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 Preliminary approach to bio-based surface healing of structural repair cement mortars

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Article

Bio-based surface healing of structural repair cement mortars

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Abstract

Mitigating the maintenance and repair costs of structures and infrastructures is a major problem in all countries. The aim of this research work is to analyse the performance of surface healing technique for crack control of cement-based mortars for structural repair in maritime environments. Microbiologically induced calcite precipitation (MICP) with ureolytic bacteria *Sporosarcina pasteuri* DSM 33 was introduced for crack-healing. Only main cracks were filled with the bioagent (bacterial spores and nutrients) for cost-saving purpose. It is intended to analyse the effectiveness of this technique for structural application in areas exposed to cyclic moisture changes. Hygric properties and their relation to durability increase were analysed through moisture buffering tests, capillary, porosity, compressive strength, SEM and microscopy analysis before and after bio-agent application to evaluate the evolution of the precipitation. For the first time, moisture buffering value (MBV) was used to evaluate the performance of the self-healed mortar and time needed for bacterial precipitation. The treated material can be classified as good in terms of MBV, and there was a general increasing trend of moisture buffering behaviour in self-healed samples. SEM analysis showed distinctive differences between the treated and non-treated cracks. The results show that bio-agent had remarkable effect on compressive strength recovery (over 87% of original value) after 21 days of healing and positively affected the initial stage of capillary absorption.

1. Introduction

According to some sources, the annual cost for maintenance and repair of concrete highway bridges due to corrosion of reinforcement has a major impact in several economies. Just in England, UK, the capital investment on renewals for structural defects, reduced load capacity or other structural needs increased 40% in the last years. In the United States in 2001 was around US $4 billion [1], with newer estimation of both direct and indirect costs being 4 times that number [2]. The figures for the repair of bridges, tunnels and other structures in the European Union are similar, being around 4 to 6 billion Euros per year [3]. It is also reported that cca. 3% of global GDP which equals to US $2.2 trillion is spent on costs related to corrosion [4]. Therefore, the idea of mitigating the maintenance and repair costs is always attractive from the financial point of view. However, the ecological benefits of reduced concrete production as a result of successful repair techniques should not be disregarded. For example, the cement production (key component of concrete) is responsible for 5-8% of global human-origin CO_2 emissions as well as considerable amount of SO_x, NO_x and other pollutants [5].

Two main causes of deterioration of reinforced concrete (RC) are: 1) the deterioration of the concrete itself, and 2) the corrosion of steel reinforcement [6]. It is obvious that cracks in the concrete play an active role in reinforcement bars corrosion, by enabling contact between the steel bars and water, which usually contains dissolved ions. Earlier studies suggested that crack widths of less than 0.3 mm presented no cause for concern, but a more recent one states that even hairline cracks influence the corrosion [7]. The main chemical reactions responsible for the corrosion process are well known:

$$Fe \longrightarrow Fe^{2+} + 2e^- \tag{1}$$
$$O_2 + H_2O + 4e^- \longrightarrow 4OH^- \tag{2}$$
$$Fe^{2+} + 2OH^- \longrightarrow Fe(OH)_2 \tag{3}$$
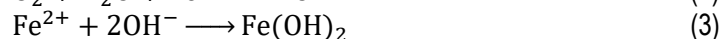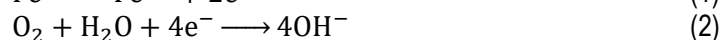
From Eq. 1-3 it is clear that the presence of water (and oxygen) is crucial. The formation of iron rust results in several negative phenomena, such as appearance of cracks in the steel cover, reduction of steel bars cross-section and deterioration of concrete-steel interface [8]–[10].

Apart from mechanical damage, the most important mechanisms of deterioration are "chloride-induced" corrosion and "carbonation". Chlorides indirectly attack the steel by reacting with the already formed $Fe(OH)_2$ deposit on the surface of the reinforcement bars producing $FeCl_2$. However, this chemical reaction is only possible if the concentration of hydroxide ions (normally present in the concrete surrounding the reinforcement bar) is sufficiently low or the concentration of chloride ions is sufficiently high. Another effect of chloride ions is neutralisation of the protective oxide layer on the surface of the steel bars, which induces a pH value decrease further propagating the corrosion reactions and overall degradation of reinforcement [11], [12]. On the other hand, carbonation is a process of neutralisation of concrete alkalinity when atmospheric CO_2 reacts with hydrated cement products. Similarly to chloride ions' effect, carbonation also destroys the protective oxide layer on steel bars, which creates electrical potential difference. As a result, anode and cathode regions are formed, where metal is dissolved and OH- ions are formed, respectively. Carbonation also reduces pH value of concrete which negatively affects the passive layer and increases the corrosion [13]–[15].

Carbonation in sense of "carbonate precipitation" can on the other hand have a positive effect on the structural integrity of the surrounding concrete. Precipitation of calcium-carbonate ($CaCO_3$) can provide efficient and compatible bonding material in the cement matrix which can result in its densification through filling of the pores and cracks. This can in turn lead to regain of mechanical properties and decrease in water permeability [16]. Microbiologically induced calcite precipitation (MICP) is a reasonably well-known technique for self-healing of concrete. Formation of $CaCO_3$ is actually a side-effect of bacterial activity inside the cement matrix. Although large majority of bacterial species can induce calcite precipitation, the carbonatogenesis depends on the metabolic pathway of precipitation as well as on external factors. Three main types of bacterial cultures used for calcite precipitation are 1) ureolytic, 2) denitrifying and 3) aerobic heterotrophic bacteria. It is important to stress that all types of cultures used for this purpose must be alkalophylic or at least alkali-tolerant, since the normal pH values of concrete are in the range of 10 and upwards.

Ureolytic bacteria decompose urea to form carbonate and ammonium ions, as shown in Equation 4:

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{bacterial urease}} 2NH_4^- + CO_3^{2-} \tag{4}$$
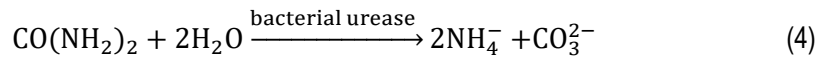
Carbonate ions formed in this way can react with calcium ions from the cement matrix to form $CaCO_3$ (Eq. 5):

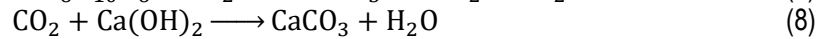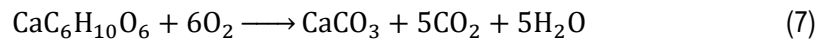$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \tag{5}$$

Main ureolytic bacterial strains used for inducing calcite precipitation in concrete are *Sporosarcina* (previously known as *Bacillus*) *pasteurii*, *Bacillus sphaericus*, *S. ureae*, *B. megaterium*, with others being *B. lentus*, *S. gingsengisoli*, *B. pseudofirmus* and others [16], [17]. In present paper authors decided to use *S. pasteurii* since it is widely used as a model-organism because of its high urease activity while being non-pathogenic alkaliresistent and sporogenic bacteria [18]. Ureolytic bacteria are more suitable for surface crack healing since they require oxygen (more readily available on the surface of the material) in order to hydrolyse urea and produce carbonate.

