the lack of cohesion observed during the demolding of bioformulated mortars prismatic specimens, a medium hardness brush was used. Weight loss from abrasion results are presented in Table 4.4.

Table 4.4 - Bioformulated mortars dry abrasion results

Specimen	Control	LB	E.coli+Fe
Weight loss (g)	3.1 ± 1.7	2.4 ± 0.6	3.3 ± 0.5

Despite the observed in the prismatic specimens, weight loss from abrasion in bioformulated mortars were similar to the ones obtained for control mortar; in fact, "LB" bioformulated even showed lower mass loss.

The difference in the surface finish between these specimens and prismatic specimens was that in these specimens a plastic float trowel was used. This finish probably helped losing the air that was on the surface of the bioformulated mortars layer, turning it more cohesive.

All mortars present higher mass loss than the maximum of 1.5 g defined by DIN 18947 (DIN, 2013).

4.3.3.4. Water absorption under low pressure by Karsten pipes

Unlike what had been observed with the water drop test, water absorption of bioformulated mortars was considerably faster when tested using Karsten pipes. Control mortars took 35 minutes to absorb the 4 mL

of water, while “LB” mortars only took 7 minutes. “E.coli+Fe” mortars were impossible to test since while the pipes were being filled the water was immediately absorbed.

This behavior might be due to the big pores observed on bioformulated mortars. While on the water drop test the surface area tested is only the small area of application of the water drop, Karsten pipes have 50 mm diameter reaching an higher area, more pores and with higher pressure.

As it can be observed on Figure 4.26, water percolates more easily through the microstructure of bioformulated mortars due to their large pores. This behavior is more visible on “E.coli+Fe” mortars and is in accordance to what occurred while the test was being performed.

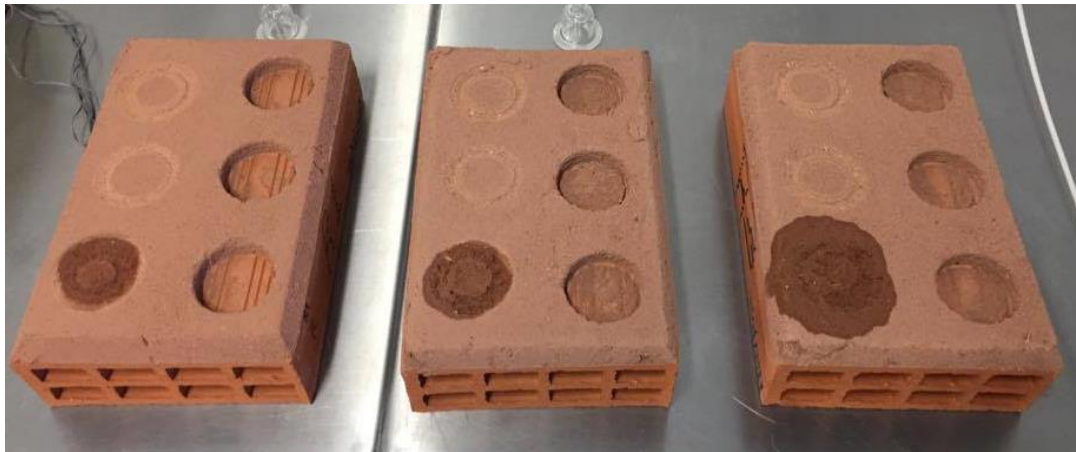


Figure 4.26 – “Control” (left), “LB” (center) and “E.coli+Fe” (right) mortars after Karsten pipe test

4.3.3.5. Surface hardness

Surface hardness of earth plasters on brick was performed after it was performed in the earth mortar cubic specimens, due to the differences observed between bioformulated mortars on these two different specimens (prismatic specimens looked more friable). Average results and respective standard deviation are presented in Table 4.5.

Table 4.5 – Formulated mortars surface hardness results

Specimen	Control	LB	E.coli+Fe
Surface hardness (-)	82 ± 5	77 ± 6	78 ± 7

Results show that bioformulated mortars have lower surface hardness than control mortars, but the obtained difference is not significant.

When compared with the results obtained on the cubic specimens, “E.coli+Fe” earth mortar layer specimens have a surface hardness almost 40% higher. This difference, that brings bioformulated mortars surface hardness closer to control mortars, may be due the application method used to apply the mortar to the brick, a more similar method to the currently used.

4.3.3.6. Surface cohesion

Bioformulated mortars show higher mass loss and, consequently, lower surface cohesion than control mortars. Average results are presented in Table 4.6.

Table 4.6 - Formulated mortars surface cohesion results

Specimen	Control	LB	E.coli+Fe
Mass loss (g/m ²)	1.4	2.3	5.2

All formulated mortars cubic specimens had considerably higher mass loss than the earth mortar layer specimens. On the other hand, when put into percentage, the difference between control and bioformulated mortars is the same. This means that, despite the application method, bioformulated mortars still have considerable lower surface cohesion than control mortars.

4.3.3.7. Water drop test

Positive results were obtained from the water drop test on earth mortars layer specimens (Table 4.7). Bioformulated mortars show a considerably higher waterproofing effect than control mortars.

Table 4.7 - Formulated mortars water drop test results

Specimen	Control	LB	E.coli+Fe
Time (s)	1.1 ± 0.5	6.0 ± 2.6	8.7 ± 0.6

On the cubic specimens, bioformulated mortars had already shown decent results, but not as good as the ones obtained for the earth plasters on brick. In this case, the application method considerably improves the capacity for the bioformulated mortars to avoid a rapid ingress of water drop.

4.3.3.8. Compressive strength

Earth plaster specimens reach compressive strengths that guarantee the minimum of 1.0 MPa defined by DIN 18947 (DIN, 2013). On the other hand, bioformulated mortars have considerably lower compressive strength than control mortars (Table 4.8).

Table 4.8 - Formulated mortars compressive strength results

Specimen	Control	LB	E.coli+Fe
Compressive strength (MPa)	3.2	1.3	1.1

Despite higher compressive strengths were obtained in these specimens for all three formulations in comparison with the prismatic ones, the difference between control and bioformulated mortars is similar to the strengths obtained on earth mortar prismatic specimens (up to 2-3 times higher).

4.4. Summary discussion

A brief qualitative analysis of the results of all biotreatments and bioformulations is presented in Tables 4.9 and 4.10, with controls as references.

Table 4.9 - Qualitative comparison of tested biotreatments

Comparison with control specimens	Surface hardness	Surface cohesion	Resistance to water absorption (water drop)
H ₂ O (1mL)	↓	↓	↓
LB+Fe (1mL)	↓	↓	↑
E.coli+Fe (1mL)	-	↓	↑
LB++Fe (1mL)	-	↓	↑
E.coli++Fe (1mL)	↓	↓	↑
H ₂ O++Fe (1mL)	↓	↓	↓
H ₂ O (2mL)	↓	↓	↓
LB++Fe (2mL)	-	↓	↑
E.coli++Fe (2mL)	-	↑	↑

Notation: Negative results in red; Positive results in green; Results equal to control in black

It is noticeable that all water-based treatments (“H₂O (1mL)”, “H₂O++Fe (1mL)” and “H₂O (2mL)”) degraded the surface of the earth mortar specimens, decreasing hardness, cohesion and water resistance. These treatments are the only ones that in which a decrease in water resistance was observed; all biotreated specimens showed an increase in water resistance.

No increase in surface hardness was noticed and only the “E.coli++Fe (2mL)” showed an increase in surface cohesion. Even if no remarkable results were obtained, it can be expected that, with further optimization, the production of a bioproduct capable of increasing surface hardness and cohesion is achievable.

The best results were obtained for the increase of resistance to water absorption because all biotreatments created a waterproofing effect. An in-depth study on the microstructure of biotreated earth mortars is necessary to understand the differences between each biotreatment and the concrete reason behind the waterproofing effect.

Stazi et al. (2016) and Aguilar et al. (2016), that also tested the effect of different surface treatments for waterproofing earth mortars (commercially available products and chitosan, respectively), obtained excellent results achieving total waterproof. The treatments tested by Stazi et al. (2016), despite reaching total waterproof, constraints earth mortars’ re-utilization. Iron-based biotreatments did not achieve such satisfactory results but do not affect earth mortars’ re-utilization, what is remarkable in terms of eco-efficiency.

