Analysis of non-axenic biomasses for self-healing concrete

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Abstract. As an alternative to the usage of axenic bacteria to achieve microbially induced calcium carbonate precipitation (MICP), this study evaluates the usage of two non-axenic biomasses as self-healing agents. A fungi-based consortium (Yeast) and heterotrophic nitrifiers (HTN) were harvested from the incubation of agricultural side streams. The characteristics of the two biomasses were identified through flow cytometry, total suspended solids and volatile suspended solids tests. The incorporation of the biomasses into concrete was evaluated in terms of compressive strength, flow and healing ability. Self-healing ability was analyzed through microscope imaging on prismatic (60x60x220 mm) samples. Cracks were induced with a three-point bending test where the widths were controlled with an LVDT sensor. A curing period of 56 days was applied to the samples and visual inspection was conducted at the start and end of the healing period with an optical microscope. Results compare and discuss the differing effects of active and autoclaved biomasses on the concrete properties and crack closure.

1 Introduction

Concrete structures suffer from the formation of microcracks which disrupts the robustness of the matrix. These cracks are difficult to detect and conventional repair methods are often ineffective. Self-healing concrete has been studied as a potential solution to this problem. This method eliminates the need for detection and manual repair of the crack due to the autonomous healing ability induced through an additive.

One of the efficient ways to obtain this healing is the addition of microorganisms. Microbially induced calcium carbonate precipitation (MICP) became a popular method due to its bio-based properties as well as effective crack-sealing ability[1]. The crack sealing is obtained through the agglomeration of the bio-minerals. The bio-mineral precipitation through MICP could be achieved either directly, where the metabolic activity of a microorganism induces the mineralization, or indirectly, where the extracellular polymeric substances (EPS) that are excreted by the bacteria interact with the available calcium ions[2]. Ureolysis and denitrification are two main factors contributing to the cost is the sporulation and encapsulation processes to increase bacterial viability inside the cementitious matrix. As a low-cost competitor, usage of self-protected cultures was attempted. In a study where the healing agent was a denitrifying non-axenic culture, the average production cost was noted as 136 €/m³ of concrete whereas the durability increase was significant[7]. Additionally, the need for sterility for axenic culture growth contributes greatly to the production cost [8].

It is clear that the bridge between academia and the market can be reinforced by exploring low-cost alternatives for bacterial self-healing. This can be realized by the usage of industrial side streams as the initiator for non-axenic cultures and the supply of a minimal feed medium. In this study, as a part of an ongoing development phase, the usage of two non-axenic biomasses in concrete was investigated. A fungi-based consortium (Yeast) and heterotrophic nitrifiers (HTN) were incorporated into the concrete matrix without additional protection and tested for their effects on the fresh and hardened properties of the matrix. Additionally, healing efficiency is evaluated through microscopic inspection of crack sealing and reduction in water absorption capacity.

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2 Materials and methods

The tests in this study were performed in triplicates to minimize error. Statistical calculations were done using SPSS.

2.1 Biomass production and characterization

The production of the non-axenic biomasses was conducted at Avecom NV, Belgium. The microbial sources were retrieved from agricultural side streams due to their rich bacterial diversity. Obtained liquors were added into aerated reactors with 10 L volume. For an initial growth period of 5 days, the media were left undisturbed. Following, a harvest-feed cycle was applied as 3 L per 24 hours until the desired volume of biomass was harvested. For both Yeast and HTN, sodium acetate was used as the carbon source. When the harvest was completed, 500 mL of both liquors were kept in cold storage as starters for the next batches. Subsequently, 100 mL per biomass was centrifuged and placed in an oven at 110°C for 24 hours to conduct total suspended solids (TSS) test. Afterwards, the remaining mass was incinerated at 600°C for 2 hours to determine the volatile suspended solids (VSS) content.

Following the tests on concrete, a flow cytometry test was conducted on both fresh HTN and Yeast liquors as well as samples collected from biomass-incorporated concrete. As control specimens, phosphate-buffered saline (PBS) and samples collected from regular concrete were used. To extract the cells from the concrete matrix, small cut prisms were placed in falcon tubes filled with PBS. The tubes were then placed in an ultrasonic bath for 12 minutes using 252 kJ of energy. A settling period of 10 minutes was allowed before collecting aliquots of the supernatant. As the last step before staining, samples were diluted 10 times with PBS to reduce noise. SybrGreen I stain was used to detect the viable bacteria through cytometry. Intact cells were determined through gate P1. Results were then calculated for the cells per mL as well as cells per gram of concrete.

