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The effect of soil incubation on bio self-healing of cementitious mortar

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Abstract

Successful implementation of bacteria-based self-healing in cracked cementitious materials requires the provision of a suitable incubation environment, which can activate the bacteria to produce e.g. calcium carbonate sealing the cracks. Research to date has focused on the self-healing process in humid air and water. However, almost all structures are built on or in the ground, thus, significant amounts of concrete are exposed to ground conditions. To investigate the effect of soil incubation on the self-healing process, laboratory experiments were conducted on mortar impregnated with *Bacillus subtilis* (encapsulated in calcium alginate). The mortar specimens were initially cracked and subdivided into three groups and each group was incubated for 28 days within different incubation environments, namely, partially-saturated soil, full-saturated soil, and water. Supported by Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray Spectrometry (EDX), the results revealed that the bio self-healing can be activated within the cracks under the saturated regime of soil as far as the matric suction is smaller than the capillary pressure of the cracks. Moreover, the results indicated there was no evidence suggesting the influence of naturally existing bacteria in the soil on the self-healing process within the considered incubation period.

Keywords

Bio self-healing; Mortars; Soils & ground conditions; Cracks.

List of notations

e	is Void Ratio
EDX	is Energy Dispersive X-Ray Spectrometry
G	is Specific Gravity
p_c	is capillary pressure
RC	is Reinforced Concrete
RH	is Relative Humidity
S	is Degree of Saturation
SEM	is Scanning Electron Microscope
u	is the pore-water pressure in soil
W_f	is the average final crack width
W_i	is the average initial crack width
γ	is the unit surface tension force per unit length for water
ρ	is the bulk (moist) density of soil
ρ_w	is the density of water

1. Introduction

Reinforced concrete used in structures such as bridges, tunnels and buildings, usually suffers from cracks leading to early deterioration and shorter service life (Mihashi and Nishiwaki, 2012). For repairing microcracks and achieving longer maintenance-free service life, significant research works have been conducted to explore techniques developed around the concepts of self-healing (Schlangen and Sangadji, 2013, van der Zwaag, 2007, Dry, 1994). A wide range of approaches is currently available (Souradeep and Kua, 2016, Gupta et al., 2017) and can be generally subdivided into two major categories: (i) Autogenous (e.g. Edvardsen, 1999), and (ii) Autonomous (e.g. Van-Tittelboom and De-Belie, 2013).

The autogenous approaches are aimed at improving the natural mechanism of cracks healing due to ongoing hydration of clinker minerals or carbonation of calcium hydroxide ($Ca(OH)_2$) (Li and Li, 2011, Ter-Heide and Schlangen, 2007), while in the autonomic approaches the concrete cracks are self-healed by adding agents. Depending on the type of added agents, autonomous approaches can be classified into two categories: (i) healing by chemical agents and (ii) healing by bacterial agents (Souradeep and Kua, 2016, Van-Tittelboom and De-Belie, 2013, Gupta et al., 2018). In particular, the precipitation of calcium carbonate ($CaCO_3$) induced by bacteria (referred to as bio self-healing throughout this paper) has been regarded as an environmental-friendly and economic solution (Gupta et al., 2017, Tziviloglou et al., 2017).

The literature has indicated that the efficiency of self-healing is highly dependent on the incubation conditions and the research have mainly focused on the self-healing process within humid air and water environments. However, almost all infrastructures are built on/in the ground, where concrete is inevitably embedded in different challenging environments. It is not clear if self-healing can be activated in soil and whether such healing is comparable with the conventional incubation environment (i.e. water). Moreover, the effect of the naturally existing bacteria within soil on the self-healing process is unknown. To start addressing these uncertainties, laboratory experiments were conducted in this study on mortar specimens (with and without added bacterial agents) and these specimens were incubated in three different environments: natural soil (saturated and unsaturated) as well as water. Based on the experimental results, the potential effect of pore-water pressure of the soil, capillary pressure of the cracks and bacteria (artificially introduced in the specimens or naturally presented in the incubation

environment) on the self-healing mechanism was discussed. Recommendations were provided to explore new directions in the application of self-healing concrete in soil, which are highly relevant to structural and ground engineering in the construction industry.

2. Laboratory incubation environments

The majority of research works on self-healing have been conducted in laboratories. After generating cracks, concrete specimens are exposed to certain incubation conditions, representing the surrounding environment of application or compatible with the healing compositions. The incubation condition for concrete specimens is a significant factor for the mechanism of bio self-healing. In most of the previous research work, successfully healed specimens have been either fully submerged in water for the entire incubation period or subjected to wet-dry cycles. Table 1 summarises the different healing incubation conditions and the healing efficiency achieved in different studies.

Wang et al. (2014a) investigated the self-healing efficiency by subjecting the cracked samples to wet-dry cycles for four weeks. The specimens were alternately immersed in tap water for 1 hour and then exposed to humid air with 60% RH for 11 hours at a temperature of 20°C. Even though the contact time of specimens with water was decreased to 2 hours per day, a complete crack sealing was achieved. Kalhori and Bagherpour (2017) studied the effect of three different incubation conditions on concrete compressive strength namely water, reactive solution and suspension of bacteria, urea and calcium chloride. The results revealed that the compressive strength at ages 7, 14, and 28 days for specimens immersed in suspension of bacteria were greater than those submerged in water and reactive solution where the compressive strength of 34.4 MPa for 28-day old specimens was approximately 30% higher than of specimens (with the same age) submerged in water.

The crack healing capacity of bacteria-based techniques has been also investigated in a low-temperature marine environment by Palin et al. (2017). The samples were submerged in artificial seawater at a temperature of 8 °C for 56 days. The results showed that the permeability of cracks of 400 μm and 600 μm width was decreased by 95% and 93%, respectively. In another study, Xu and Yao (2014) investigated the efficiency of self-healing process for specimens submerged in a medium containing bacterial spores, yeast extract and calcium source. The results showed that crack width ranging from 100 to 400 μm was completely sealed by calcium carbonate.