Table 4.10 - Qualitative comparison of tested bioformulations

Comparison with control specimens	LB	E.coli+Fe
Consistency	↓	↓
Dynamic modulus of elasticity	↓	↓
Flexural strength	↓	↓
Compressive strength	↓	↓
Thermal conductivity	↓	↓
Adhesive strength	↓	-
Dry abrasion resistance	↓	↑
Durability in water	↑	↑
Water absorption under low pressure	↑	↑
Resistance to water absorption (water drop)	↑	↑
Surface hardness	↓	↓
Surface cohesion	↓	↓

Bioformulated mortars proved to be considerably weaker and more brittle than control mortars. These results are not satisfactory for current plastering mortars but a more in-depth study on the bioproducts may surpass this behavior. On the other hand, promising results were obtained for durability in water.

Bioformulated mortars showed a high durability in water, specially the "E.coli+Fe" formulated ones. As mentioned above, a study on the microstructure of these bioformulated mortars is essential to understand what is contributing to improve the durability in water of earth mortars.

In addition to the improvement on the behavior towards water absorption, thermal conductivity of bioformulated mortars considerably lowered. This improvement might probably be due to higher porosity of bioformulated mortars: despite reducing strengths it also reduces thermal conductivity, creating the possibility of applying these mortars as thermal insulation.

Formulations tested by Stazi et al. (2016) also lead to the decrease of compressive strength, while chitosan formulated earth mortars tested by Aguilar et al. (2016) showed an increase of compressive strength. Both Stazi et al. (2016) and Aguilar et al. (2016) tested formulations showed an improvement on their behavior towards water.

Comparing the results obtained for the tested iron-based bioproducts with the ones obtained by Stazi et al. (2016), it seems more advantageous to use the bioproducts for surface biotreatment than on the bioformulation of earth mortars.

5. Conclusions

5.1. Final remarks

An iron-based bioproduct was produced and used as surface biotreatment and in the bioformulation of earth mortars. The consolidative and waterproofing effects of the bioproduct were assessed in both of the used applications.

Inspiring results were obtained specially on biotreated mortars. The most notorious achievement was the increase of water resistance throughout a water repelling effect.

Biotreated mortars did not show a significant increase on their consolidation. Compared with control mortar (no treatment applied), surface hardness and surface cohesion did not significantly increase. On the other hand, if compared with H₂O mortars (water treated mortars), the negative effect of the application of a liquid on the surface of the earth mortar specimens is nullified by some consolidation resulting from the biotreatment.

E. coli culture based biotreatments were the ones achieving the best results, in particular when iron concentration was increased. In this case, the resistance towards water absorption, assessed with resort to the water drop test, increased more than 4500%.

The results obtained for bioformulated mortars do not seem very promising, unlike the ones obtained for biotreated mortars, even though a higher resistance towards water and a lower thermal conductivity were obtained. This brings to conclusion that bioformulated mortars need in fact a more in depth study namely in order to control the porous structure. Since significant improvements were obtained on biotreated mortars, similar results may possibly be reached on bioformulated mortars.

The use of iron-based bioproducts for the bioformulation of earth mortars lead to a substantial decrease on mechanical strengths due to a harmful effect created by the addition of LB medium. In order to achieve positive mechanical results, the effect created by the LB medium must be avoided on future bioproducts. Nevertheless, the same effect can be further studied for lightweight and/or thermal insulation mortars.

A review paper on bioconsolidation of construction materials was submitted to a scientific journal and another paper collecting the results obtained with the biotreatment and bioformulation of the earth plastering mortar is being prepared for submission.

5.2. Proposals for future research

As great potential for improvements have been shown, a more extensive research on bioconsolidation of earth mortars is required to improve and optimize both biotreatments and bioformulations, including the bioproducts but also the application techniques and their influence on durability. Alternative bioproducts should also be developed and tested.

In both, biotreatment and bioformulation, a study on the microstructure is imperative in order to understand if the consolidative and the waterproofing effects come from the precipitation of iron-oxide.

Hygroscopic tests to assess sorption-desorption behavior must be performed in order to understand how the waterproofing effect may affect the hygroscopic characteristics of the earth mortars.

A more in-depth comparison with other researchers should be performed when more studies on biotreatments and optimized formulations of earth mortars are published.

Biotreatments

Different components concentration and volumes to be applied can be tested in order to improve biotreatment consolidation effect.

The method of application must be adapted to achieve a more practical application. Spraying is one application methods that needs to be tested to understand how the pressure applied to the biotreatment may damage the bacterial culture and if the bioproducts are adequate for this type of application.

All tested biotreatments were applied in a controlled environment. The application in harsh environments should be tested, specially under high temperatures since bacterial culture should be conserved under low temperatures and bioproducts characteristics may change.

Biotreatments were always applied moments after their production. Different conservation times of the biotreatment should be tested to better understand how storage affects the bioconsolidative effect.

More test procedures should be conducted, primarily to test the effect of the biotreatment on non-tested materials characteristics, namely on long term durability.

Alternative construction materials can and should be tested with this biotreatment, specially earth-based materials for comparison, namely with different types of clays but also other materials that take part of built heritage needing consolidation and protection.

Life cycle assessment (LCA) and life cycle cost (LCC) of biotreated and control mortars should be evaluated.

Bioformulation

Different volume percentages of bioproduct or different dilutions in water need to be tested. Different mixing conditions and sample preparation should also be tested.

One of the main problems of bioformulated mortars was the behavior that the bioproduct broth to fresh state mortars, namely a fluffy consistence, which in turn justified a significant decrease of mechanical strengths. Therefore, a deconstruction of the LB medium can be performed in order to determine the component or mixture of components that produce the fluffy consistency of bioformulated mortars.

The effect of LB medium can be assessed on different mortars, namely when low thermal conductivity and/or low weight is the main aim.

Biotreatments can also be tested on bioformulated mortars.

After achieving a more resistant mortar, more tests can be performed to evaluate the possible improvement on other earth mortar weaknesses, namely on susceptibility for development of molds and fungus, resistance to abrasion on dry and humid conditions and long term durability.

Embodied energy, LCA and LCC of bioformulated and control mortars should be assessed.

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Appendix

A.1. Biotreated mortars - 1st screening

Table A.1 - Surface hardness test results: 1st Screening – 1st Treatment

24/10/2016	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Surface hardness	Avg. surface hardness	S.D.
Control	0_1	88	82	85	91	81	89	84	85	74	84	87	5
	0_2	94	90	90	89	89	91	93	93	91	91		
	0_3	88	89	85	82	78	84	87	87	95	86		
H ₂ O	1_1	82	78	84	54	-	76	65	74	64	72	70	10
	1_2	83	59	89	55	-	56	69	71	75	70		
	1_3	78	73	70	52	-	61	74	66	82	70		
LB	2_1	78	87	84	80	-	82	85	78	92	83	84	5
	2_2	75	80	83	85	-	81	86	83	87	83		
	2_3	88	84	80	77	-	79	89	91	90	85		
LB+Fe	3_1	89	80	80	73	-	85	82	80	85	82	83	6
	3_2	90	86	83	70	-	75	95	82	84	83		
	3_3	87	84	79	74	-	76	88	86	95	84		
E.coli+Fe	4_1	85	84	83	88	-	85	84	88	82	85	83	4
	4_2	84	76	76	80	-	83	90	85	80	82		
	4_3	83	80	84	74	-	89	87	82	84	83		
E.coli+DPS+Fe	5_1	81	90	86	75	-	84	86	87	88	85	85	4
	5_2	90	86	90	85	-	84	79	79	83	85		
	5_3	86	84	82	88	-	86	88	88	88	86		

Table A.2 - Surface hardness test results: 1st Screening – 1st Treatment (144 hours after)