Denitrifying bacteria on the other hand are able to use nitrate ions as electron acceptors if deprived of oxygen [19], and the mechanism of carbonate production follows Equation 6:

$$5HCOO^- + 2NO_3^- \longrightarrow N_2 + 3HCO_3^- + 2CO_3^{2-} + H_2O \tag{6}$$

with the next chemical reaction being the same as Eq 5. This characteristic makes them adequate for deeper cracks, where oxygen may be scarce. Some strains used for this purpose are *Pseudomonas aeruginosa*, *P. denitrificans*, *Diaphorobacter nitroreducens* and *Castellaniella denitrificans* [20]–[23]. An interesting fact is that denitrifying bacteria can also produce nitrite (NO_2^-) [24], a known corrosion inhibitor [25].

Aerobic heterotrophic bacteria metabolically converse organic compounds under aerobic conditions, which can result in $CaCO_3$ precipitation. Organic compounds can be introduced with bacterial culture (self-healing bio agent) in form of calcium salts of organic acids (lactate, glutamate, acetate etc.) [16], [26]–[28]. These compounds are then consumed by bacteria during which process precipitation of $CaCO_3$ is induced. Equations 7 and 8 provide an example of how calcium lactate is transformed into calcium carbonate and carbon dioxide, which can further react with $Ca(OH)_2$ from cement matrix:

$$CaC_6H_{10}O_6 + 6O_2 \longrightarrow CaCO_3 + 5CO_2 + 5H_2O \qquad (7)$$
$$CO_2 + Ca(OH)_2 \longrightarrow CaCO_3 + H_2O \qquad (8)$$

Apart from metabolic production of carbonate ions and consequential $CaCO_3$ formation, bacteria can also induce the precipitation of the calcite by acting as nucleation sites. Namely, the bacterial cell wall contains negatively charged groups due to which calcium cations can bond to it. Afterwards this bonded calcium reacts with carbonate to form $CaCO_3$ [29] (Fig. 1).
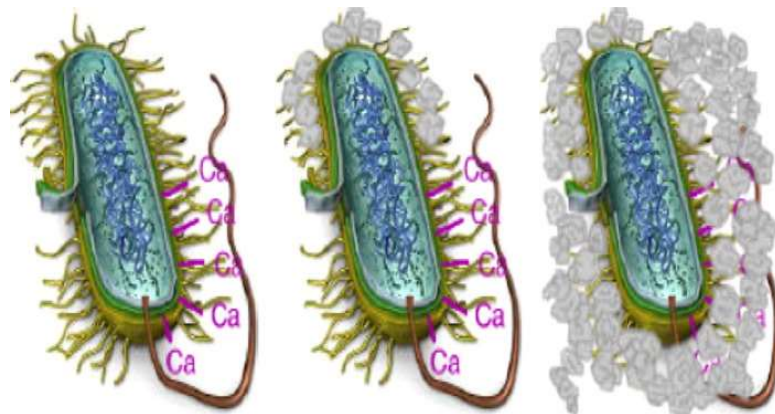
Figure 1 - Bacteria as nucleation sites for calcium-carbonate precipitation [30].

The studies mentioned in the previous paragraphs generally relied on a technique where the bio-agent was added during the manufacture of the cement/concrete. The method of cement crack healing used in this paper – externally added bio-agent – is still in the early stage of research. Comparatively fewer studies on this type of crack remediation was done as opposed to the 'internal' autonomous self-healing. Although 'internal' method has proven to be effective in crack repair of future cracks evolving in the material, it is not adequate for already present cracks on structures and buildings in service. Therefore, the externally applied bio-agent must be used. This method is also known as 'crack closing method' [31].

Two main techniques for external application of healing agent have so far been studied, 1) immersion or soaking of material in bio-agent suspension and 2) dropping of bio-agent directly on cracks. Immersion technique was studied by Choi *et al.* (2017) showing good results in regard to crack closure (up to 80% closure of cracks with widths between 0.5 and 1.1 mm) and water permeability (decrease from 3.03x10^{-3} m/s to 8.25x10^{-5} m/s), although there was not much improvement regarding mechanical properties. The main drawback of this technique remains its impracticality in real life application. On the other hand, the dropping technique has the potential to have a wider practical significance, since it could be used on present structures needing crack maintenance. Results from a study by Jongvivatsakul *et al.* (2019) indicate that the external application of healing agent by dropping technique has more efficiency than the internal self-healing since the localised point of repair gets a high concentration of remediation agent and therefore exhibits higher rate of crack closure [31]. There was also a study in which a mix of bacteria (with nutrients) and filler was used (silica fume and sand) which demonstrated promising results [32].

The analysis of hygrothermal and structural behaviours gives an indication of the performance and durability of these types of cement-based materials and their suitability when exposed to high level of moisture such as in maritime environments. Therefore, in this study we focused on the effectiveness of this repair technique for structural application in areas exposed to cyclic moisture changes. For cost saving purpose, only the main cracks

were filled with the bio-agent, and to the authors' best knowledge, the moisture buffering test was done for the first time on cement mortar treated with bacteria-based repair agent.

2. Materials and sample preparation
2.1. Materials, mortar mix proportions and crack preparation

In total, 11 Portland cement mortar CEM I 42.5 beams (with standard dimensions: 4 cm x 4 cm x 16 cm) were prepared according to standard EN 196-1 (Methods of testing cement – Part 1: Determination of strength [33]) and cured for 28 days. Table 1 shows the composition of mortar beams. The beams were then subjected to flexural strength test in accordance with the same standard during which 2 cracks were developed at the sites of point loading on each mortar beam. These cracks were the subject of external healing repair (in 6 out of 11 mortars) as described in 2.2. It should be noted that the samples investigated were actually the remaining parts of the original beams, which had various dimensions after the beams were broken by the flexural testing. Figure 2 shows an example of mortar following exposure to the flexural test.