Luo et al. (2015) investigated three different incubation conditions namely: (i) water (ii) humid air with 90% RH; (iii) wet-dry cycles. The temperature for the three incubation conditions was 25°C. For the incubation of wet-dry cycles, the specimens were alternately immersed in water for 12 hours and exposed to air for 12 hours. The healed cracks were observed for specimens incubated in water and dry-wet cycles, whereas no clear healing was noticed for the specimens incubated in dry condition.

Table 1. Overview of crack healing in cementitious materials in different incubation conditions

Type of specimen / Incubation condition	Incubation period (weeks)	Crack width healed (μm)	Remarks	References
Mortar / incubated in Water with wet-dry cycles	4	Up to 500	Water permeability was decreased by 68%	(Wang et al., 2014a)
Cement paste / Tap water exposed to atmosphere with an adequate supply of CO2	-	400	Precipitate formed at an early age of 7 days showing excellent healing ability.	(Qian et al., 2015)
Mortar / Water with wet-dry cycle	8	Up to 970	No self-healing was observed for specimens incubated at 95% RH.	(Wang et al., 2014b)
Mortar / Closed jar with RH more than 90%	-	Not Applicable	-	(Wang et al., 2012)
Concrete / Tap water	4	Up to 790	The optimum healing was achieved after 28 days of incubation.	(Zhang et al., 2017)
Mortar / Wet-dry cycles	4	Up to 417	Water tightness was increased by 50%.	(Xu and Wang, 2018)
Mortar / Urea and demineralized water	4	450	-	(Da Silva et al., 2015)
Concrete / Tap water at room temperature.	2	150	-	(Jonkers, 2011)
Mortar / Medium containing bacterial spores, calcium source and yeast extract.	-	100 - 400	-	(Xu and Yao, 2014)

Mortar / Artificial seawater	8	400 - 600	Water permeability decreased by up to 95%	(Palin et al., 2017)

As can be seen from Table 1, the technique of bio self-healing concrete has been tested in different incubation conditions but largely in water and humid air. The best results have been achieved in water (Luo et al., 2015) and this might be attributed to a number of factors included the presence of moisture, dissolved oxygen, and partial pressure of carbon dioxide.

3. Environmental exposures of concrete structures

Reinforced concrete (RC) is used for a broad range of structures including tunnels, bridges, buildings, retaining walls and dames (Figure 1) and these structures can be exposed to different environments. Some structures such as tunnels are completely built within soil, while other structures (such as buildings and bridges) typically have two parts, super-structures exposed to air as well as sub-structures located below ground level. Coastal and offshore structures are typically exposed to the seawater environment.

The environments surrounding these structures have different physical and chemical properties, which may pose a risk of corrosion affecting the RC durability. The risk of corrosion or attack associated with environmental conditions is considered in construction regulations and concrete design codes. In the UK, the Construction (Design and Management) Regulation (Summerhayes, 2016) states that chemical exposure risks must be assessed and managed. Therefore, it is essential for any future development of the bacterial based self-healing concrete to consider the effect of environmental conditions.

Carbonate precipitation and biogenic mineral formation, in general, is highly influenced by environmental conditions such as pH, temperature, and availability of nucleation sites. Microbes can influence many relevant parameters and in the case of superstructures (above the ground), the deliberately introduced microbes may exert considerable control over physicochemical conditions in small water-filled fissures. However, below ground surface, the situation is likely to be much more complex and influenced by both the surrounding soil chemistry and the organisms within the soil. Therefore, it is not clear how bio self-healing of concrete will work below ground, and the relative contributions of deliberately encapsulated bacteria compared to indigenous soil microbes is unknown.

Although research to date has focused on the self-healing process in humid air and water environments, in reality almost all structures are built on or in the ground (see Figure 1). Thus, a significant amount of concrete structural elements is exposed to a wide range of ground conditions, e.g. different soil types, groundwater regimes, chemical and bacterial compositions naturally existed within the ground. Moreover, these ground conditions might change during construction and over the entire design life of the structures. Research in this area is necessary because in concrete structures below ground surface, cracks are invisible, surrounded by soil and their location cannot be accessed. In particular, research is required to understand how ground conditions could influence the bio self-healing process and whether an adjustment should be adopted to achieve the required efficiency.

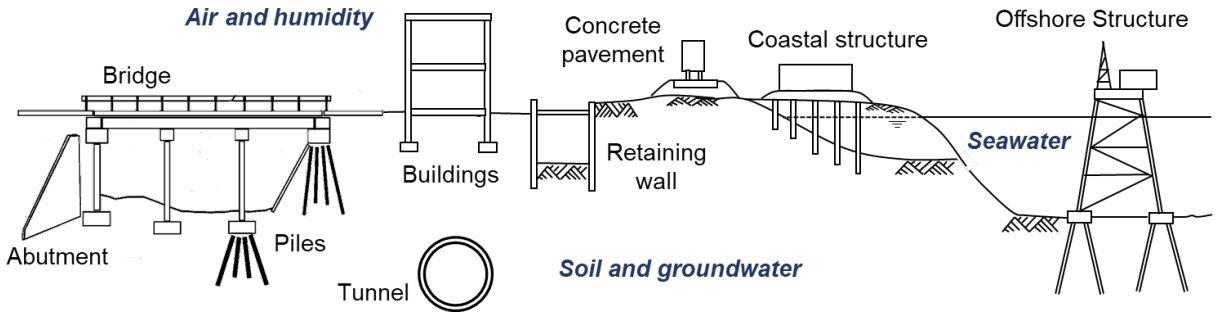

Figure 1. Structures and their environmental exposures

4. Experiments of bio self-healing mortar incubated in soil: Method and Materials

4.1. Types of specimens and incubation environments

In this study, six different types of mortar specimens were prepared as shown in Table 2. The first three types (A1, A2, and A3) were typically mixed with bio self-healing agents but incubated in different mediums i.e. partially-saturated natural soil (A1), fully-saturated natural soil (A2) and tap water (A3). The aim of these particular experiments was to examine if the bio self-healing process can be activated within pre-cracked mortar specimens under partially and fully saturated natural soil and whether the healing efficiency is comparable with conventional incubation environment i.e. within water.