2.2 Mix design and specimen preparation

Table 1 summarizes the mix design which was used for this study. The composition was calculated regarding the XS3 exposure class[9] and the w/c ratio was adjusted to 0.45. The dimensions for the prisms were 60x60x220 mm³. To supply samples with flexural resistance, two reinforcement bars (Ø = 3mm) were placed 15 mm above the bottom of the moulds. Fresh properties of concrete, being air content, flow and slump, were determined during casting. The compressive strength of the concrete was determined by cubic samples (150x150x150 mm³) at 7 and 28 days. The cast prisms were demoulded after 24 hours and placed in the curing room until the crack creation.

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM I 52.5</td>
<td>368</td>
</tr>
<tr>
<td>Sand 0-4 mm</td>
<td>725</td>
</tr>
<tr>
<td>Gravel 2-8 mm</td>
<td>1119</td>
</tr>
<tr>
<td>Limestone filler</td>
<td>58</td>
</tr>
<tr>
<td>Water</td>
<td>166</td>
</tr>
<tr>
<td>Superplasticizer</td>
<td>2.00</td>
</tr>
<tr>
<td>Yeast/HTN</td>
<td>0.45/0.14</td>
</tr>
</tbody>
</table>

Through the analysis of the initial (CW₀) and final (CW₁) crack width measurements, healing efficiency (HE) was calculated as follows:

\[
HE = \left( \frac{CW_0 - CW_1}{CW_0} \right) \times 100\% \quad (1)
\]

A capillary water absorption test was applied before immersing the specimens in the water for 2 months as the healing period. The test was conducted with respect to RILEM TC 116-PCD[10]. An epoxy coating was applied to the prisms where the opening around the crack zone was 14 mm wide. Next, specimens were put in an oven at 40°C until the mass change was stabilized. Following a day of acclimatization, beams were placed on spacers at the water level 3 mm above the lower surface. Periodic weight measurements were taken for up to 24 hours and the sorptivity index (SI) was calculated as the difference between the initial mass (M₀) and the current mass (M₁) divided by the square root of time(√t):

\[
SI = \frac{M_1 - M_0}{\sqrt{t}} \quad (2)
\]

For self-healing to occur, prisms were submerged at ambient temperature. At the end of the healing period, both optical inspection and capillary absorption evaluations were repeated as described above.
3 Results and discussions

3.1 Biomass characteristics and viability

Following the production of the biomasses, they were tested in terms of their total (TSS) and volatile (VSS) suspended solids contents. Respectively for Yeast and HTN their TSS was 2.7 g/L and 0.84 g/L. For VSS, it was 2.26 g/L for Yeast and 0.71 g/L for HTN. The solid content for both biomasses was in a fine powder state. The initial pH was arranged as 5 and 7 for Yeast and HTN whereas their final pH during harvest was 7 and 9 respectively. The pH change can be associated with the excreted metabolites by the microorganisms as well as the consumption of nutrients which affects the pH through growth.

To evaluate the intact cell numbers in biomass-incorporated concrete as well as in pure HTN and Yeast, a flow cytometry analysis was conducted. To understand the effects of being exposed to the cementitious matrix for 6 months, cells/mL of fresh biomasses and ones that were extracted from concrete were compared. As controls, PBS solution and samples extracted from control prisms were used. The intact cell number for Control and PBS was $1.1 \times 10^6$ cells/mL and $7.6 \times 10^4$ cells/mL. For the pure biomasses Yeast (Y0) and HTN (H0), the count was $1.4 \times 10^9$ cells/mL and $4.2 \times 10^7$ cells/mL. When the microorganisms were immersed in the harsh environment of concrete, a decrease in the intact cell number was expected, especially since the biomasses were added with no additional protection. Roughly 10 times reduction was present for the Yeast and HTN incorporated 6-month-old prisms. For Yeast, the cell count was $8.9 \times 10^6$ cells/g. However, since the consortium Yeast does include types of fungi, the test should be repeated with specified channels and gates to better analyse the cells of Yeast. For HTN, $5.4 \times 10^6$ cells/g was calculated as the present cells. In previous studies, cell concentration to achieve MICP was determined as $10^7$ cells/cm$^3$ for Sporosarcina pasteurii [11] and $2.2 \times 10^6$ cells/mL for Bacillus megaterium [12]. It should be noted that these cell concentrations were analysed for the ureolytic bacteria specifically, thus, the concentrations that were detected for Yeast and HTN cannot be interpreted accordingly. Furthermore, relatively cost-effective tests such as plating to count CFU per gram of concrete should be integrated to increase the sample size hence the accuracy. Additionally, the noise that is picked up by the equipment should be minimised through further dilution to increase precision.