Figure 2 – Cement mortar beam with developed cracks after flexural testing.

The 6 samples subjected to the external healing bio-agent are named '**bio samples**' and the 5 without the bio-agent applied are named '**non-bio samples**'.

Table 1 – Composition of mortar samples.

| Ingredients	Mass per 3 cement beams (g)
Cement	450 ± 2
Sand	1350 ± 5
Water	225 ± 1
Water/cement ratio – 0.50	

2.2. Microbiological crack repair

6 cement mortar beams were selected as samples for external healing experiment. The procedure of application of the liquid bio-agent onto surface consisted of several steps. Firstly, the pH value of the beams was determined

and since it was deemed too high (~12) the pH value was lowered to a more acceptable one, according to *Sporosarcina pasteurii* viable pH zone. The pH lowering process consisted of keeping the beams in tap water for at least 6 hours, then drying them in dryer for 3h and then repeating the tap water submerging (this was done in several cycles until the pH value dropped below 11).

After that, the beams were sterilised in autoclave and bacterial suspension was prepared. Sporosarcina pasteurii was chosen as high eddiciency ureolitic bacteria which can survive extreme conditions such as high pH value or low aw value. The culture was incubated on TSA (Trypticase Soy Agar, HiMedia, Mumbai, India) with addition of 20% urea during 7 days at 30 °C. The bacterial suspension of S. pasteuri DSM 33 was freshly prepared in sterile distilled water. The concentration of bacteria was $8.8 \cdot 10^7$ cfu/mL. In order not to overload the system with extra ions, modified Urea broth was used (nutrient broth 4 g, NH_4Cl 10 g, $naHCO_3$ 2.15 g, urea 20 g, distilled water 100 mL.

Every mortar beam sample selected for external healing testing had 2 relatively prominent cracks on the lateral sides in regard to the side that was in contact with the flexural strength measuring device (points of loading). On both of these cracks 100 µL of nutrient solution was applied by dropping, using a sterile micropipette. Then, on one of the cracks (marked 'bacteria', Fig. 3) 100 µL of bacterial suspension was dropped, and on the other (marked 'blank', Fig. 3) 100 µL of sterile distilled water was dropped and the process was repeated for all 6 samples. This was done in order to visually compare the differences in carbonate precipitation with and without bacterial culture on the exact same sample. After the application of bio-agent and blank suspensions, the beams were put in a dish filled with distilled water up to 1/3 of the beams' height (the side with the treated cracks being on top) and incubated at 30 °C for 21 days. The non-bio samples were kept in the same conditions as the bio samples.
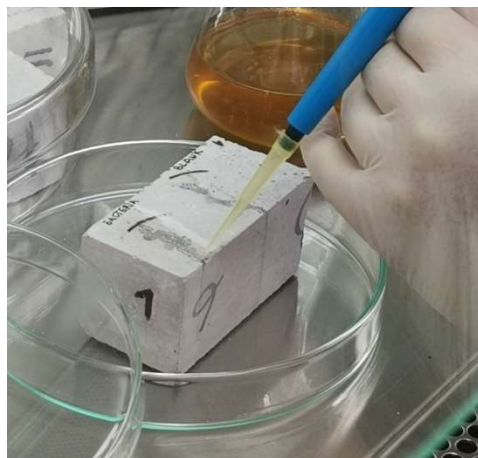
Figure 3 - Application of bio-agent to the 'bacteria' crack ('blank' crack can be seen to the right).

3. Tests and methods

The performance of MICP method for external crack healing was investigated using both destructive and non-destructive tests. In addition, the SEM analysis was used to confirm the presence of the MICP product and other structures that indicate bacteria induced precipitation.

3.1. Crack observation

Cracks were investigated using a portable digital microscope Pro10-3 (ViTiny, USA). The position of objective lenses was marked with permanent marker to facilitate the search for the same position of the crack to be viewed and recorded. Crack widths were measured using integrated ViTiny software.

3.2. Moisture buffering test

A combination of NORDTEST protocol [34] and ISO 21453 (ISO, 2008) was employed in order to determine the ability of the material to adsorb and desorb water vapour from the environment. All but one surface area of the samples were covered using aluminium tape, laid horizontally with the exposed surface pointing upwards. The samples were put in artificial conditions chamber and were stabilised for 24 hours at 23°C at 60% RH. Afterwards, they were exposed to a cyclical step change in RH of 75% for 8 hours and 53% for 16 hours, in accordance with test conditions defined by Romano *et al*. [35]. It should be noted that during the 3rd and 4th cycles measurements were not made due to inaccessibility of the laboratory. Nevertheless, this did not crucially affect the overall results. Moisture buffering value (MBV) is defined as in Equation 9.

$$MBV = \frac{m_a - m_d}{A\Delta\varphi}$$
(9)

where
m_a = Mass of sample at end of moisture adsorption stage (g)
m_d = Mass of sample at end of moisture desorption stage (g)
A = Exposed surface area of sample (m^2)
$\Delta\varphi$ = Difference in RH between adsorption and desorption stage (%)

3.3. Porosity
Open porosity measurements were done in accordance with EN 1936:2006 (CEN, 2007) to determine the void space inside the samples, which can be used as an indicator of the microstructure of the sample.

3.4. Capillary test
The bio samples and non-bio samples were subjected to capillary action test in accordance with EN 1015-18: 2002 (CEN, 2002), to investigate if there is an influence of the bio agent on the material's capacity to absorb water in short term.

3.5. Compressive strength test
Compressive strength test was done in accordance with standard EN 1015-11 (CEN, 1999) to determine the change and recovery of the mechanical properties of the material after the application of bio-agent.