The other three specimens' types (B1, B3 and B3) were similarly incubated in the partially-saturated soil (B1), fully-saturated soil (B2) and tap water (B3) however these specimens did not include any self-healing agent. The aim of this part of testing was to examine the natural mechanism of cracks healing

which might be developed by the naturally existing bacteria within the soil as well as the ongoing hydration of clinker minerals within the mortars.

Table 2. Types of specimens used for the experimental study

Group ID	Specimens ID	Bio self-healing agent	Incubation medium	Purpose of the test
1	A1	added	Partially sat. soil	Main tests
	B1	Not added	Partially sat. soil	Main tests
2	A2	added	Fully saturated soil	Main tests
	B2	Not added	Fully saturated soil	Main tests
3	A3	added	Water	Control tests
	B3	Not added	Water	Control tests

Among the six different types of specimens, four of them (A1, A2, B1, B2) represented the main tests (soil incubation), while the remaining specimens (A3 and B3) served as control tests (water incubation). Three specimens for each type were produced making a total number of eighteen. More specimens could have been produced and tested in this study; however as the experiments generated consistent results, the number of specimens was found to be adequate.

4.2. General procedures

The methodology adopted for the experiments generally followed the procedures shown in Figure 2. After casting and curing the mortar/cementitious specimens (mixed with the selected self-healing agents), a preliminary level of pre-cracking (damage) is induced by a controlled way. The specimens were placed in different incubation environments for 4 weeks. The efficiency of the self-healing was evaluated by means of quantitative and qualitative techniques, where the cracks were scanned by SEM/ EDX and measured under microscope before and after incubation. Particular aspects of the selection process and experimental procedures are highlighted as follow.

4.3. Preparation of bacterial spores

Bacillus subtilis H50620/9 (supplied by Philip Harris, UK) was selected for the study since this genus has the ability to form resistant long-lived spores (Kalhori and Bagherpour, 2017, Pei et al., 2013) as

well as the ability to produce calcium carbonate. To produce the spores, the bacterial strains were cultivated in Basal medium 121 and its derivatives 121A and 121B as described by Sonenshein et al. (1974). The composition of bacteria growth was 8 g of nutrient broth (Difco), 1 g of Potassium chloride (KCl), and 0.25 g of magnesium sulphate (MgSO4 7H2O) per litter adjusted to pH 7.0 to 7.2. The culture was incubated in a shaker at 120 rpm at a temperature of 36°C for 72 hours until the formation of spores was observed. Spore formation was confirmed under a microscope (LABOPHOT-2, Nikon) using the gram and spore Stain methods. To minimize the presence of vegetative cells, spores were harvested with the use of a centrifuge machine, where the culture was spun at very high speed (3390 RCF) for 10 min and then washed twice using distilled water. The centrifugal force causes heavier particles to move away from the axis of rotation, resulting in the deposition of spores at the bottom of the test tube forming what is known by a pellet. The spores were then encapsulated with nutrition in calcium alginate as follows.

