INDUSTRY CORNER
CRACK HEALING UTILISING BACTERIAL SPORES IN CONCRETE

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ABSTRACT
This self repair system is based upon harmless ground borne bacteria as the self-healing agent. The bacteria are activated after the concrete is cracked and the bacterial spores are exposed to moisture and air. The bacterial reproduction process creates a calcite by-product which fills the cracks in the concrete. By sealing the cracks in concrete, an effective barrier to air or liquid borne deleterious materials are formed and as a consequence of this, enhanced durability is achieved in the structure, resulting in lower life cycle costs.

The concrete/mortar prisms were cracked and tested for water flow. They were then left for 56 days to heal and were subject to a test for water tightness. Healing was observed and a reduced water flow (74\% and 32\% healed) measured with the healed samples when compared to the specimens that were cracked and subjected to a water flow test without any healing agent. The number of samples were limited and a larger scale test is recommended for further work; however, this is a proof of concept of the process of healing and testing.

KEYWORDS
self-healing, bio-based mortar, micro-cracking, light weight aggregates, liquid-tightness

1. INTRODUCTION
Bacteria are abundant, incredibly diverse, and conduct precipitation of mineral carbonates across a spectrum of natural environments (Siddique and Chahal, 2011). The majority of bacteria are either spheres and known as cocci or rod shaped and referred to as bacilli (Siddique and Chahal, 2011).

In nature, it is common for microbial mineral plugging to occur in porous media (Stocksfischer et al., 1999). Bio-calcification or microbiologically induced calcite precipitation (MICP) is a phenomenon concerning the urease enzyme (Sarda et al., 2009). Microbial CaCO\textsubscript{3} has wide scope, as it has a varied range of environmentally friendly applications. It can consolidate damaged materials, especially ones bearing cracks (Wang et al., 2014). MCIP is a natural phenomenon which is associated with a range of bacteria species given the right conditions, in particular, an alkaline environment rich in Ca\textsuperscript{2+} ions (Achal and Pan, 2011). The calcite deposition is able to consolidate media and potentially reduce moisture ingress.
The purpose of this paper is to investigate the effectiveness of the self-healing properties of bacterial mortar specimens. The original round robin tests carried out by Delft, Ghent, Bath and Northumbria Universities examined the test method for consistency and effectiveness of the self-healing process. This paper focusses upon the self-healing properties of the bacterial concrete (BAC).

Water flow through cracked bacterial concrete specimens was the basis of the testing programme using a paired comparison test to determine the degree of self-healing that took place over a 56 day healing period. The healed specimens were not water tested prior to the post healed condition in case the initial water flow washed away the bacterial spores, as these are the protagonist healing agent.

“The mortar prisms were prepared at the Technical University Delft (TUD) and were shipped to the other four laboratories at the age of 7 days. Three types of mortar mixtures were investigated: one reference mixture (REF) with normal weight aggregates, one control mixture (CTRL) with non-impregnated lightweight aggregates (LWA) and one mixture (BAC) with impregnated LWA with the bacteria based self-healing agent. For the three mixtures used: ordinary Portland cement (CEM I 42.5N, ENCI, The Netherlands), 0/4 mm sand or 0.125/1 mm sand and 1/4 mm LWA (expanded clay particles, Liapor 0/4 mm, Liapor GmbH Germany) were used. Bacterial spores were impregnated into diatomaceous earth and when the diatomaceous earth ruptures the spores are exposed to the air and moisture. This activates the spores and the resultant alkaphilic bacteria create a sealing medium of calcite. All specimens were cast in polystyrene moulds and were kept in sealed plastic bags until they were tested.” (Tziviloglou et al 2016a).

1.1. Mix design
The detailed mixture proportions are presented in Table 1. (Tziviloglou et al 2016)

1.1.1. Aggregate preparation
The bio-based healing agent consisted of spores derived from alkaliophilic bacteria of the genus *Bacillus* and organic mineral compounds. The healing agent was embedded in expanded clay particles, Liapor 1/4 mm (Liapor GmbH Germany) via impregnation under vacuum with calcium lactate (nutrient for the bacteria), yeast extract (vitamins for bacteria) and bacterial spores in a solution. The healing agent solution contained: calcium lactate (200g/L), yeast extract (4g/L) and bacteria spores (108 spores/L). (Tziviloglou et al 2015).

### Table 1. Mixing proportions of mortar specimens.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>CEM I (kg/m³)</th>
<th>Water (kg/m³)</th>
<th>0/1mm Sand (kg/m³)</th>
<th>1/4 mm Sand (kg/m³)</th>
<th>LWA 1/4 mm (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>463</td>
<td>231.5</td>
<td>810</td>
<td>810</td>
<td>0</td>
</tr>
<tr>
<td>CTRL</td>
<td>463</td>
<td>231.5</td>
<td>810</td>
<td>0</td>
<td>257</td>
</tr>
<tr>
<td>BAC</td>
<td>463</td>
<td>231.5</td>
<td>810</td>
<td>0</td>
<td>283*</td>
</tr>
</tbody>
</table>

*weight of LWA after the impregnation with the healing agent (assuming weight increase of 10%).
1.2. Test moulds and samples preparation
The specimens were manufactured using a mould in accordance with Figure 1.