27/10/2016	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.	
144 hours after 5th feeding (1st Treatment)	Control	0_1	80	87	81	82	75	79	76	89	88	68	82	86	81	85	6
		0_2	91	91	89	91	89	88	84	88	82	90	86	88	88		
		0_3	84	85	92	90	89	84	91	80	78	81	86	92	86		
	H ₂ O	1_1	81	76	88	50	55	59	49	60	71	65	40	66	63	62	13
		1_2	71	47	73	46	36	52	58	81	74	80	45	68	61		
		1_3	74	61	72	51	50	60	65	54	60	75	49	80	63		
	LB	2_1	79	84	84	75	92	81	81	82	82	80	79	84	82	82	5
		2_2	90	83	88	86	69	73	80	81	82	77	76	88	81		
		2_3	86	80	85	85	85	75	75	81	78	83	86	89	82		
	LB+Fe	3_1	90	86	89	86	84	75	81	82	75	79	76	83	82	82	5
		3_2	80	79	90	87	74	80	87	85	82	87	85	86	84		
		3_3	84	78	83	78	86	83	75	77	75	82	88	91	82		
	E.coli+Fe	4_1	85	85	86	86	90	81	88	88	89	90	86	83	86	86	3
		4_2	89	82	90	84	89	90	88	87	85	89	81	88	87		
		4_3	87	78	84	86	81	82	79	87	88	84	86	92	85		
	E.coli+DPS+Fe	5_1	88	80	83	91	95	86	83	85	87	87	82	90	86	86	4
		5_2	94	80	92	84	86	86	82	81	85	84	85	84	85		
		5_3	86	88	84	87	89	85	93	89	77	91	86	92	87		

Table A.3 - Surface hardness test results: 1st Screening – 2nd Treatment

03/11/2016	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.	
72 hours after 1st feeding (2nd Treatment)	Control	0_1	94	87	80	82	86	75	74	81	83	80	75	82	83	6	
		0_2	87	89	83	90	91	88	81	90	84	91	84	86			87
		0_3	76	80	81	89	83	75	69	74	81	79	85	78			79
	H ₂ O	1_1	78	90	85	56	66	51	52	57	70	67	50	71	66	62	15
		1_2	83	67	85	56	40	30	38	49	59	75	61	53	58		
		1_3	79	53	85	60	40	43	54	53	55	70	60	76	61		
	LB	2_1	83	82	85	83	88	78	88	82	82	95	85	86	85	84	5
		2_2	85	82	82	82	77	84	80	82	86	93	83	84	83		
		2_3	93	78	84	85	86	78	83	77	81	97	87	88	85		
LB+Fe	3_1	89	83	81	86	85	82	77	78	79	80	70	84	81	82	6	
	3_2	84	80	92	83	74	84	86	75	84	84	82	80	82			
	3_3	95	84	87	70	84	81	88	78	77	81	88	92	84			
E.coli+Fe	4_1	94	86	89	88	87	80	91	81	84	88	90	81	87	87	4	
	4_2	92	82	89	83	83	94	88	92	84	88	81	81	86			
	4_3	88	86	83	87	82	88	90	84	88	88	90	91	87			
E.coli+DPS+Fe	5_1	85	90	82	82	90	82	83	84	90	91	85	81	85	85	3	
	5_2	88	87	91	81	88	85	79	86	83	85	82	85	85			
	5_3	83	84	89	82	82	83	89	83	86	87	91	90	86			

Table A.4 - Surface hardness test results: 1st Screening – 2nd Treatment (7 days after)

07/11/2016	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.	
168 hours after 1st feeding (2nd Treatment)	Control	0_1	83	79	80	86	77	83	75	86	84	82	86	87	82	85	5
		0_2	94	87	85	85	94	84	81	91	83	93	92	91	88		
		0_3	85	92	92	82	85	79	83	78	77	86	86	77	84		
	H ₂ O	1_1	75	82	84	61	54	48	42	42	63	71	44	69	61	59	17
		1_2	85	54	84	36	34	36	48	49	60	74	56	67	57		
		1_3	91	64	77	87	39	51	40	42	46	78	50	56	60		
	LB	2_1	85	84	91	83	87	85	91	89	87	89	82	81	86	85	4
		2_2	88	81	87	83	74	81	90	84	80	86	84	85	84		
		2_3	93	81	81	84	91	86	86	80	82	86	90	87	86		
	LB+Fe	3_1	90	86	87	85	76	81	80	84	83	86	80	89	84	83	5
		3_2	84	79	91	90	78	82	72	79	80	89	92	78	83		
		3_3	91	82	83	80	78	82	80	84	77	80	86	90	83		
	E.coli+Fe	4_1	89	89	89	88	88	88	86	88	85	89	89	84	88	88	3
		4_2	92	86	86	87	94	82	91	87	85	85	91	87	88		
		4_3	90	89	83	92	86	88	90	86	84	90	87	91	88		
	E.coli+DPS+Fe	5_1	89	81	83	91	93	89	89	83	90	89	85	82	87	86	4
		5_2	90	86	93	85	90	84	84	86	85	87	86	83	87		
		5_3	91	88	83	83	78	81	92	83	83	83	92	90	86		

Table A.5 - Surface hardness test results: 1st Screening – 2nd Treatment (115 days after)

22/02/2017	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.		
115 days after 1st feeding (2nd Treatment)	Control	0_1	87	79	79	90	82	73	81	88	90	91	81	89	84	85	5	
		0_2	82	82	84	85	82	89	82	82	85	81	90	84	95			85
		0_3	86	89	89	89	96	92	85	77	87	85	74	90	87			
	H ₂ O	1_1	79	80	66	56	56	60	31	39	66	61	36	58	57	58	16	
		1_2	84	64	78	70	41	44	47	48	68	69	51	69	61			
		1_3	77	62	78	77	41	38	38	56	31	76	37	52	55			
	LB	2_1	81	82	87	81	90	92	71	84	87	88	85	83	84	84	5	
		2_2	89	75	85	80	81	88	84	75	86	80	82	78	82			
		2_3	94	81	85	86	79	82	86	84	75	95	86	88	85			
	LB+Fe	3_1	90	90	85	92	77	82	75	85	90	80	78	83	84	82	8	
		3_2	86	88	84	82	70	86	56	72	81	89	80	88	80			
		3_3	93	86	89	79	65	75	81	84	84	83	85	90	83			
	E.coli+Fe	4_1	96	85	89	94	93	89	85	84	90	93	90	84	89	88	4	
		4_2	92	87	91	92	93	91	83	88	92	86	99	81	90			
		4_3	88	86	86	81	84	81	84	86	88	83	83	89	85			
	E.coli+DPS+Fe	5_1	92	87	83	86	89	89	86	88	87	86	87	92	88	88	3	
		5_2	94	86	93	86	85	88	88	82	93	90	89	85	88			
		5_3	93	89	84	83	88	85	92	87	85	87	88	87	87			

Table A.6 - Ultrasound propagation speed test results: 1st Screening – 1st Treatment

24/10/2016	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
72 hours after 5th feeding (1st Treatment)	Control	0_1	25,5	39,18	1536	1563
		0_2	24,9	39,79	1598	
		0_3	25,1	39,04	1555	
	H ₂ O	1_1	26,4	39,44	1494	1518
		1_2	26,2	39,46	1506	
		1_3	25,6	39,79	1554	
	LB	2_1	24,5	39,33	1605	1637
		2_2	23,3	39,04	1676	
		2_3	24,1	39,27	1629	
	LB+Fe	3_1	24,4	39,43	1616	1626
		3_2	24,3	39,42	1622	
		3_3	23,9	39,19	1640	
	E.coli+Fe	4_1	24,6	39,32	1598	1585
		4_2	25,2	39,62	1572	
		4_3	25,1	39,77	1584	
	E.coli+DPS+Fe	5_1	25,9	39,58	1528	1592
		5_2	24,1	39,39	1634	
		5_3	24,6	39,71	1614	

Table A.7 - Ultrasound propagation speed test results: 1st Screening – 2nd Treatment