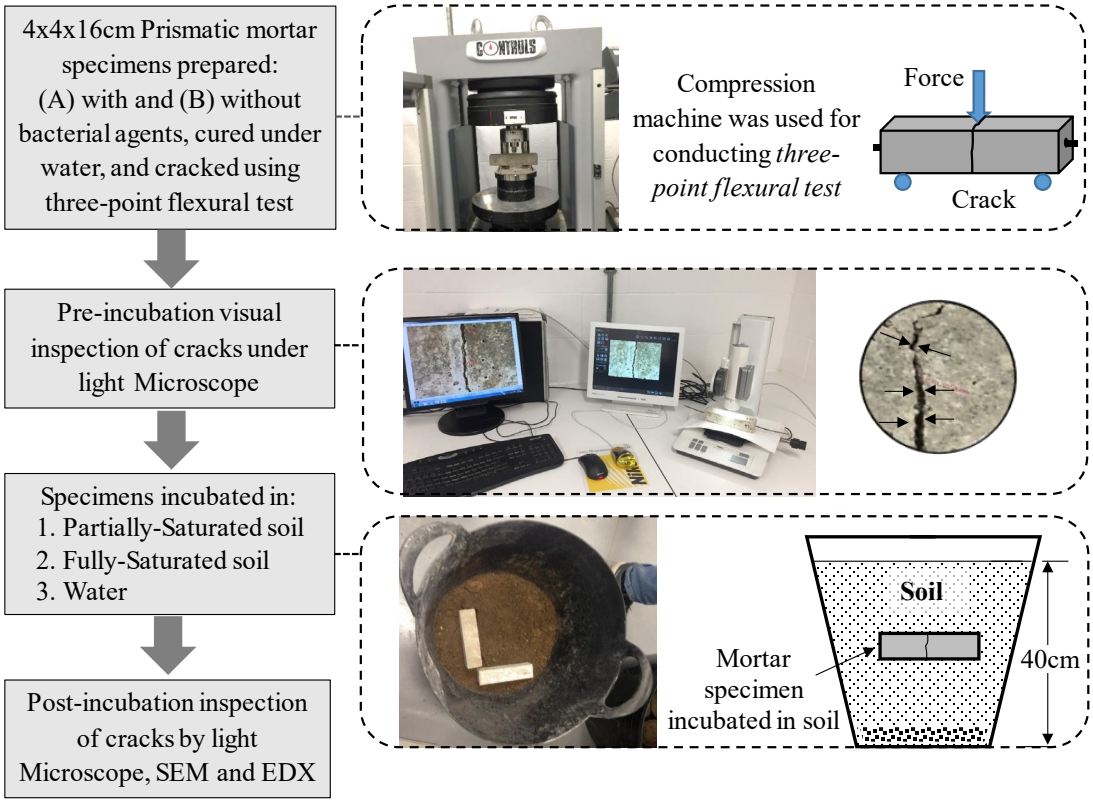

Figure 2. The process of the experimental work adopted in this study

4.4. Encapsulation process

To protect the bacterial spores from the harsh environment of fresh concrete such as high pH and temperature, bacterial spores have been encapsulated in most studies conducted on self-healing. In our experimental work, calcium alginate was used in a similar way adopted by Palin et al. (2016) to encapsulate the bacteria and nutrition. A solution was prepared using 7.5 g of sodium alginate, 0.5 g of yeast extract, 7.8 g of hydrochloride (as alkali buffer - 0.1 mol L^{-1}) and the bacterial spores (6.1 x 10^6 CFU mL^{-1}). The solution was mixed homogeneously using a magnetic stirrer to form a 1.5 % bacterial sodium alginate solution. The solution was manually dropped via syringe into a coagulate solution consisting of 8 g of calcium chloride and 4 g of calcium lactate to form the calcium alginate beads as shown in Figure 3. After 20 minutes, the formed beads were removed from the calcium chloride solution, washed twice using sterilized water, and dried at 37℃ for 24 h. The particle size of the produced capsules was approximately 150 µm.

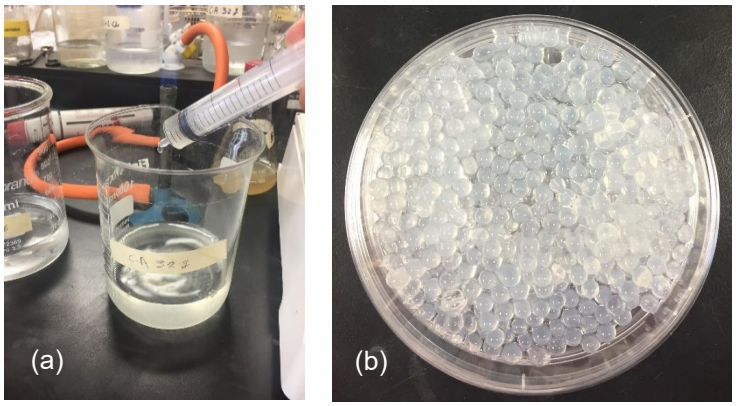

Figure 3. (a) Encapsulation process of Calcium Alginate Beads (CAB), (b) Calcium Alginate Beads (CAB)

4.5. Mixture of mortar specimens

The mortar specimens were prepared by mixing Portland cement (CEM II/B-V 32.5R), sand, and tap water in accordance with the mixture proportions indicated in Table 3. For specimens A1, A2 and A3, the self-healing agent (i.e. bacteria encapsulated in calcium alginate beads) was added afterwards and mixed by a digital mortar mixer until the mixture became homogenous. The mixture was cast in prismatic moulds, with dimensions of 4 x 4 x 16 cm, and placed on a vibrating table to remove any trapped air. During this process, each specimen was reinforced by a single axial steel bar (5mm diameter) to prevent

full breakage during the creation of crack in the next stage. Similar mixture and preparation procedures were adopted for specimens B1, B2 and B3 but without adding the self-healing agent.

Table 3. Mixture proportions (mass-ratio) of the mortar specimens A1-A3

Cement	Sand	Water	Self-healing agent*(%)
20 %	65 %	10 %	5.0 %

* bacteria encapsulated in Calcium alginate beads

All specimens were removed from the moulds after 24 hrs and cured in water for a duration of 28 days. However, in usual field conditions, such prolonged water curing may not be available for subsurface structural elements. Moreover, concrete might develop cracks at early stage due to soil pressure or other mechanical and environmental factors. Nevertheless, the curing approach adopted for this study may resemble the curing condition of pre-cast concrete.

4.6. Generation of crack and evaluation of healing ratio

After the curing period, all specimens were lightly cracked using three-point flexural test (see Figure 2) and the generated cracks were inspected under a light microscope (Nikon, Japan) to measure the average initial width (W_i). In preparation for the microscopic imaging, each crack was marked up at 6-8 positions distributed uniformly along the crack length. In each image, crack width at each position nearby the marker were measured using Shuttlepix Editor software. The average value of the initial cracks' widths for all specimens was approximately 300μm with a maximum standard deviation of 120μm.

Further visual inspection of the cracks was conducted at the end of the incubation period to measure the final crack widths and evaluate the crack healing performance. The efficiency of crack healing for each specimen was evaluated using the healing ratio calculated by the following equation:

$$Healing\ Ratio\ \% = \frac{W_i - W_f}{W_i} \times 100 \qquad (1)$$

where W_i and W_f are the average initial and final crack width, respectively.

4.7. Characteristics of the incubation environments

The pre-cracked specimens were subdivided into three groups and each group was tested in different incubation environments (see Table 2): (i) partially-saturated soil, (ii) fully-saturated soil, and (iii) water.

(i) Specimens incubated in partially-saturated soil

The first group of the pre-cracked specimens (A1- with bacteria and B1- without bacteria) were incubated in the middle of 40 cm thick fine-grained soil as shown in Figure 2 for a total duration of 4 weeks (28 days). The soil contained mainly natural alluvial deposits, which was manually sourced from *Sturgess Field* (Derby, UK). The natural moisture content (m) was approximately 24±2 (%) as determined by conducting oven-dry tests on 5 representative samples.