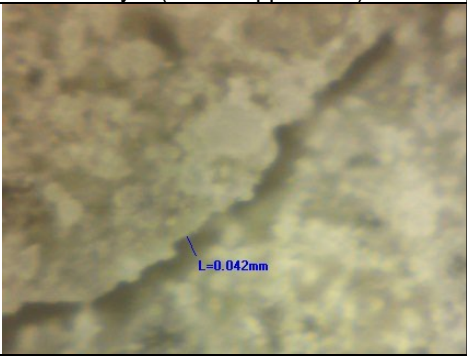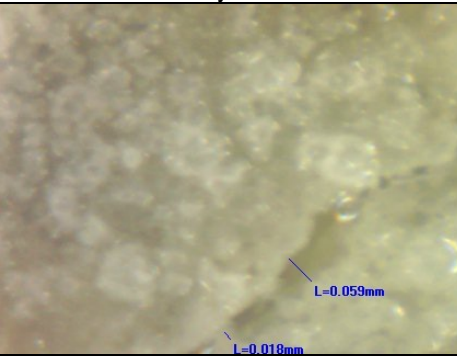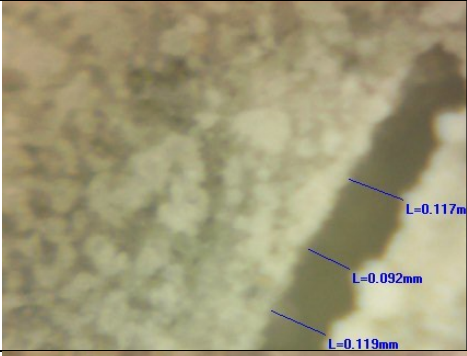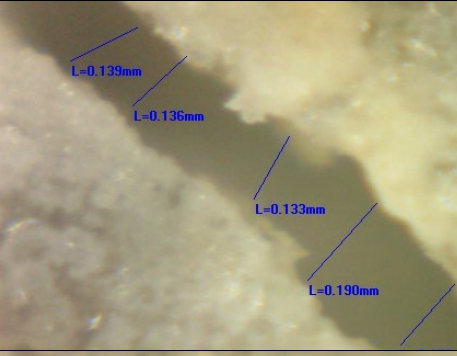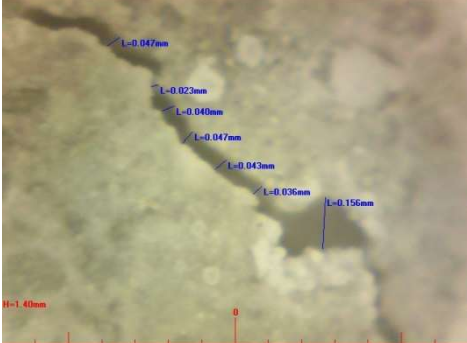### 3.6. Scanning Electron Microscopy
SEM analysis was done using INSPECT S50 Scanning Electron Microscope (FEI, USA) with 20 kV accelerating voltage and 21 pA current. Samples for SEM analysis were prepared by breaking the bio samples along the treated and investigated cracks ('bacteria' and 'blank') and in case of non-bio samples along one of the cracks created during the initial flexural testing (See Section 2.2). After this, the inner surface area of the crack was revealed and a slice of approximately 0.5 cm thickness was cut with a diamond circular knife. Then, these slices were further reduced to samples sized approximately 1 cm x 1 cm x 0.5 cm. Samples were analysed without sputtering (direct observation).

4. Results and discussion

4.1. Crack observation

Table 2 shows selected images of cracks before application of the repair bio-agent – day 0 – ('bacteria' cracks) and 21 days after the application. 'Blank' cracks are also shown for comparison.

Table 2 - Images of cracks on bio and non-bio samples.

|		Day 0 (before application)	Day 21
Bio sample	«bacteria» crack	L=0.042mm	L=0.059mm / L=0.018mm
	«blank» crack	L=0.117m / L=0.092mm / L=0.119mm	L=0.139mm / L=0.136mm / L=0.133mm / L=0.190mm
Non-bio sample		L=0.047mm / L=0.023mm / L=0.040mm / L=0.047mm / L=0.043mm / L=0.036mm / L=0.156mm / H=1.40mm / 0	L=0.019mm / L=0.021mm / L=0.021mm / L=0.024mm / L=0.029mm / L=0.015mm / L=0.147mm

Images of 'bacteria' cracks show what seems to be the initial stage of microbiologically induced calcite precipitation. This preliminary evaluation is based on noticed morphology of the cracks before and after the treatment and on measurements of the crack widths. Although microscope and software used had technical limitations in regard to precision of the measurements, it can be noticed that the crack widths reduced in size between 20 and 40%. On the other hand, in most of the 'blank' cracks there are no observable reduction of widths, and there is even some increase of widths, probably due to sample handling.

Non-bio samples showed various phenomena regarding the cracks. It can be noticed that autogenous healing took place on some of the cracks, but it is non-systematic. Some of the cracks almost completely filled up and

some were almost unaffected. It could be concluded that the autogenous healing depended on the locally developed micro-conditions.

4.2. Moisture buffering test
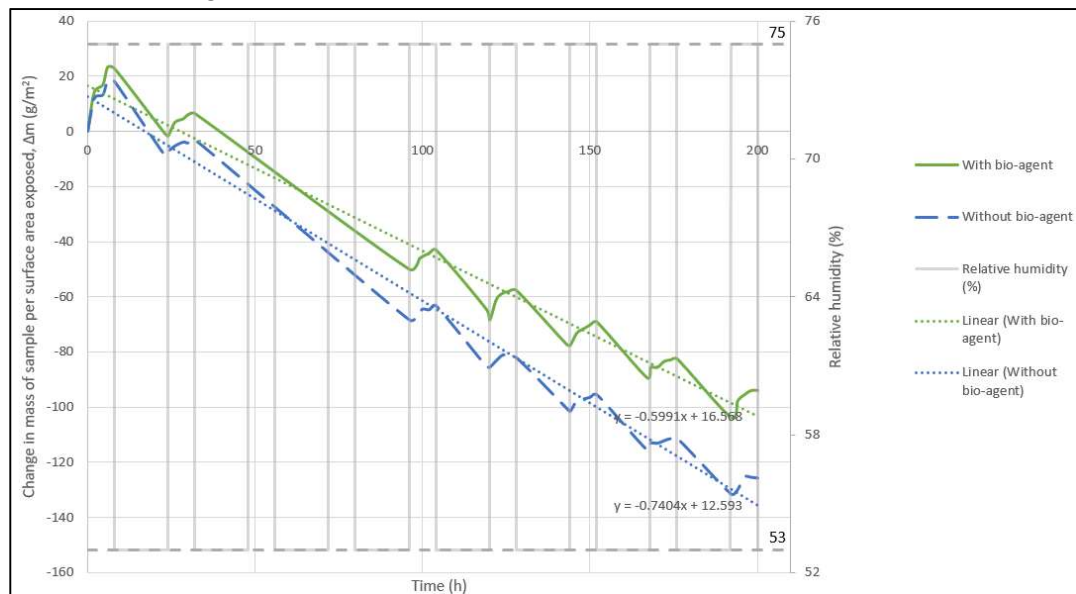
Figure 4 - Adsorption/desorption graphs for samples with and without bio-agent applied, after 1 month.