03/11/2016	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
72 hours after 1st feeding (2nd Treatment)	Control	0_1	25,2	39,18	1555	1572
		0_2	24,6	39,79	1617	
		0_3	25,3	39,04	1543	
	H ₂ O	1_1	25,8	39,44	1529	1540
		1_2	25,9	39,46	1524	
		1_3	25,4	39,79	1567	
	LB	2_1	25,3	39,33	1555	1611
		2_2	23,5	39,04	1661	
		2_3	24,3	39,27	1616	
	LB+Fe	3_1	24,2	39,43	1629	1629
		3_2	23,7	39,42	1663	
		3_3	24,6	39,19	1593	
	E.coli+Fe	4_1	24,2	39,32	1625	1598
		4_2	25,1	39,62	1578	
		4_3	25,0	39,77	1591	
	E.coli+DPS+Fe	5_1	25,6	39,58	1546	1589
		5_2	24,4	39,39	1614	
		5_3	24,7	39,71	1608	

Table A.8 - Ultrasound propagation speed test results: 1st Screening – 2nd Treatment (7 days after)

07/11/2016	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
168 hours after 1st feeding (2nd Treatment)	Control	0_1	24,2	39,18	1619	1663
		0_2	23,3	39,79	1708	
		0_3	23,5	39,04	1661	
	H ₂ O	1_1	24,2	39,44	1630	1625
		1_2	25,2	39,46	1566	
		1_3	23,7	39,79	1679	
	LB	2_1	23,8	39,33	1653	1711
		2_2	22,2	39,04	1759	
		2_3	22,8	39,27	1722	
	LB+Fe	3_1	22,7	39,43	1737	1749
		3_2	22,1	39,42	1784	
		3_3	22,7	39,19	1726	
	E.coli+Fe	4_1	22,7	39,32	1732	1721
		4_2	23,2	39,62	1708	
		4_3	23,1	39,77	1722	
	E.coli+DPS+Fe	5_1	23,1	39,58	1713	1720
		5_2	22,9	39,39	1720	
		5_3	23,0	39,71	1727	

Table A.9 - Ultrasound propagation speed test results: 1st Screening – 2nd Treatment (115 days after)

22/02/2017	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
115 days after 1st feeding (2nd Treatment)	Control	0_1	24,1	39,18	1626	1723
		0_2	22	39,79	1809	
		0_3	22,5	39,04	1735	
	H ₂ O	1_1	24,2	39,44	1630	1658
		1_2	23,7	39,46	1665	
		1_3	23,7	39,79	1679	
	LB	2_1	23,9	39,33	1646	1723
		2_2	22,8	39,04	1712	
		2_3	21,7	39,27	1810	
	LB+Fe	3_1	21,7	39,43	1817	1825
		3_2	21,7	39,42	1817	
		3_3	21,3	39,19	1840	
	E.coli+Fe	4_1	22,2	39,32	1771	1744
		4_2	22,4	39,62	1769	
		4_3	23,5	39,77	1692	
	E.coli+DPS+Fe	5_1	22,7	39,58	1744	1718
		5_2	23,1	39,39	1705	
		5_3	23,3	39,71	1704	

Table A.10 - Surface cohesion test results: 1st Screening – 1st Treatment

24/10/2016	Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	MI _{avg} (g/m ²)	S.D.	Average mass loss variation
72 hours after 5th feeding (1st Treatment)	Control	0_1	0,0264	-	0,0015	17,5	22,7	8,7	-
		0_2	0,0467		0,0014	32,8			
		0_3	0,0248		0,0014	17,7			
	H ₂ O	1_1	0,0679	112%	0,0016	41,3	44,2	2,7	95%
		1_2	0,0680		0,0015	44,8			
		1_3	0,0718		0,0015	46,6			
	LB	2_1	0,0368	16%	0,0016	23,0	24,2	2,0	7%
		2_2	0,0353		0,0015	23,0			
		2_3	0,0413		0,0016	26,4			
	LB+Fe	3_1	0,0397	17%	0,0016	24,7	24,4	4,4	8%
		3_2	0,0311		0,0016	19,8			
		3_3	0,0435		0,0015	28,7			
	E.coli+Fe	4_1	0,0206	-20%	0,0016	12,7	16,8	5,3	-26%
		4_2	0,0351		0,0015	22,8			
		4_3	0,0228		0,0015	15,0			
	E.coli+DPS+Fe	5_1	0,0321	18%	0,0015	20,9	24,5	3,9	8%
		5_2	0,0458		0,0016	28,7			
		5_3	0,0373		0,0016	24,0			

Table A.11 - Surface cohesion test results: 1st Screening – 2nd Treatment

03/11/2016	Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	MI _{avg} (g/m ²)	S.D.	Average mass loss variation
72 hours after 1st feeding (2nd Treatment)	Control	0_1	0,0680	-	0,0015	45,1	49,5	4,0	-
		0_2	0,0754		0,0014	52,9			
		0_3	0,0709		0,0014	50,6			
	H ₂ O	1_1	0,0921	35%	0,0016	56,0	61,6	6,2	24%
		1_2	0,0919		0,0015	60,5			
		1_3	0,1052		0,0015	68,3			
	LB	2_1	0,0466	-39%	0,0016	29,2	27,9	1,2	-44%
		2_2	0,0412		0,0015	26,8			
		2_3	0,0433		0,0016	27,7			
	LB+Fe	3_1	0,0393	-44%	0,0016	24,4	25,7	1,2	-48%
		3_2	0,0422		0,0016	26,9			
		3_3	0,0389		0,0015	25,7			
	E.coli+Fe	4_1	0,0322	-49%	0,0016	19,8	23,3	3,7	-53%
		4_2	0,0419		0,0015	27,2			
		4_3	0,0348		0,0015	22,9			
	E.coli+DPS+Fe	5_1	0,0325	-52%	0,0015	21,1	21,8	0,9	-56%
		5_2	0,0344		0,0016	21,5			
		5_3	0,0354		0,0016	22,8			

Table A.12 - Surface cohesion test results: 1st Screening – 2nd Treatment (7 days after)

07/11/2016	Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	M _{l,avg} (g/m ²)	S.D.	Average mass loss variation
168 hours after 1st feeding (2nd Treatment)	Control	0_1	0,0610	-	0,0015	40,5	41,0	3,5	-
		0_2	0,0638		0,0014	44,7			
		0_3	0,0529		0,0014	37,8			
	H ₂ O	1_1	0,0914	93%	0,0016	55,5	73,2	16,6	79%
		1_2	0,1145		0,0015	75,4			
		1_3	0,1364		0,0015	88,6			
	LB	2_1	0,0447	-34%	0,0016	28,0	24,9	7,2	-39%
		2_2	0,0255		0,0015	16,6			
		2_3	0,0469		0,0016	30,0			
	LB+Fe	3_1	0,0385	-32%	0,0016	23,9	25,8	1,7	-37%
		3_2	0,0425		0,0016	27,1			
		3_3	0,0400		0,0015	26,4			
	E.coli+Fe	4_1	0,0286	-53%	0,0016	17,6	17,6	0,3	-57%
		4_2	0,0277		0,0015	18,0			
		4_3	0,0265		0,0015	17,4			
	E.coli+DPS+Fe	5_1	0,0238	-40%	0,0015	15,5	22,7	6,4	-45%
		5_2	0,0441		0,0016	27,6			
		5_3	0,0390		0,0016	25,1			

Table A.13 - Surface cohesion test results: 1st Screening – 2nd Treatment (115 days after)

22/02/2017	Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	M _{l,avg} (g/m ²)	S.D.	Average mass loss variation
115 days after 1st feeding (2nd Treatment)	Control	0_1	0,0734	-	0,0015	48,7	38,1	11,7	-
		0_2	0,0572		0,0014	40,1			
		0_3	0,0359		0,0014	25,6			
	H ₂ O	1_1	0,0941	67%	0,0016	57,2	63,2	8,4	54%
		1_2	0,1106		0,0015	72,8			
		1_3	0,0919		0,0015	59,7			
	LB	2_1	0,0452	-25%	0,0016	28,3	28,5	1,1	-31%
		2_2	0,0423		0,0015	27,5			
		2_3	0,0463		0,0016	29,6			
	LB+Fe	3_1	0,0368	-46%	0,0016	22,9	20,6	5,8	-50%
		3_2	0,0221		0,0016	14,1			
		3_3	0,0379		0,0015	25,0			
	E.coli+Fe	4_1	0,0201	-56%	0,0016	12,3	16,9	4,3	-59%
		4_2	0,0324		0,0015	21,0			
		4_3	0,0262		0,0015	17,2			
	E.coli+DPS+Fe	5_1	0,0354	-46%	0,0015	23,0	20,4	2,8	-50%
		5_2	0,0279		0,0016	17,5			
		5_3	0,0324		0,0016	20,8			