In addition to the moisture content (m), it was necessary to determine the degree of saturation (S) as this would give a better indication of the water content (in terms of the volume) at a scale ranging from zero to 100%, i.e. S is equal 0 when the soil is dry and 100% when it is fully saturated. For calculation purposes, the degree of saturation (S) is given as a function of other soil properties, including the void ratio (e) and specific gravity (G) using Equation (2) (Craig (2004):

$$S = \frac{m\,G}{e} \qquad (2)$$

Furthermore, the void ratio (e) can be given by the following equation (Craig (2004):

$$e = G(1+m)\frac{\rho_w}{\rho} - 1 \qquad (3)$$

where ρ_w = the density of water and ρ = the bulk density of soil. By incorporating Equation (3) into Equation (2), the degree of saturation can be calculated from:

$$S = \frac{m\,G}{G\,(1+m)\frac{\rho_w}{\rho} - 1} \qquad (4)$$

To determine the degree of saturation (S) of the soil, the moisture content (m), bulk density (ρ), and specific gravity (G) were experimentally examined. Summary of these properties is shown in Table 4. The experiments were conducted on samples recovered by Bulk Sampling Method and tested according to the British Standard BS1377 (BSI, 1990).

Table 4. The properties of the natural soil used for incubating the mortar specimens

Soil properties	**Ave Value (unit)**	**Method of testing**
Moisture content (m)	24 ± 2 (%)	Oven-dry method*
Bulk density (ρ)	1835 (kg/m^3)	Core cutter method*
Specific gravity (G)	2.67	Bulk density divided by density of water
Degree of saturation (S)	79.7 (%)	Based on Equation (4)
pH value	7.05	Measured by pH meter

Content of sand	61.2 (%)	Particle Size Distribution determined by Sieve test*.
Content of silt	17.3 (%)	
Content of clay	19.6 (%)	
Organic content**	1.9 (%)	

** according to BS1377 (BSI, 1990)*; ***decaying plant and animal material*

Alluvial deposits typically contain a variety of materials, including fine particles of silt and clay and larger particles of sand and gravel in addition to some organic matter (Fookes, 1997). This was confirmed by conducting Particle Size Distribution (PSD) testing as presented in Table 4. The alluvial soil used for the experiments comprised soft to firm dark brown silty sandy clay with a small portion of organic matters. The alluvial clay would contain a wide range of bacteria naturally existed within the ground and had a neutral pH value ranging between 6.5 and 7.6, which was confirmed by using a pH meter.

(ii) Specimens incubated in fully-saturated soil

The second group of the pre-cracked specimens (A2 and B2) were also incubated in the same type of soil but in different containers where the degree of saturation was increased to 100% to create fully-saturated incubation condition – in accordance with the experimental programme (Table 2). To bring the soil from the partial to full saturation, tap water was gradually added to the soil container in a controlled manner. The soil container was covered and left overnight to allow the water to dissipate and distribute evenly. A thin layer of granular material was placed at the bottom of the soil containers to allow any excess water to drain freely creating a reserve which can be re-used by the soil if it starts to dry.

(iii) Specimens incubated in water

The third group of specimens (A3 and B3) were incubated in tap water to serve as control tests and this water had pH values ranging between 6.6 and 7.5. Previous research conducted by (Zhang et al., 2017) has shown that the optimum healing in tap water was achieved after 28 days of incubation. Therefore, this incubation period was used for all tested specimens.

4.8. Soil moisture content and suction stress

In general, the water in the voids of saturated soil is under pressure (positive pore-water pressure, *u*), whereas partially-saturated soils (particularly fine-grained soils such as clay and silt) can develop negative pore-water pressure i.e. suction stress. The suction value depends primarily on the moisture content and increases with soil hydration (Hamza and Ikin, 2019). For some of the tests, the soil was

planned to be fully-saturated during the experiment, therefore it was important to find out the minimum moisture content at which the pore-water pressure would be positive or approximately within the normal, 'equilibrium', values (u = 0). This was determined by examining the soil-water characteristic curve (SWCC). Although this has been considered as a rough guide, a tensiometer was used in the test to measure the pore-water pressure (u) and ensure the soil has no suction, i.e. u = almost zero. The soil suction was monitored using UMS Miniature-Tensiometer T5 connected to a compatible data logger. The probe was positioned within close proximity to the cracks where it could measure the soil matric potential. In addition, damp cloth and plastic sheet were placed on the top of the soil container to avoid surface evaporation.

4.9. SEM and EDX scanning

At the end of the incubation period of 4 weeks, the specimens were removed from the incubation environments (soil and water) to visually inspect the cracks' healing under the microscope. The precipitated material (in the cracks) was further inspected by Scanning Electron Microscope (SEM) and its chemical composition was examined by Energy Dispersive X-Ray Spectrometry (EDX). In preparation for the scanning works of specimens incubated in soil, it was necessary to conduct ultrasound cleaning (within the water) to remove any remaining soil particles. These specimens were then dried and broken along their cracks to scan the cracks' lips.

5. Result and analysis

5.1. SEM and EDX

Figure 4 shows the results obtained from the SEM and EDX scanning of one of the crack's lips taken from a specimen incubated in soil (A2). The thickness of the product precipitated on cracks' lips was measured during SEM scan (Figure 4) and it varied from 40 to 100μm with an average value of 65μm. Based on these measurements, the average total thickness of the materials precipitated on a single crack was about 170μm which is comparable with the results obtained from the specimens incubated in water (A3).