The Fig. 4 shows that the change of mass of material follows change of humidity in the testing chamber. After 18 h of experiment non-bio mortar's mass change went into negative. Same thing happened only after 40 h in case of bio mortar which indicates different porous structure of the two materials. It took longer time for bio mortar to lose mass (i.e. the trapped water) due to desorption phenomena, which can be explained by a more complex internal porous structure developed by the bio-agent. Different behaviour is also evident from the slope values of respective trend lines for each graph (-0.599 and -0.740 for bio and non-bio mortar, respectively). The results show that there is a decrease of the impact of moisture cyclic change by 20% for the bio mortars in comparison with the conventional ones. Desorption phenomena are clearly the more dominant factor in the mass change of both materials, as can be seen from the graphs which have general downward trend. However, cement mortar with bio agent is less affected by the change of environmental humidity in respect to the non-bio mortar – the adsorption and desorption phenomena (shown as change of mass per surface area) are generally less expressed. Further study should prolong the experiment to determine the final mass change and the behaviour differences of respective materials after final stabilisation.
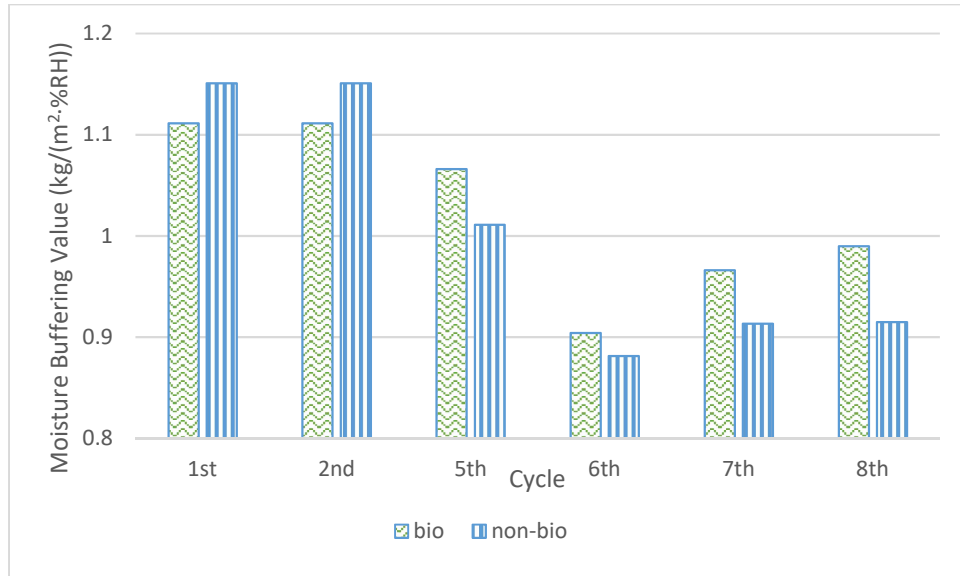
Figure 5 - Moisture buffering values of bio and non-bio cement mortars, after 1 month.

Moisture buffering value (MBV) is a quantitative representation of a material's ability to absorb and desorb water vapour from the air when the air humidity is high and low, respectively. It is generally a desirable property for a material to have high MBV. Fig. 5 shows that MBVs for both types of mortars are at least 15% higher in the first 2 days of cycles. After the 2nd cycle, there is a switch in the MBVs, where MBVs for bio mortars tend to be 3% to 7% bigger than for non-bio mortars. This could be associated with the time needed for bacterial precipitation. It is also noticed that there is a general rising trend of MB values for bio samples from the 6th cycle onwards, while the values for non-bio samples are comparatively lower and stagnating. Based on classification given by Rode *et al*. [36] the tested samples can be categorised as 'Good' (MBV 0.8 to 1.0). Further study should encompass longer exposure of samples to the humidity cycles in the testing chamber in order to determine the final MBV improvement of the bio samples.

4.3. Porosity
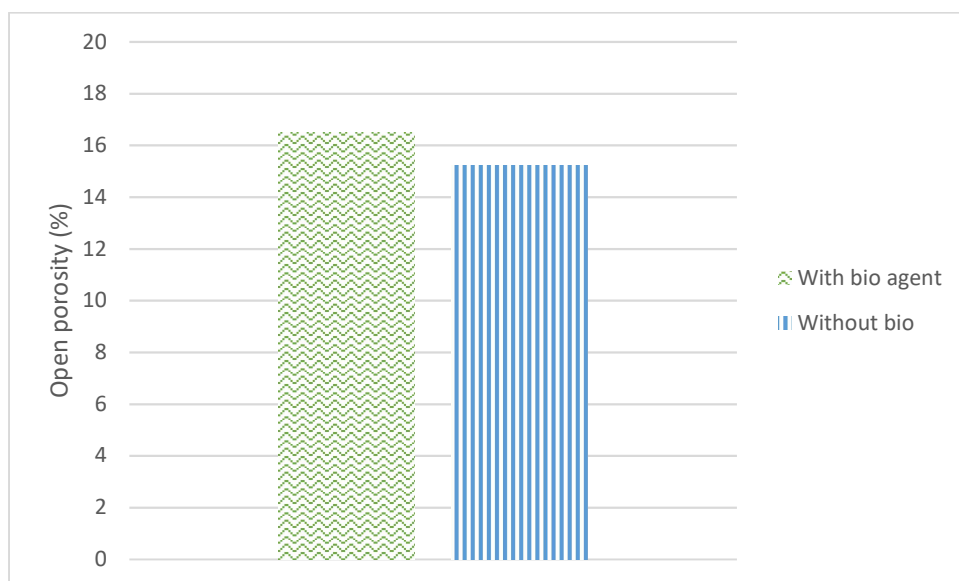
Figure 6 - Average open porosity of the selected samples with and without bioagent, after 2 months (coefficient of variation obtained for the bio mortars was 2% and for the non-bio mortar was 1.5%).

Fig. 6 shows that open porosity of the bio-samples is somewhat larger than samples without bioagent applied. However, the results are quite similar (16% and 15%) indicating that this test is not adequate, at least for the first 2 months after mortar preparation. Probably, the newly formed carbonate bridges were not yet sufficiently stable relative to the surrounding cement matrix and were washed out with the water used as a measuring fluid.