Table A.14 - Water drop test results: 1st Screening – 1st Treatment

24/10/2016		Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
72 hours after 5th feeding (1st Treatment)	Control	0_1	0,6	0,7	0,1
		0_2	0,6		
		0_3	0,8		
	H ₂ O	1_1	0,0	0,0	0,0
		1_2	0,0		
		1_3	0,0		
	LB	2_1	1,2	1,3	0,1
		2_2	1,4		
		2_3	1,2		
	LB+Fe	3_1	1,4	1,3	0,1
		3_2	1,2		
		3_3	1,4		
	E.coli+Fe	4_1	2,0	2,0	0,0
		4_2	2,0		
		4_3	2,0		
	E.coli+DPS+Fe	5_1	1,6	1,7	0,1
		5_2	1,6		
		5_3	1,8		

Table A.15 - Water drop test results: 1st Screening – 2nd Treatment

03/11/2016		Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
72 hours after 1st feeding (2nd Treatment)	Control	0_1	0,2	0,5	0,4
		0_2	0,4		
		0_3	0,9		
	H ₂ O	1_1	0,0	0,0	0,0
		1_2	0,0		
		1_3	0,0		
	LB	2_1	1,5	1,6	0,2
		2_2	1,4		
		2_3	1,8		
	LB+Fe	3_1	1,4	1,6	0,3
		3_2	1,5		
		3_3	2,0		
	E.coli+Fe	4_1	5,9	6,8	1,1
		4_2	6,4		
		4_3	8,0		
	E.coli+DPS+Fe	5_1	3,2	2,8	0,5
		5_2	2,3		
		5_3	2,8		

Table A.16 - Water drop test results: 1st Screening – 2nd Treatment (7 days after)

07/11/2016		Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
168 hours after 1st feeding (2nd Treatment)	Control	0_1	0,3	0,5	0,2
		0_2	0,5		
		0_3	0,6		
	H ₂ O	1_1	0,0	0,0	0,0
		1_2	0,0		
		1_3	0,0		
	LB	2_1	0,7	1,3	0,7
		2_2	2,0		
		2_3	1,3		
	LB+Fe	3_1	1,6	1,5	0,4
		3_2	1,9		
		3_3	1,1		
	E.coli+Fe	4_1	8,0	6,4	1,4
		4_2	5,4		
		4_3	5,9		
	E.coli+DPS+Fe	5_1	2,2	2,3	0,1
		5_2	2,3		
		5_3	2,3		

Table A.17 - Water drop test results: 1st Screening – 2nd Treatment (115 days after)

22/02/2017		Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
115 days after 1st feeding (2nd Treatment)	Control	0_1	0,9	0,7	0,3
		0_2	0,4		
		0_3	0,9		
	H ₂ O	1_1	0,0	0,0	0,0
		1_2	0,0		
		1_3	0,0		
	LB	2_1	1,1	1,1	0,1
		2_2	1,2		
		2_3	1,0		
	LB+Fe	3_1	1,0	1,3	0,4
		3_2	1,2		
		3_3	1,7		
	E.coli+Fe	4_1	2,6	3,1	0,5
		4_2	3,1		
		4_3	3,6		
	E.coli+DPS+Fe	5_1	2,7	2,1	0,6
		5_2	2,0		
		5_3	1,6		

A.2. Biotreated mortars - 2nd screening

Table A.18 - Surface hardness test results: 2nd Screening - 96 hours

17/02/2017	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.	
96 hours after feeding	Control	0_4	81	73	90	78	88	91	89	87	91	89	85	74	85	85	6
		0_5	81	90	86	83	82	87	88	91	90	70	81	90	85		
	H ₂ O (1mL)	1_4	68	84	78	71	82	76	74	70	74	80	81	85	77		
		1_5	69	84	84	74	86	78	70	59	85	82	78	72	77	78	7
		1_6	80	61	90	80	80	87	77	82	69	84	80	80	79		
	LB+Fe (1mL)	3_4	88	81	86	80	76	66	78	79	81	88	83	75	80	83	5
		3_5	88	90	88	95	85	81	88	83	86	81	79	81	85		
		3_6	78	89	86	83	78	83	77	82	85	80	83	84	82		
	E.coli+Fe (1mL)	4_4	77	79	90	81	82	86	90	88	81	81	79	82	83	83	4
		4_5	86	91	75	76	80	77	84	85	84	83	83	87	83		
		4_6	85	85	76	86	80	84	88	84	75	85	79	79	82		
	LB++Fe (1mL)	6_1	86	83	88	89	80	84	88	84	88	86	85	87	86	85	3
		6_2	90	83	81	85	81	82	91	89	88	86	84	90	86		
		6_3	88	86	81	82	83	79	83	86	80	86	81	84	83		
	E.coli++Fe (1mL)	7_1	91	91	92	90	81	87	93	85	85	86	89	85	88	86	3
		7_2	86	86	89	85	85	84	84	84	85	84	85	82	85		
		7_3	89	84	88	88	84	88	85	80	89	84	92	87	87		
	H ₂ O++Fe (1mL)	8_1	84	83	88	76	72	81	79	70	80	76	76	66	78	79	6
		8_2	76	86	85	75	75	85	81	82	79	75	65	89	79		
		8_3	86	84	80	74	78	84	75	73	75	85	89	79	80		
	H ₂ O (2mL)	9_1	73	76	76	78	74	78	81	77	77	69	63	73	75	78	6
		9_2	76	81	86	63	85	77	82	83	76	80	80	80	79		
		9_3	78	89	86	74	72	81	75	82	83	82	70	77	79		
	LB++Fe (2mL)	10_1	85	74	84	78	88	84	86	83	84	83	82	89	83	83	5
		10_2	87	91	77	80	81	80	91	88	85	82	88	84	85		
		10_3	68	82	88	87	78	74	81	75	87	88	83	85	81		
	E.coli++Fe (2mL)	11_1	86	84	83	78	86	87	85	84	83	85	85	84	84	84	4
		11_2	83	87	80	83	81	83	87	84	78	79	85	83	83	84	
		11_3	89	93	80	83	81	82	79	81	85	75	89	86	84		

Table A.19 - Surface hardness test results: 2nd Screening – 65 days

19/04/2017	Specimen	65 days after treatment												Avg. surface hardness	S.D.
		Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12		
Control	0_4	89	75	91	77	83	77	79	85	94	87	89	89	85	6
	0_5	96	86	91	82	81	91	91	82	83	76	74	90	85	
H ₂ O (1mL)	1_4	71	82	84	72	84	80	65	73	81	79	76	78	77	6
	1_5	74	76	71	79	82	81	75	74	84	76	76	73	77	
	1_6	87	82	86	71	83	76	62	74	84	76	78	85	79	
LB+Fe (1mL)	3_4	92	85	85	82	74	74	84	86	81	85	83	81	83	4
	3_5	80	90	89	80	94	86	85	85	86	80	81	77	84	
	3_6	82	84	84	79	84	85	79	87	81	86	91	86	84	
E.coli+Fe (1mL)	4_4	90	79	86	82	88	84	84	94	93	79	84	87	86	4
	4_5	82	89	87	85	86	84	77	80	86	85	84	86	84	
	4_6	88	87	79	75	83	86	79	86	83	94	85	87	84	
LB++Fe (1mL)	6_1	84	80	85	86	87	86	84	83	84	87	83	84	84	4
	6_2	83	87	79	85	91	86	78	98	89	84	82	87	86	
	6_3	89	83	79	87	82	88	86	85	83	78	79	84	84	
E.coli++Fe (1mL)	7_1	86	90	88	82	86	89	81	81	80	84	89	82	85	4
	7_2	94	82	86	91	91	87	87	86	84	86	86	82	87	
	7_3	84	83	81	79	82	79	80	82	87	75	74	82	81	
H ₂ O++Fe (1mL)	8_1	64	72	71	60	73	71	76	73	83	73	45	71	69	9
	8_2	81	82	83	68	75	85	82	85	79	81	77	81	80	
	8_3	85	86	79	75	75	71	66	77	84	88	80	84	79	
H ₂ O (2mL)	9_1	75	64	81	69	84	63	59	73	82	62	57	68	70	9
	9_2	88	85	83	70	66	69	83	80	86	80	75	74	78	
	9_3	75	76	75	79	86	77	79	72	87	86	61	72	77	
LB++Fe (2mL)	10_1	82	86	84	79	86	85	85	90	85	82	84	87	85	4
	10_2	87	83	82	89	88	83	86	89	89	80	86	92	86	
	10_3	80	78	76	84	86	79	88	86	87	84	85	93	84	
E.coli++Fe (2mL)	11_1	94	81	95	81	95	83	87	84	87	78	83	84	86	5
	11_2	96	87	93	88	85	84	84	85	80	86	81	79	86	
	11_3	85	92	84	81	80	83	81	79	86	87	86	85	84	