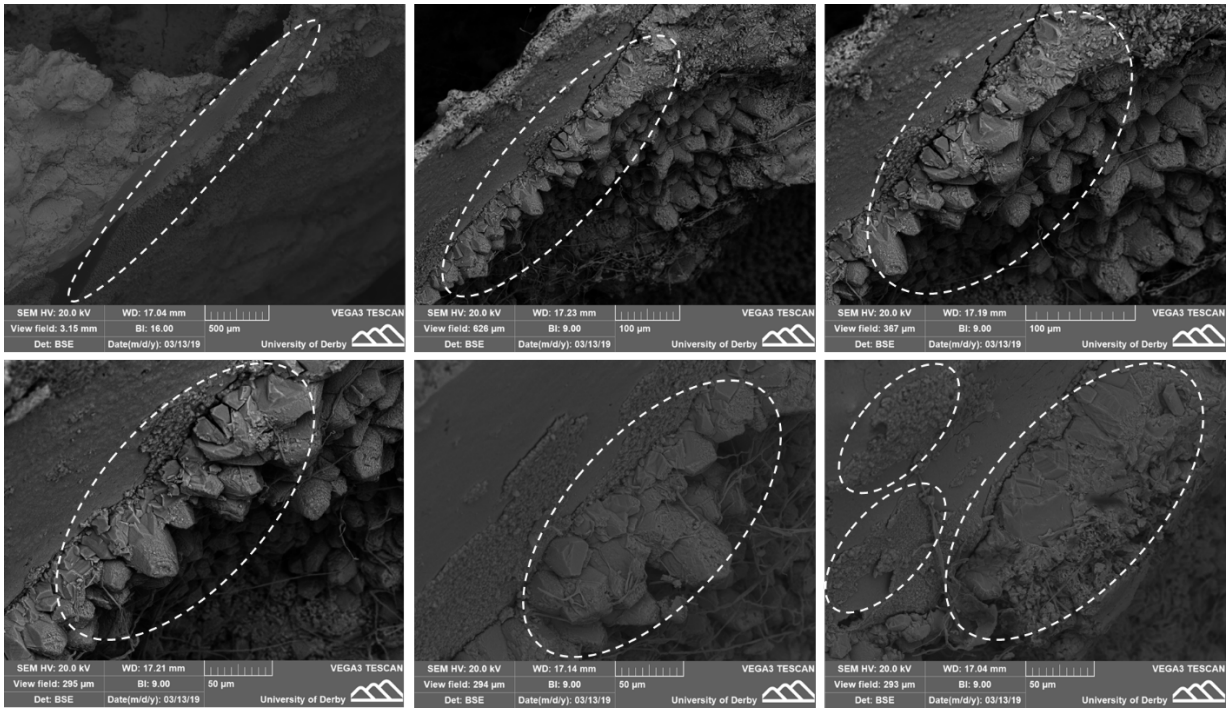

Figure 4. SEM results taken from specimens A2 (incubated in saturated soil) showing zones of precipitates at different scales and locations. These materials were confirmed by EDX analysis to be limestone crystals (see Figure 5)

Results of EDX analysis from specimen A2 (shown in Figure 5-a) demonstrated that the major elements of the precipitate are calcium, carbon, and oxygen. In contrast, the EDX results obtained from different spectrum located within the concrete showed different signature (Figure 5-b). It can, therefore, be concluded that cracks were sealed by precipitated calcium carbonate product ($CaCO_3$). The mineral precipitations of $CaCO_3$ is likely to be formed due to chemical equilibrium processes resulting from microbial respiration releasing carbon dioxide into a confined space (De-Muynck et al., 2010).

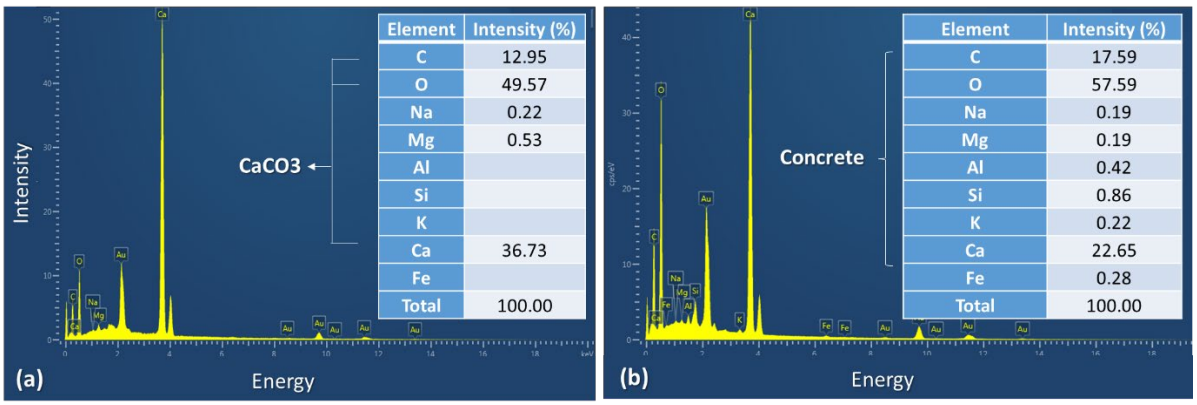

Figure 5. Typical EDX results obtained from different spectrums: (a) located within the precipitated materials, (b) located within the concrete

Distinct crystal sizes and shapes of hexagonal structure, and other phases with spherical shapes of the calcium carbonate were observed in both types of specimens A2 and A3 as shown in Figure 6. According to De-Muynck et al. (2010), the crystallinity and the size of the crystal phase can be influenced by the concentration of urea and calcium carbonate in the culture medium, where the crystal size and crystallinity become lower as the concentration of urea and calcium increases.