4.4. Capillary test
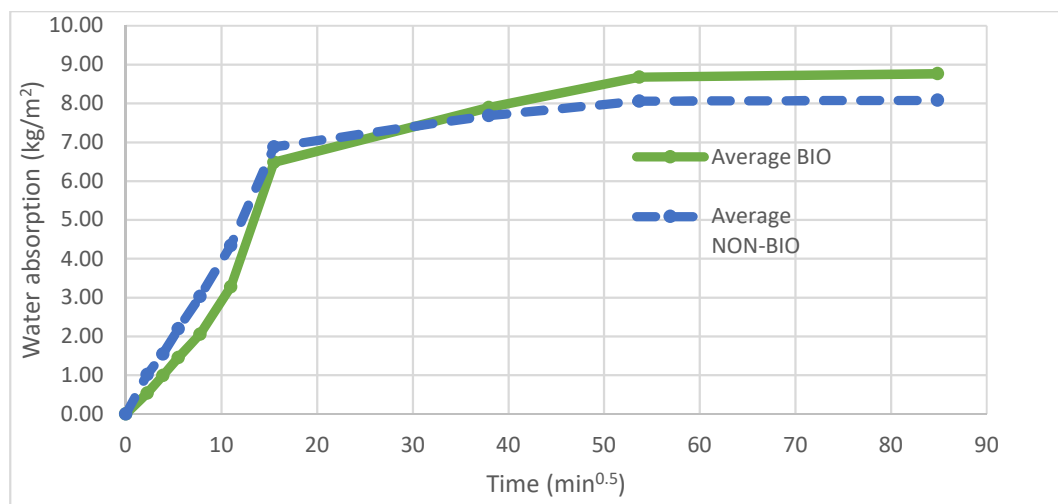
Figure 7 - Capillary action curve for bio and non-bio cement mortar samples, after 2 months.

Fig. 7 shows the average values of capillary action curves for bio and non-bio samples. Initial water absorption is generally lower for the bio samples than the non-bio samples, which is a desirable result. Only after 900 min of testing does the water absorption of the bio samples become somewhat higher than non-bio samples. This presents an interesting result as it is obvious that the bio-agent affected the initial stage of the capillary absorption in a positive way (i.e. preventing water migration) but had little effect afterwards, when the samples tend to become saturated. From practical point of view, the behaviour presented in the first part of the capillary curve (water absorption in a non-saturated medium) is associated with the behaviour of a maritime structure area exposed to the tidal-splash. The second region of the curve is related to a saturated medium as it happens in structures under water. Therefore, it seems that this bioproduct presents benefits to minimise migration of water-soluble ions in tidal-splash zones.

It is known that *S. pasteurii* favours oxygen, so it can be presumed that the highest activity was at the surface, which may be related with the obtained results – capillary action was impeded by the microstructure formed near the surface. To completely explain this phenomenon further study is required.

4.5. Compressive strength test
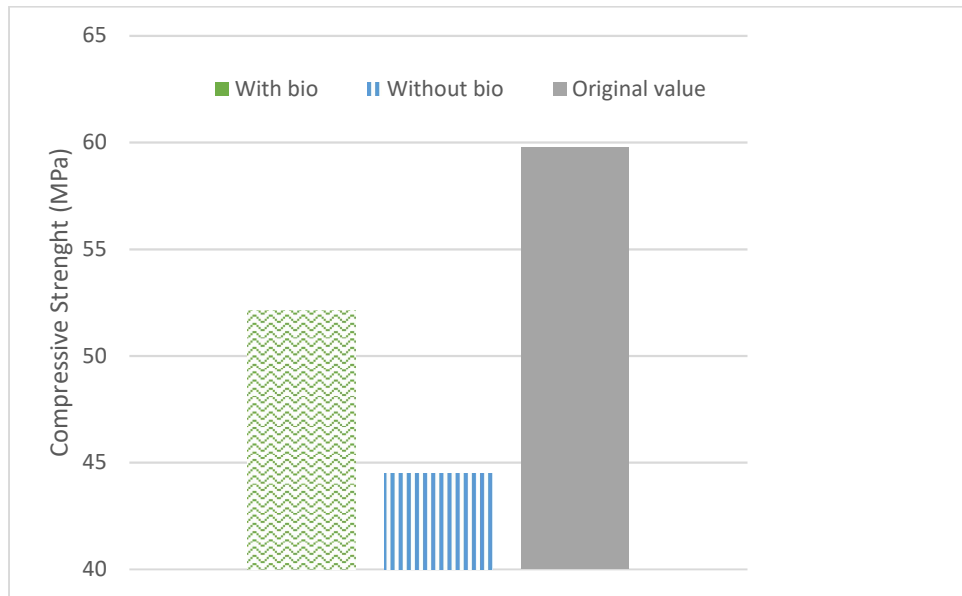
Figure 8 - Compressive strength values of bio (waving lines) and non-bio cement mortar (vertical lines) samples after 2 months, and original value of the samples after 1 month.

The 'original value' of the samples seen on Fig. 8 represents the compressive strength value of the cement mortar samples obtained after 28 days of curing. It represents the average value for all samples, to some of which the bio-agent was added afterwards (as described in 2. Materials and Methods). The bio mortar samples showed remarkable recovery of compressive strength of over 87% (52.14 MPa out of original 59.77 MPa), while the non-bio samples presented only 74% of the original value (44.50 MPa). Coefficient of variation was well within tolerated values for building materials (4% for bio and 9% for non-bio).