Table A.20 - Ultrasound propagation speed test results: 2nd Screening - 96 hours

17/02/2017	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
96 hours after feeding	Control	0_4	25,2	39,14	1553	1572
		0_5	24,1	38,36	1592	
	H ₂ O (1mL)	1_4	23,3	38,79	1665	1656
		1_5	23,3	39,35	1689	
		1_6	24,3	39,25	1615	
	LB+Fe (1mL)	3_4	24,3	39,69	1633	1655
		3_5	22,9	38,81	1695	
		3_6	23,4	38,30	1637	
	E.coli+Fe (1mL)	4_4	25,1	39,31	1566	1573
		4_5	24,7	38,75	1569	
		4_6	24,3	38,52	1585	
	LB++Fe (1mL)	6_1	25,3	39,36	1556	1596
		6_2	24,5	39,37	1607	
		6_3	23,7	38,54	1626	
	E.coli++Fe (1mL)	7_1	23,8	39,36	1654	1656
		7_2	22,3	39,03	1750	
		7_3	24,5	38,32	1564	
	H ₂ O++Fe (1mL)	8_1	23,3	38,73	1662	1649
		8_2	23,7	39,56	1669	
		8_3	24,2	39,08	1615	
	H ₂ O (2mL)	9_1	25,2	39,55	1569	1608
		9_2	24,0	39,13	1630	
		9_3	24,2	39,34	1626	
	LB++Fe (2mL)	10_1	23,2	39,41	1699	1664
		10_2	23,5	39,24	1670	
		10_3	24,3	39,44	1623	
	E.coli++Fe (2mL)	11_1	24,0	39,32	1638	1694
		11_2	22,7	38,62	1701	
		11_3	22,5	39,18	1741	

Table A.21 - Ultrasound propagation speed test results: 2nd Screening - 65 days

19/04/2017	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
65 days after treatment	Control	0_4	25,5	39,14	1535	1570
		0_5	23,9	38,36	1605	
	H ₂ O (1mL)	1_4	22,3	38,79	1739	1735
		1_5	22,0	39,35	1789	
		1_6	23,4	39,25	1677	
	LB+Fe (1mL)	3_4	22,8	39,69	1741	1751
		3_5	22,3	38,81	1740	
		3_6	21,6	38,30	1773	
	E.coli+Fe (1mL)	4_4	22,8	39,31	1724	1725
		4_5	22,7	38,75	1707	
		4_6	22,1	38,52	1743	
	LB++Fe (1mL)	6_1	22,3	39,36	1765	1758
		6_2	22,6	39,37	1742	
		6_3	21,8	38,54	1768	
	E.coli++Fe (1mL)	7_1	22,3	39,36	1765	1752
		7_2	22,2	39,03	1758	
		7_3	22,1	38,32	1734	
	H ₂ O++Fe (1mL)	8_1	21,7	38,73	1785	1795
		8_2	21,7	39,56	1823	
		8_3	22,0	39,08	1776	
	H ₂ O (2mL)	9_1	22,1	39,55	1790	1770
		9_2	22,4	39,13	1747	
		9_3	22,2	39,34	1772	
	LB++Fe (2mL)	10_1	20,6	39,41	1913	1855
		10_2	21,4	39,24	1834	
		10_3	21,7	39,44	1818	
	E.coli++Fe (2mL)	11_1	21,5	39,32	1829	1894
		11_2	19,8	38,62	1951	
		11_3	20,6	39,18	1902	

Table A.22 - Surface cohesion test results: 2nd Screening - 96 hours

17/02/2017		Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	MI _{avg} (g/m ²)	S.D.	Average mass loss variation
96 hours after feeding	Control	0_4	0,0074	0,0107	-	0,0014	5,3	7,2	-	-
		0_5	0,0139			0,0015	9,1			
	H ₂ O (1mL)	1_4	0,0206	0,0200	87%	0,0015	13,9	13,4	1,4	86%
		1_5	0,0218			0,0015	14,5			
		1_6	0,0175			0,0015	11,9			
	LB+Fe (1mL)	3_4	0,0171	0,0117	10%	0,0015	11,7	7,9	3,4	9%
		3_5	0,0076			0,0015	5,1			
		3_6	0,0103			0,0015	6,9			
	E.coli+Fe (1mL)	4_4	0,0191	0,0141	32%	0,0015	12,8	9,6	4,7	32%
		4_5	0,0063			0,0015	4,1			
		4_6	0,0169			0,0014	11,8			
	LB++Fe (1mL)	6_1	0,0055	0,0082	-23%	0,0015	3,7	5,5	1,9	-24%
		6_2	0,0113			0,0015	7,5			
		6_3	0,0079			0,0015	5,3			
	E.coli++Fe (1mL)	7_1	0,0122	0,0098	-8%	0,0015	8,3	6,6	3,5	-9%
		7_2	0,0134			0,0015	8,9			
		7_3	0,0039			0,0015	2,6			
	H ₂ O++Fe (1mL)	8_1	0,0429	0,0289	171%	0,0014	29,6	19,5	8,8	169%
		8_2	0,0225			0,0015	14,6			
		8_3	0,0212			0,0015	14,2			
	H ₂ O (2mL)	9_1	0,0341	0,0358	236%	0,0015	22,4	24,0	1,6	232%
		9_2	0,0353			0,0015	24,1			
		9_3	0,0379			0,0015	25,5			
	LB++Fe (2mL)	10_1	0,0077	0,0125	18%	0,0015	5,3	8,4	2,9	16%
		10_2	0,0132			0,0015	9,1			
		10_3	0,0167			0,0015	10,9			
	E.coli++Fe (2mL)	11_1	0,0096	0,0054	-50%	0,0016	6,1	3,5	2,3	-51%
		11_2	0,0030			0,0014	2,1			
		11_3	0,0035			0,0015	2,4			

Table A.23 - Surface cohesion test results: 2nd Screening - 65 days

19/04/2017		Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	MI _{avg} (g/m ²)	S.D.	Average mass loss variation
65 days after treatment	Control	0_4	0,0099	0,0162	-	0,0014	7,1	10,9	-	-
		0_5	0,0224			0,0015	14,7			
	H ₂ O (1mL)	1_4	0,0439	0,0360	238%	0,0015	29,7	24,3	7,7	123%
		1_5	0,0233			0,0015	15,5			
		1_6	0,0409			0,0015	27,7			
	LB+Fe (1mL)	3_4	0,0217	0,0210	97%	0,0015	14,8	14,1	1,2	29%
		3_5	0,0190			0,0015	12,7			
		3_6	0,0223			0,0015	14,9			
	E.coli+Fe (1mL)	4_4	0,0222	0,0184	72%	0,0015	14,9	12,4	3,5	14%
		4_5	0,0129			0,0015	8,4			
		4_6	0,0200			0,0014	13,9			
	LB++Fe (1mL)	6_1	0,0169	0,0198	86%	0,0015	11,4	13,2	1,6	21%
		6_2	0,0220			0,0015	14,5			
		6_3	0,0204			0,0015	13,8			
	E.coli++Fe (1mL)	7_1	0,0153	0,0180	69%	0,0015	10,4	12,0	6,5	10%
		7_2	0,0097			0,0015	6,5			
		7_3	0,0289			0,0015	19,2			
	H ₂ O++Fe (1mL)	8_1	0,0443	0,0334	214%	0,0014	30,6	22,5	7,5	106%
		8_2	0,0323			0,0015	20,9			
		8_3	0,0236			0,0015	15,8			
	H ₂ O (2mL)	9_1	0,0373	0,0363	241%	0,0015	24,5	24,3	3,7	122%
		9_2	0,0300			0,0015	20,5			
		9_3	0,0415			0,0015	27,9			
	LB++Fe (2mL)	10_1	0,0149	0,0166	56%	0,0015	10,2	11,2	1,8	3%
		10_2	0,0193			0,0015	13,3			
		10_3	0,0156			0,0015	10,2			
	E.coli++Fe (2mL)	11_1	0,0116	0,0105	-1%	0,0016	7,4	7,0	4,4	-36%
		11_2	0,0035			0,0014	2,4			
		11_3	0,0164			0,0015	11,2			