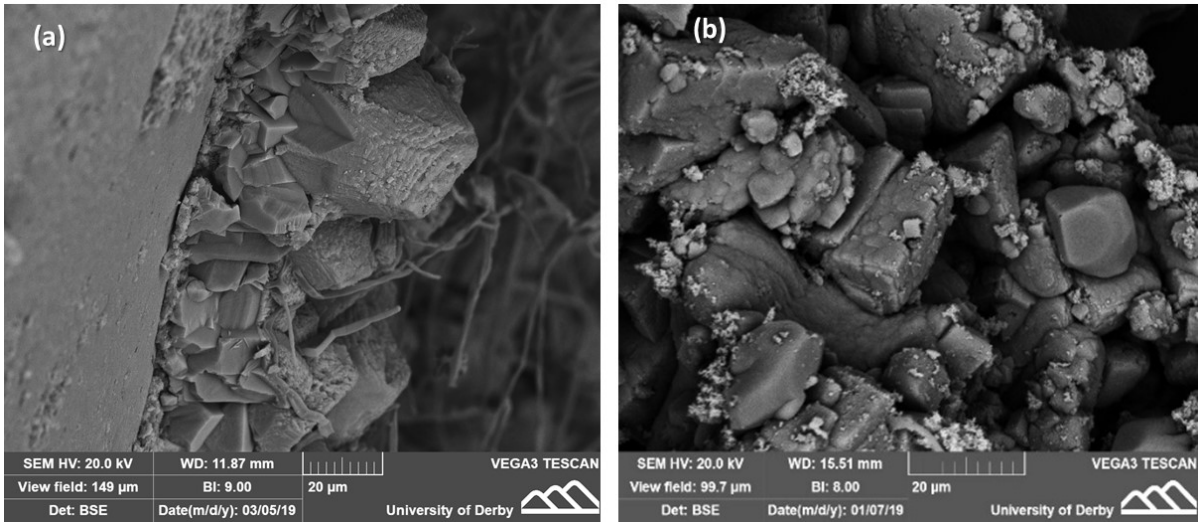

Figure 6. Result of SEM of the precipitated product in two healed crack taken from (a) Specimen A2 and (b) Specimen A3

5.2. Evaluation of healing ratio

(i) Bacteria-doped specimens

The efficiency of crack healing was evaluated for all specimens using Equation (1) and presented in Figure 7. For the mortar specimens with self-healing agents (i.e. specimens A1, A2 and A3), the healing ratio for fully-saturated soil incubation (A2) was found to be comparable with the results obtained for water incubation (A3). Specimens A1, which was incubated in partially-saturated soil, did not show any significant healing despite being doped with bacteria. However, it was also noticed that the healing ratio in the other bacteria-doped specimens (A2 and A3) did not reach 100%, particularly along the larger cracks. This might be attributed to the limited incubation time as well as the encapsulation method of calcium alginate, which has been reported in some cases with a similar result (Palin et al., 2017, Alazhari, 2017). Further future trials should assess the impact of different encapsulation methods and time scale on healing efficiency.

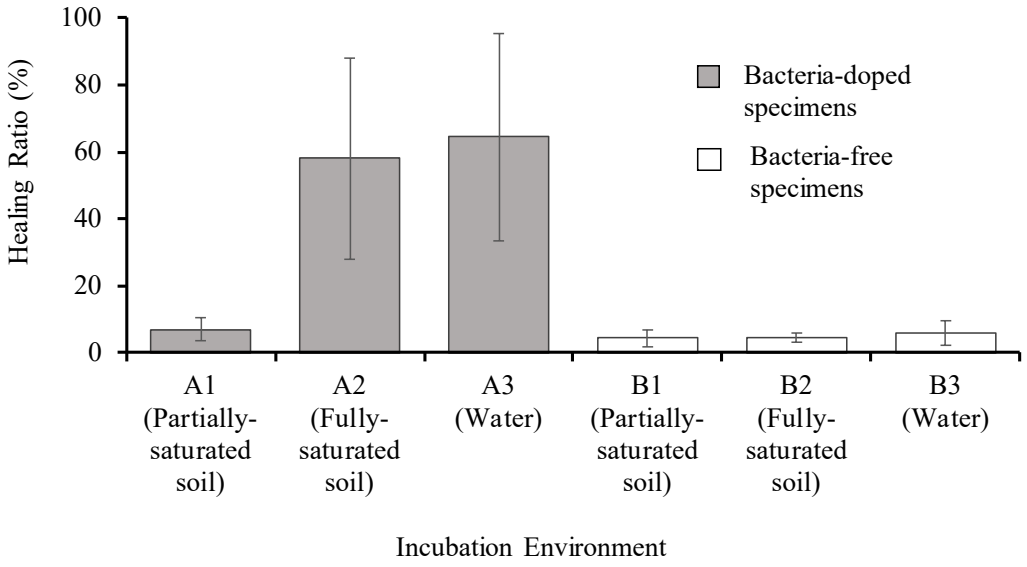

Figure 7. The evaluated healing ratio of the six types of specimens

(ii) Bacteria-free specimens

The bacteria-free specimens (B1, B2 and B3) did not show any significant crack sealing improvement after the 4 weeks of incubation in soil and water. The observed slight healing can be attributed to the autogenous healing process, particularly along narrow cracks. This implies that only the specimens impregnated with bacteria and provided with fully-saturated incubation environment had experienced much notable self-healing within the incubation period.

5.3. Healing mechanism in soil

(i) The effect of soil suction and crack capillary pressure

The healing mechanism in specimens A2 (incubated in fully-saturated soil) can be explained as follow. As the bacteria-based beads were broken along the pre-existing crack, bacteria and nutrition were expected to be concomitantly freed up. Therefore, the bacteria would be exposed at the crack surfaces and activated as soon as they became in contact with water ingress from the surrounding fully-saturated soil. This is supported by the Tensiometer measurements (Figure 8) where the values of matric suction in the soil were generally around the equilibrium (i.e. $u \approx 0$). In contrast, there was significant suction in the partially-saturated soil of specimens A1. In such condition, the soil might have attracted water molecules which became less available for the bacteria in cracks to maintain the calcium carbonate production. This might explain the significantly smaller healing ratio experienced by specimens A1 in

comparison with specimens A2 (Figure 7) where water is expected to be readily available throughout the test.