4.6. SEM analysis

Fig. 9 ('bacteria' crack sample) shows typical morphology ascribed to calcite – grainy crystals, which are absent from 'blank' and 'non-bio' samples. Fig. 10 ('bacteria' crack sample) shows what appear to be bacterial spores (marked with purple circles and letter S). As it is hard to claim that these are in fact spores (Without new-born $CaCO_3$ crystals in surrounding, these spherical forms can be bacterial spores which were not metabolically active during incubation time), a good indication toward that statement is the fact that there were no similar morphologies detected inside the cracks where no bio-agent was applied. The same micrograph also show forms typical for ettringite (blue marker and letter E) and calcium silicate hydrates (red marker and letters CSH) [37]. It should be noted that the hydration products are much more prominent inside the 'bacteria' crack sample than in other samples, which could be a positive side-effect of bio-agent application and explains the better mechanical characteristics of the bio samples. Furthermore, calcite crystal and an amorphous film, most likely bacterial biofilm, can be seen in Fig. 11 (marked with white marker and letter C, and green arrow and letter B respectively). Figures 12 and 13 show 'blank' and 'non-bio' samples, where absence of calcite phase is obvious. Observed morphologies of these samples are remarkably similar to control samples of cement mortar found in literature (e.g. [38]).
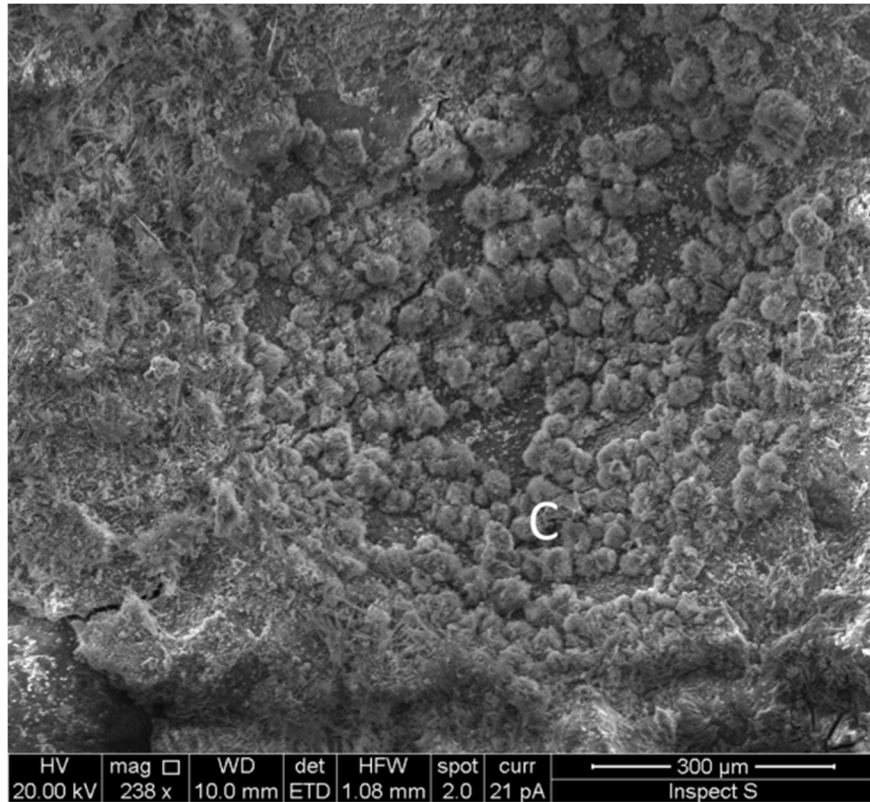
Figure 9 - SEM image of 'bacteria' crack cross-section, magnification 238 x.
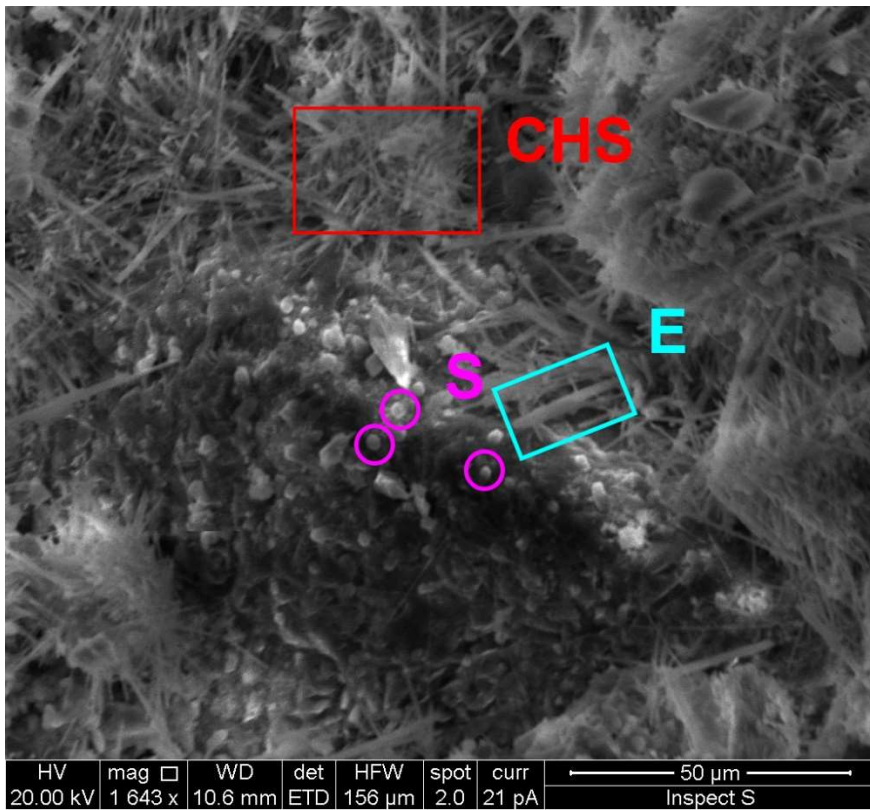

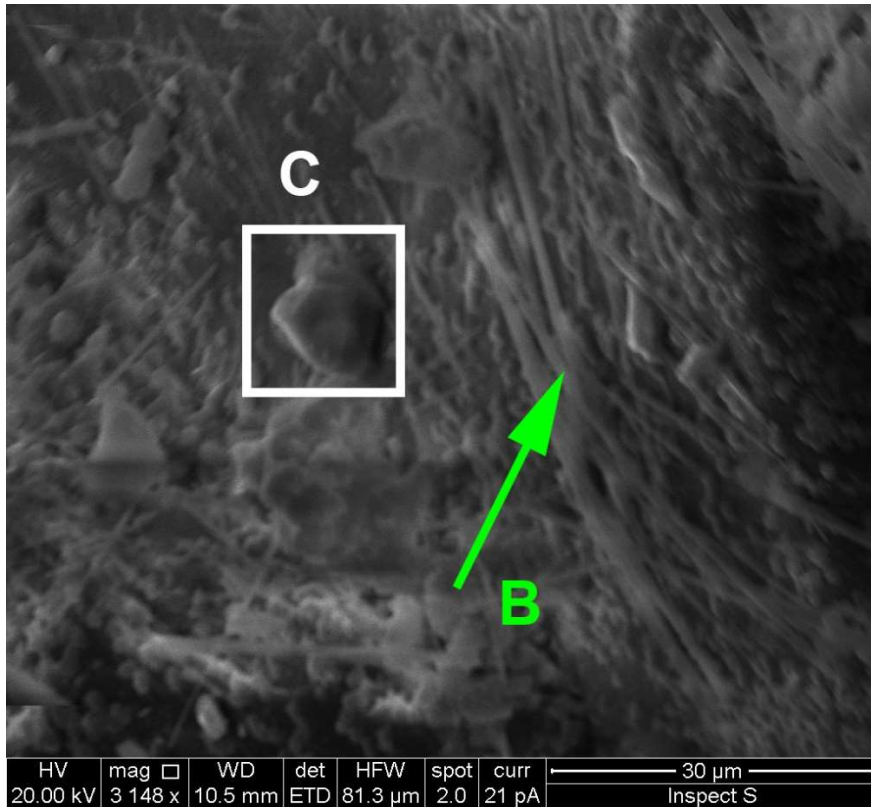**Figure 10 -** SEM image of 'bacteria' crack cross-section, magnification 1643 x.