2. TEST PROGRAMME AND METHODOLOGY
A series of test options were considered for the test procedure; however, the water flow test was deemed to be the most appropriate test available to illustrate the self-healing effect of bacteria in concrete. The pH level of the concrete needed to be in the region of 8–10 to activate the bacterial spores effectively. The test regime will create a material that has a pH level in excess of 11 initially when the initial casting is carried out. The pH level will reduce in a short period of time after casting to permit microbiological activity. It should be noted that an alkaline environment is essential for the bacteria to work effectively.

2.1 Figure 2 displays the test programme as carried out.

2.2 Upon crack formation, the weak lightweight aggregate break and the healing agent activates (Tziviloglou Wiktor, Jonkers, Schlangen, 2016).
The samples were then tested for water flow, and a further three samples were cracked and then submerged horizontally (on spacers of 5 to 10 mm) in a plastic bucket filled with 5 litres of tap water per three specimens in order to heal the cracks. The samples were left to heal for 56 days prior to the water flow test.
2.3 Water test
Figure 3 displays the schematic arrangement of the water flow test. This test is currently being developed as a new test standard by the RILEM technical committee 253-MCI.

The constant head water supply was fed into the centre of the concrete prism and the water passing through the crack was weighed after a test period of five minutes. The original test specimens were discarded in case the water flow had washed out the bacterial spores and cracked samples were used for the healed water flow test.

2.4 Specimen formation
The specimens were formed as displayed in Figure 4.

2.5 Alicona measurements
A 3D optical measurement system (Alicona Infinite Focus G4) was used to examine the surface of the cubes. Two-dimensional surface roughness ($R_a$, $R_s$), waviness ($W_a$, $W_s$), dimension and volume of surface pores were measured. The system has a resolution of 40 nm. The used measurement system is capable of providing complete 3D information through measurements of X, Y, Z coordinates for millions of points on the scanned samples.
**FIGURE 3.** Water flow test apparatus (Tziviloglou et al 2016a)

Nomenclature:
1—Constant head water container  
2—Plastic feed tube  
3—Connector  
4—Resin seal  
5—Electronic scale

**FIGURE 4.** Test specimen displaying wire reinforcement and a 5mm diameter central hole for water test. (Tziviloglou et al 2016).
3. RESULTS AND DISCUSSION
The results are listed in the chronological order that the tests were carried out, as displayed in Tables 2–7.

3.1 Flexural strength at 28 days
The flexural beam 3 point bending test broke the beams centrally and provided two specimens for the compressive test. Each broken specimen was labelled with the original beam number first followed by the half remaining, e.g. beam number one became broken halves 1.1 and 1.2. The standard deviation remains very low given the variable nature of concrete manufacture.

The flexural strength as displayed in Table 2 is not representative of the cementitious material, as the reinforcing wires have provided a flexural strength of 25% of the compressive strength. This is outside the normal range expected on unreinforced concrete where the flexural strength is in the region of 10–15% of the compressive strength.

3.2 Compressive strength at 28 days
The characteristic strength of the concrete (Table 3) given a 5% defective is 17.8 N/mm².

The compressive strength of the mortar mix as displayed in Table 3 is on the low side of commercially used concrete. This was due to the inclusion of clay aggregates.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Failure load (kN)</th>
<th>Flexural strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1948</td>
<td>5.14</td>
</tr>
<tr>
<td>2</td>
<td>2.2881</td>
<td>5.36</td>
</tr>
<tr>
<td>3</td>
<td>2.067</td>
<td>4.84</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>5.12</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>0.26</td>
</tr>
</tbody>
</table>

TABLE 2. Flexural strength.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Failure load (kN)</th>
<th>Compressive strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>68.8</td>
<td>20.39</td>
</tr>
<tr>
<td>1.2</td>
<td>54.7</td>
<td>18.12</td>
</tr>
<tr>
<td>2.1</td>
<td>63.8</td>
<td>19.74</td>
</tr>
<tr>
<td>2.2</td>
<td>66.2</td>
<td>21.18</td>
</tr>
<tr>
<td>3.1</td>
<td>62.1</td>
<td>18.63</td>
</tr>
<tr>
<td>3.2</td>
<td>63.9</td>
<td>20.92</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>19.83</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>1.24</td>
</tr>
</tbody>
</table>

TABLE 3. Compressive strength.
3.3 Crack initiation to prisms at 29 days
Displacement was measured and converted into a crack mouth opening value to attempt to create a 300 micron crack. W1 is the initial reading and W2 is the value once the load was released. W2 was confirmed with a crack width microscope.