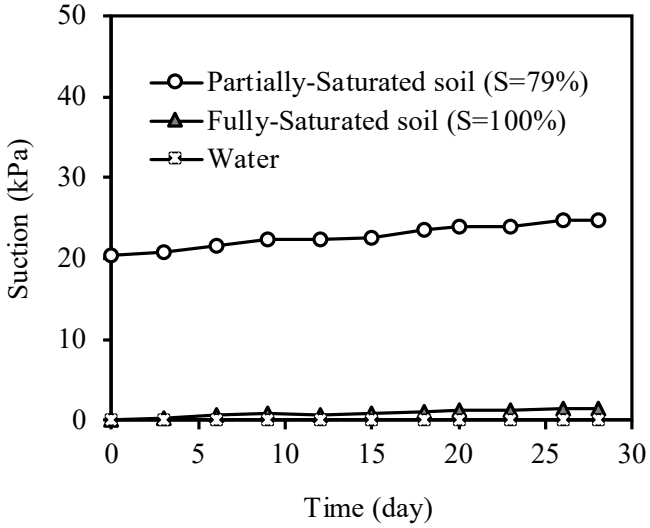

Figure 8. Tensiometer measurements showing the suction (negative pore-water pressure) in the incubation environments. S is the degree of saturation of soil.

The availability of water for the bacteria in cracks are likely to be controlled by the balance between two pressures: (i) matric suction of the soil and (ii) capillary pressure generated in the specimens' cracks. Based on the simplified system illustrated in Figure 9, the capillary pressure (p_c) can be approximately estimated using the following equation (Schneider et al., 2017):

$$p_c = \frac{\gamma \cos\theta}{d} \qquad (5)$$

where γ is the unit surface tension force per unit length for water (i.e. typically 0.073 N/m at 20°C), and θ is the contact angle of the wet surface with the crack surface. It is important to note that this equation has originally been developed for *cylindrical* capillary in a tube where d is the radius of the tube. Using d equal to the average cracks' width, the estimated value of capillary pressure (p_c) can reach up to 2.0 kPa, which is far less than the matric suction recorded in the partially-saturated soil (A1). This indicates that the cracks in specimens A1 might have failed to obtain an adequate amount of water to maintain the healing process as evidenced in the poor healing ratio of less than 5% (Figure 7).

In contrast, the measured suction in the fully-saturated soil (A2) was much smaller than the capillary pressure (p_c) of the cracks. Therefore, the availability of water for the healing process in specimens A2 did not seem to be affected by suction. However, during the last week of testing, the soil experienced a

slight increase in suction as the soil would be expected to lose some moisture through evaporation. This might have slightly affected the healing in specimens A2 in comparison with A3 (Figure 7), where water was readily available.

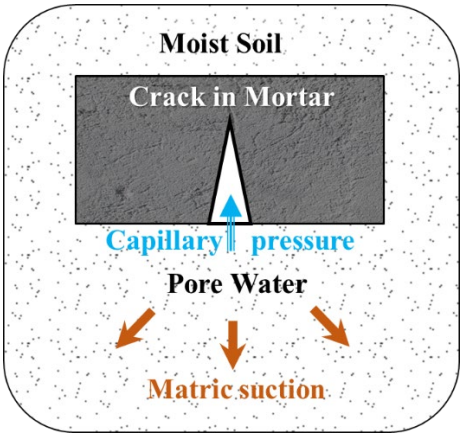

Figure 9. Schematic diagram of the pressures controlling the soil pore-water adjacent to the mortar crack.

(ii) The effect of bacteria naturally presented in the incubation environment

Bacillus subtilis (introduced in the mortar specimens) is one the species of genus *Bacillus*, which is the most commonly encapsulated bacteria in self-healing concrete formulations. *Bacillus* also includes *B. sphaericus* and *B. cohnii* (Wang et al., 2012, Wang et al., 2014a, Zhang et al., 2017) although other genera are used. *Bacillus* species are also common spore-forming bacteria found in all natural soils and are not unique in their ability to precipitate minerals, however, based on our experimental results there was no evidence suggesting the influence of such bacteria on the specimens incubated in soil (specimens B1 and B2).

The experimental results indicated that only the mortar specimens impregnated with bacteria had notably experienced self-healing within the testing duration. The potential healing effect of the naturally existing bacteria in soil might require longer incubation time and additional nutrient source, which could be provided within the cementitious mixture. However, due to the scope of our experimental work, it was not possible to prove such a hypothesis.

6. Conclusion

The study has shown that bio self-healing of concrete generally proceeds in a similar fashion within saturated natural soil as has been previously reported for concrete incubated in humid air and water. However, this was found to be particularly applicable when the soil pore-water pressure is positive or near-equilibrium. The study revealed that partially-saturated fine-grained soil can develop suction, which may overcome the capillary pressure of the cracks and hence disrupt the water ingress through cracks slowing down the bacterial production of calcium carbonate.

The experimental work used mortar specimens impregnated with *Bacillus subtilis* spores in order to be comparable with the existing knowledge on bio self-healing. However, since *Bacillus* species are naturally occurring soil microbes, it was initially anticipated that this impregnation would not be necessary in order to realise the self-healing for the mortar specimens incubated within natural soil. Nevertheless, the experimental results did not support this hypothesis, which remains to be further investigated.

This study provides a good basis for future investigation into the bio self-healing of concrete for underground structures, which can be exposed to a wide range of ground conditions. The scope of this experimental work focused on a specific type of soil with two saturation regimes. However, further experimental investigations are required to determine the efficiency of bio self-healing in different ground conditions. In particular, research is required to understand how such ground conditions could influence the bio self-healing process and whether an adjustment should be adopted to achieve the required efficiency. The study might also benefit from further research investigating the possible interaction between the naturally existing bacteria in the soil and the bacteria artificially introduced in the cementitious material.

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