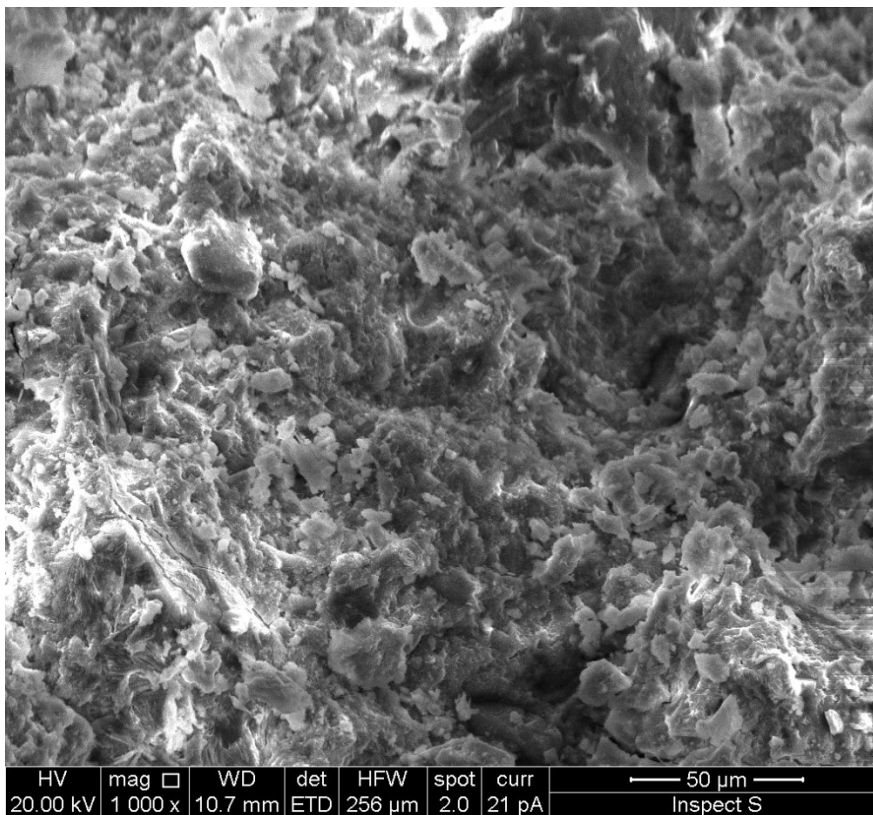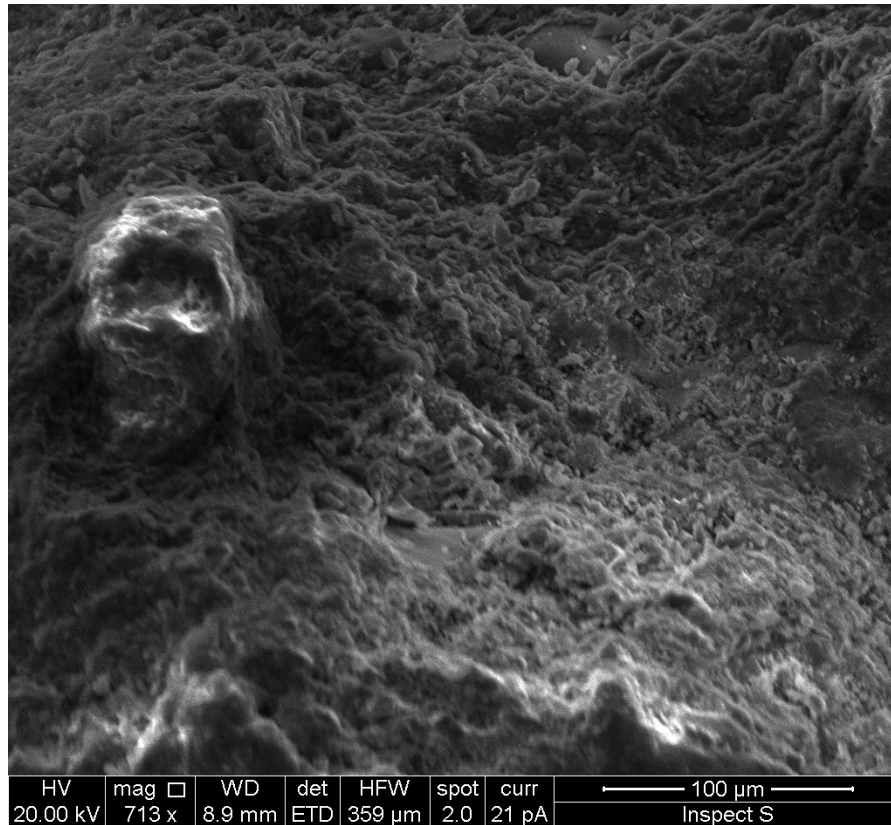**Figure 11 -** SEM image of 'bacteria' crack cross-section, magnification 3148 x

Figure 12 - SEM image of 'blank' crack cross-section, magnification 1000 x.

Figure 13 - SEM image of 'non-bio' crack cross-section, magnification 713 x.

5. Conclusions and recommendations

Preliminary study of the external healing bio-agent based on *Sporosarcina pasteurii* bacterial culture showed a promising path to further investigation of dropping technique as a potential application method for repair of concrete structures and buildings already in service. Investigated bio-agent had remarkable effect on compressive strength recovery (over 87% of original value) and positively affected the initial stage of capillary absorption. To the authors' knowledge, moisture buffering value (MBV) was determined for the first time for cement mortars externally treated with bio agent in this paper. The treated material can be classified as good in terms of MBV, and there was a general increasing trend of MBV closer to the end of experiment. SEM analysis showed distinctive differences between the treated and non-treated cracks – apart from calcite formation in the treated cracks, there were more developed morphologies of hydration products (calcium silicate hydrates and ettringite). This is the probable explanation of significantly better mechanical properties of the bio samples. Further study should include microbiological experiments to confirm the viability of the bacteria and to optimise the parameters for application of the bio-agent. Furthermore, the MBV experiment should be prolonged to determine the final mass change and the behaviour differences of respective materials after final stabilisation.

Acknowledgements

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