3.4 Water flow permeability test at 29 days
The variability of the crack width at the surface did not necessarily reflect a constant crack width at the central hole running through the specimen. The aperture at the central hole was not directly measured and could only be assumed from the surface measurements. The crack was created from a central force creating a rotational movement which created an arc shaped crack being wider at the surface than in the centre. Table 5 displays water passing through the cracked specimen prior to healing taking place.

3.4.1 Water flow permeability test at 85 days
Table 6 displays water passing through the cracked specimen after a period of micro induced calcite precipitation.
Due to the differential between the initial cracked sample crack width and the initial healed sample crack width, a method of relating equal water flow performance to the crack width was required to standardise the results. This has been achieved by examining the water flow per micron as displayed in Table 7.

The water flow sealing capacity was calculated using Equation (1) and using the water flow per micron

\[ SE_{wf} = \frac{W_{ref\ cracked} - W_{healed}}{W_{ref\ cracked}} \]

Equation (1)

### 3.4.2 Rationalised flow data

Examining the average values determined a crack width for the 85 day specimens at 55% of the 29 day old specimens.

The 85 day specimens water flow is 53% less than the 29 day old specimens. Given that the sample size is small, it cannot be taken as a representative example of what might be considered a normal distribution. Examining the average values, it can be stated that there is no significant healing taken place.

However, the microscope observations clearly show a large degree of crack sealing/healing has taken place. Specimen 7 displays values which are not in keeping with the surrounding data as for a relatively modest crack width there is a large water flow. If specimen 7 is removed from the results, then the relative crack width and water flow are 46% and 17% reduced. These values are more in keeping with the observable results.

### Table 6. Water flow through healed samples.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Measured crack width (μm)</th>
<th>Mass of water passing through the crack after 5 minutes (g)</th>
<th>Water flow per micron (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>245.20</td>
<td>290.57</td>
<td>1.1850</td>
</tr>
<tr>
<td>8</td>
<td>107.64</td>
<td>25.40</td>
<td>0.2360</td>
</tr>
<tr>
<td>9</td>
<td>194.48</td>
<td>51.91</td>
<td>0.2669</td>
</tr>
<tr>
<td>Avg</td>
<td>182.44</td>
<td>122.63</td>
<td>0.6722</td>
</tr>
</tbody>
</table>

### Table 7. Sealing efficiency.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>SE_{wf}</th>
<th>Results/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.7443</td>
<td>This was removed as an outlier</td>
</tr>
<tr>
<td>8</td>
<td>0.3188</td>
<td>74% healed</td>
</tr>
<tr>
<td>9</td>
<td>0.3188</td>
<td>32% healed</td>
</tr>
</tbody>
</table>
The water flow per micron for each test is 0.7049 g/μ for 29 days and 0.67722 g/μ for 85 days. Taking out the considered outlier of sample 7, the values are 0.7049 g/μ and 0.2515 g/μ respectively. This data is more representative of the observable crack healing and sealing and is displayed in Figure 5.

### 3.5 Healed mortar prisms

All Figures produced were taken on the Alicona 3D scanning microscope, unless otherwise stated. Figure 6 displays a healed crack on beam 7. The deposition of bacterial formed calcite is self-evident. Section 3.5 relies heavily upon the graphical representation of healed cracks to inform the reader as to the observable effectiveness of the bacterial repair action.

Figure 8 displays the healed crack on beam 7 and the 2D and 3D details are taken across the line shown on the plan view of the healed beam.

Figure 9 displays the 3D build-up of calcite formed from bacterial action, filling the cracked beam (8).

Figure 10 was taken using a crack inspection microscope and it clearly shows a degree of self-healing has taken place on beam 8.

The sealed crack displayed on Figure 10 has been highlighted for ease of identification.

Figure 11 was taken using a crack inspection microscope and it clearly shows a degree of self-healing having taken place on beam 9, however the healing taken place at the surface is granular in texture and not totally sealed.

### 4.0 CONCLUSION

The water flow results show a marked reduction in water flow due to the sealing action of the bacteria. This result is confirmed with the Alicona scans and the optical crack microscope plates. The results concur with work by Tziviloglou Wiktor, Jonkers, Schlangen, (2016b) although the small number of samples was considered to be an area of improvement when the test is repeated.
FIGURE 6. Displays the healed surface profile of beam 7.

FIGURE 7. This figure was prepared by using a crack inspection microscope, and it clearly shows a degree of self-healing has taken place on beam 7 as the crack can be seen to be largely filled with calcite.
**FIGURE 8.** Healed crack.

**FIGURE 9.** The 3D build-up of calcite formed from bacterial action.

**FIGURE 10.** Displays healed crack on beam 8.
Another short coming of the test, was not testing the original water flow samples with the healed samples. This test procedure was deemed too random and a new procedure was sought to evaluate the effects of MICP as a self-healing mechanism. The second series of tests will use the same initial water flow samples as the final healed samples, thus reducing the variability.

ACKNOWLEDGEMENTS:
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5.0 REFERENCES