Table A.24 - Water drop test results: 2nd Screening - 96 hours

17/02/2017	Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
96 hours after feeding	Control	0_4	0,9	-
		0_5		
	H ₂ O (1mL)	1_4	0,2	0,1
		1_5		
		1_6		
	LB+Fe (1mL)	3_4	1,7	0,2
		3_5		
		3_6		
	E.coli+Fe (1mL)	4_4	4,5	0,5
		4_5		
		4_6		
	LB++Fe (1mL)	6_1	1,8	0,3
		6_2		
		6_3		
	E.coli++Fe (1mL)	7_1	45,8	3,0
		7_2		
		7_3		
	H ₂ O++Fe (1mL)	8_1	0,2	0,1
		8_2		
		8_3		
	H ₂ O (2mL)	9_1	0,2	0,1
		9_2		
		9_3		
	LB++Fe (2mL)	10_1	2,9	0,8
		10_2		
		10_3		
	E.coli++Fe (2mL)	11_1	57,9	15,1
		11_2		
		11_3		

Table A.25 - Water drop test results: 2nd Screening - 65 days

19/04/2017	Specimen	Time until water drop	Average time until water drop	S.D.
65 days after treatment	Control	0_4	1,4	-
		0_5		
	H ₂ O (1mL)	1_4	0,5	0,1
		1_5		
		1_6		
	LB+Fe (1mL)	3_4	2,3	0,6
		3_5		
		3_6		
	E.coli+Fe (1mL)	4_4	2,4	0,1
		4_5		
		4_6		
	LB++Fe (1mL)	6_1	2,2	0,4
		6_2		
		6_3		
	E.coli++Fe (1mL)	7_1	20,9	9,2
		7_2		
		7_3		
	H ₂ O++Fe (1mL)	8_1	0,4	0,2
		8_2		
		8_3		
	H ₂ O (2mL)	9_1	0,4	0,0
		9_2		
		9_3		
	LB++Fe (2mL)	10_1	2,8	1,1
		10_2		
		10_3		
	E.coli++Fe (2mL)	11_1	19,4	2,1
		11_2		
		11_3		

A.3. Bioformulated mortars

Table A.26 - Fresh state tests results: bioformulated mortars

Formulation		Flow diameter (mm)			Slump height (mm)	Penetration depth (mm)	Wet bulk density (kg/dm ³)
		D1	D2	D3			
Control (R1)	1 st	163,0	167,0	166,0	20	12	1,9761
	2 nd	164,5	165,0	166,0	19	14	1,9765
	3 th (72h)	136,0	139,5	138,0	27	8	-
LB (R2)	1 st	178,0	179,0	181,0	15	36	1,6144
	2 nd	186,0	185,0	183,0	15	36	1,6098
	3 th (72h)	141,0	143,0	144,0	26	11	1,8833
E.coli+Fe (R3)	1 st	188,0	190,0	189,5	15	51	1,5510
	2 nd	182,0	184,5	182,5	14	48	1,5557
	3 th (72h)	144,0	142,5	143,0	25	12	1,8641

Table A.27 - Adhesive strengths test results: bioformulated mortars

Specimen	Diameter (mm)	Area (mm ²)	Load (N)	Adhesive strength (MPa)
Control	48,81	1870,76	73,757	0,04
	49,11	1894,22	51,882	0,03
LB	48,50	1847,45	44,311	0,02
E.coli+Fe	48,77	1868,08	70,112	0,04
	48,34	1834,90	77,684	0,04
	48,60	1855,08	80,208	0,04

Table A.28 - Dry abrasion resistance test results: bioformulated mortars

Specimen	Before (kg)	After (kg)	Abrasion weight loss (g)	Average abrasion weight loss (g)	S.D.
Control	4,7264	4,7239	2,5	3,1	1,7
	4,7239	4,7221	1,8		
	4,7239	4,7188	5,1		
LB	4,5423	4,5399	2,4	2,4	0,6
	4,5399	4,5381	1,8		
	4,5381	4,5351	3,0		
E.coli+Fe	4,6205	4,6173	3,2	3,3	0,5
	4,6173	4,6144	2,9		
	4,6144	4,6106	3,8		

Table A.29 - Dynamic modulus of elasticity results: bioformulated mortars

Specimen	Mass (kg)	Length (mm)	Width (mm)	Height (mm)	Dynamic modulus of elasticity (MPa)				Avg. Modulus (Mpa)	S.D.	Avg. Modulus (Mpa)	S.D.
R 1_1	0,4366	160,62	39,65	40,69	3558	3560	3578	3569	3566	9	3527	45
R 1_2	0,4312	160,19	39,01	40,78	3569	3575	3563	3584	3573	9		
R 1_3	0,4287	160,00	40,08	40,35	-	-	-	-	-	-		
R 1_4	0,4344	159,90	39,85	40,85	3448	3462	3527	3543	3495	47		
R 1_5	0,4289	160,02	39,77	40,58	3434	3501	3529	3483	3487	40		
R 1_6	0,4260	160,25	39,84	40,26	3509	3504	3531	3503	3512	13		
R 1_7	0,4449	159,87	39,88	41,14	3735	3749	3733	3698	3729	22	3729	22
R 2_1	0,3355	160,78	40,12	40,40	1595	1595	1582	1586	1590	7	1838	287
R 2_2	0,3290	160,97	38,70	40,10	1622	1626	1619	1621	1622	3		
R 2_3	0,3378	161,32	39,76	40,48	1543	1569	1555	1570	1559	13		
R 2_4	0,3746	161,43	39,83	40,45	2127	2131	2116	2135	2127	8		
R 2_5	0,3833	161,39	40,08	40,51	2287	2292	2304	2279	2291	10		
R 2_6	0,3591	161,35	39,99	40,27	1831	1853	1839	1844	1842	9		
R 2_7	0,3903	160,29	39,37	40,88	2727	2735	2319	2324	2526	236	2526	236
R 2_8	0,3818	160,15	39,83	41,13	-	-	-	-	-	-	1958	142
R 3_1	0,3558	165,14	40,58	40,33	1713	1721	1743	1740	1729	15		
R 3_2	0,3545	164,75	39,90	40,70	1836	1839	1847	1861	1846	11		
R 3_3	0,3604	164,79	40,02	40,53	2082	1847	1891	1974	1949	103		
R 3_4	0,3665	160,10	39,67	40,75	2058	2041	2060	2054	2053	9		
R 3_5	0,3698	159,84	39,88	40,85	2103	2116	2105	2109	2108	6		
R 3_6	0,3695	159,91	39,84	40,81	2061	2066	2064	2059	2063	3		
R 3_7	0,3653	159,65	38,61	40,89	-	-	-	-	-	-	-	-

Table A.30 - Flexural strength test results: bioformulated mortars

Specimen	b (mm)	h (mm)	I (mm ⁴)	e (mm)	Load (N)	Flexural strength (MPa)	Average flexural strength (MPa)	S.D.
R 1_1	40,7	39,7	211366	19,8	121,853	0,3	0,3	0,0
R 1_2	40,8	39,0	201663	19,5	116,800	0,3		
R 1_3	40,3	40,1	216467	20,0	129,153	0,3		
R 1_4	40,9	39,9	215425	19,9	111,745	0,3		
R 1_5	40,6	39,8	212635	19,9	126,905	0,3		
R 1_6	40,3	39,8	212047	19,9	135,610	0,3		
R 1_7	41,1	39,9	217336	19,9	122,414	0,3	0,3	-
R 2_1	40,4	40,1	217412	20,1	60,645	0,1	0,2	0,1
R 2_2	40,1	38,7	193661	19,4	46,325	0,1		
R 2_3	40,5	39,8	212004	19,9	49,695	0,1		
R 2_4	40,5	39,8	212914	19,9	75,245	0,2		
R 2_5	40,5	40,1	217244	20,0	119,606	0,3		
R 2_6	40,3	40,0	214505	20,0	46,885	0,1		
R 2_7	40,9	39,4	207782	19,7	81,142	0,2	0,1	-
R 2_8	41,1	39,8	216493	19,9	35,377	0,1		
R 3_1	40,3	40,6	224503	20,3	60,084	0,1	0,2	0,0
R 3_2	40,7	39,9	215443	20,0	69,911	0,2		
R 3_3	40,5	40,0	216403	20,0	55,592	0,1		
R 3_4	40,8	39,7	211918	19,8	83,388	0,2		
R 3_5	40,8	39,9	215885	19,9	89,565	0,2		
R 3_6	40,8	39,8	215026	19,9	85,353	0,2		
R 3_7	40,9	38,6	196126	19,3	89,284	0,2	0,2	-

Table A.31 - Compressive strength test results: bioformulated mortars

Specimen	Load (N)	Compressive strength (MPa)	Average compressive strength (MPa)	S.D.
R 1_1	1118,3	0,7	0,7	0,0
R 1_2	1108,4	0,7		
R 1_3	1032,4	0,6		
R 1_4	1200,3	0,8		
R 1_5	1232,6	0,8		
R 1_6	1126,7	0,7		
R1_7	1103,7	0,7	0,7	-
R 2_1	444,5	0,3	0,3	0,1
R 2_2	377,9	0,2		
R 2_3	325,7	0,2		
R 2_4	454,0	0,3		
R 2_5	892,0	0,6		
R 2_6	415,5	0,3		
R 2_7	557,8	0,3	0,3	-
R 2_8	419,5	0,3		
R 3_1	401,2	0,3	0,4	0,2
R 3_2	458,2	0,3		
R 3_3	388,0	0,2		
R 3_4	813,9	0,5		
R 3_5	884,1	0,6		
R 3_6	865,3	0,5		
R 3_7	392,5	0,2	0,2	-
R1 (brick)	6883,8	3,4	3,2	-
	6302,2	3,1		-
R2 (brick)	2708,0	1,3	1,3	-
R3 (brick)	2900,4	1,4	1,1	-
	1669,7	0,8		-

Table A.32 - Surface hardness test results: bioformulated mortars

06/03/2017	Specimen	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10	Point 11	Point 12	Surface hardness	Avg. surface hardness	S.D.
Control	R 1_2	86	90	89	90	96	91	90	90	89	85	90	88	90	86	5
	R 1_4	79	80	75	82	78	92	83	83	89	95	83	86	84		
	R 1_6	82	84	80	86	90	86	90	78	87	79	79	89	84		
Control (72h)	R1_7	79	87	92	87	79	81	78	90	81	76	74	88	83	83	6
Control (brick)	R1	84	79	85	77	85	82	84	83	82	84	70	86	82	82	5
LB	R 2_1	57	67	64	81	64	75	64	63	69	61	72	65	67	78	9
	R 2_4	87	82	86	81	85	86	82	87	80	86	83	89	85		
	R 2_6	81	82	86	80	85	79	82	89	78	75	85	78	82		
LB (72h)	R 2_8	80	76	83	66	79	69	80	75	90	85	79	81	79	79	7
LB (brick)	R2	74	73	85	70	76	85	76	75	86	78	70	76	77	77	6
E.coli+Fe	R 3_2	57	63	53	62	60	64	60	60	68	79	53	71	63	57	11
	R 3_5	64	53	56	65	54	56	73	37	61	40	41	58	55		
	R 3_6	64	46	39	64	59	43	68	55	36	40	65	58	53		
E.coli+Fe (72h)	R 3_7	71	64	64	73	56	55	60	47	63	75	72	68	64	64	8
E.coli+Fe (brick)	R3	81	79	73	70	85	73	92	76	70	75	83	77	78	78	7

Table A.33 - Ultrasound propagation speed test results: bioformulated mortars

06/03/2017	Specimen	Time (μ s)	Height (mm)	v_{US} (m/s)	v_{US} (m/s)	S.D.
Control	R 1_2	34,5	40,06	1161	1138	24
	R 1_4	34,9	38,88	1114		
	R 1_6	34,5	39,26	1138		
Control (72h)	R1_7	34,1	39,83	1168	1168	-
LB	R 2_1	41,2	39,98	970	1021	45
	R 2_4	38,0	40,16	1057		
	R 2_6	37,1	38,41	1035		
LB (72h)	R 2_8	35,3	39,36	1115	1115	-
E.coli+Fe	R 3_2	37,4	39,43	1054	1050	13
	R 3_5	38,5	39,84	1035		
	R 3_6	37,3	39,55	1060		
E.coli+Fe (72h)	R 3_7	36,4	39,46	1084	1084	-

Table A.34 - Water drop test results: bioformulated mortars

06/03/2017	Specimen	Time until water drop absorption (s)	Average time until water drop absorption (s)	S.D.
Control	R 1_2	1,8	1,4	0,4
	R 1_4	1,0		
	R 1_6	1,3		
Control (72h)	R1_7	1,4	1,4	-
Control (brick)	R1	1,7	1,1	0,5
		0,7		
		1,0		
LB	R 2_1	2,0	2,8	0,9
	R 2_4	2,6		
	R 2_6	3,7		
LB (72h)	R 2_8	2,7	2,7	-
LB (brick)	R2	7,8	6,0	2,6
		3,1		
		7,2		
E.coli+Fe	R 3_2	1,4	1,9	0,6
	R 3_5	2,5		
	R 3_6	1,7		
E.coli+Fe (72h)	R 3_7	2,9	2,9	-
E.coli+Fe (brick)	R3	9,5	8,7	0,6
		8,3		
		8,4		

Table A.35 - Surface cohesion test results: bioformulated mortars

06/03/2017	Specimen	Mass (g)	M _{avg} (g)	Mass loss variation	Surface area (m ²)	Mass loss (g/m ²)	MI _{avg} (g/m ²)	S.D.	Average mass loss variation
Control	R 1_2	0,0116	0,0194	-	0,0016	7,2	12,1	4,7	-
	R 1_4	0,0268			0,0016	16,7			
	R 1_6	0,0197			0,0016	12,4			
Control (72h)	R1_7	0,0152	0,0152	-22%	0,0016	9,5	9,5	-	-21%
Control (brick)	R1	0,0036	0,0036	-81%	0,0025	1,4	1,4	-	-88%
LB	R 2_1	0,0267	0,0197	2%	0,0016	17,0	12,5	4,0	3%
	R 2_4	0,0179			0,0016	11,1			
	R 2_6	0,0145			0,0016	9,3			
LB (72h)	R 2_8	0,0140	0,0140	-8%	0,0016	8,9	8,9	-	-6%
LB (brick)	R2	0,0057	0,0057	58%	0,0025	2,3	2,3	-	58%
E.coli+Fe	R 3_2	0,0464	0,0657	239%	0,0016	29,6	40,6	11,1	236%
	R 3_5	0,0636			0,0016	40,4			
	R 3_6	0,0870			0,0017	51,8			
E.coli+Fe (72h)	R 3_7	0,0465	0,0465	206%	0,0015	30,2	30,2	-	217%
E.coli+Fe (brick)	R3	0,0129	0,0129	258%	0,0025	5,2	5,2	-	258%