All in all, these results show a significant difference in terms of cell count between the control and biomass-added samples proving that the incorporation was successful.

3.2 Fresh and hardened properties

Evaluations were done during casting for the slump, flow and air content of the mixtures and the results are summarized in Figure 1. In terms of slump, there was a 10% increase for HTN compared to control and an 11% decrease for Yeast. It should be noted that, during the casting, only half of the initially planned superplasticizer was used for HTN. The reason was to keep the batches in the same flow and slump class, F4 and S3 respectively.

![Fig. 1. Slump and flow values for the mixtures](image)

The air content of Yeast and HTN were 2.7% and 2.4% respectively whereas, for the reference, it was 2.8%. It is known that the addition of bacteria could increase the air voids through bubbling during casting[13]. However, for this study, this was not the case and no significant change was observed.

The compressive strength of the batches was determined on the 7th and 28th days and the results are presented in Figure 2. During the early strength gain, HTN showed a decrease of about 9% when compared to the other two mixes. On day 28, there were no significant differences between the batches.

![Fig. 2. Compressive strength test results for 7 and 28 days](image)
3.3 Self-healing evaluation

The self-healing evaluation was done based on two parameters, visual crack sealing and capillary water absorption reduction. The microscopic images were analysed and the average crack widths were determined before and after the healing period. Figure 3 presents the initial crack width versus the healing efficiency. HTN had an initial crack width of 294 µm where after two months it reduced to 20 µm with a 93% of HE. For Yeast, the average width was measured as 276 µm and the cracks were completely sealed. As a surprising outcome, Control specimens showed 62% of healing. This width reduction can be attributed to the further cement hydration at the early age even though the crack creation date was selected as the 56th day.

Fig. 3. Healing efficiency of the samples plotted versus initial crack widths

To visualize the effects of HTN and Yeast addition, Figure 4 presents the initial (Day 0) and final (Day 56) images of the samples. The agglomerated material in the cracks is being analysed and will be reported in future studies. However, the precipitated product is predicted to be various polymorphs of calcium carbonate (CaCO₃).

For Yeast and HTN the level of HE can be attributed to the MICP. However, the underlying mechanisms still need to be elucidated to reveal the role of both direct and indirect MICP achieved through biomass addition. Heterotrophic nitrifiers can convert ammonium (NH₄⁺) into nitrate (NO₃⁻) and nitrate (NO₂⁻) which can both directly and indirectly contribute to MICP. For Yeast, the pathway is still being investigated although traces of ureolytic bacteria were previously detected.

To further analyse the healing performance of the samples, water absorption tests were applied. Figures 5 and 6 show the initial and final absorbed water plotted as a function of the square root of time. It should be noted that the used capillary water absorption test has been originally designed for uncracked samples. However, a modified version of this test has been used for self-healing concrete studies.

The MICP product, CaCO₃, occurs in different polymorphs. Calcite, vaterite, aragonite and amorphous calcium carbonate (ACC) can be listed as the main variants. Some of the factors affecting this variety in MICP can be listed as the bacterial strain, mineral source and temperature[15], [16]. Durability and the robustness...
of the precipitated product also change with the differing polymorphs. Previously calcite has been shown to be the most stable polymorph when compared to others[17]. In the context of this study, further assessment is needed to evaluate the effects of morphology on absorption reduction. Scanning electron microscopy (SEM) and X-ray crystallography (XRD) assessments should be conducted for further exploration.

A decrease was also observed in SI for the uncracked (UNCN) specimens after the healing period. For Control, Yeast and HTN the decrease was 49%, 36% and 58% respectively. The further cement hydration and carbonation of the concrete can fill up and seal the pores on the surface. The reduction of the SI in the cracked samples can be explained by the healing that occurred both through autogenous and autonomous mechanisms. However, it should also be noted that the initial pore structure of the specific prism could affect this result.

4 Conclusions

This paper is the result of an ongoing study where the focus is on the development of two non-axenic self-healing biomasses. The research has been focused on the potential upscaling and integration of these biomasses into concrete. The question of compatibility was then tested through effects on fresh and hardened concrete properties, crack sealing ability and permeability reduction. The following conclusions from this paper can be summarized below:

1. A significant increase of cells/g of concrete was observed when the Yeast and HTN biomasses were incorporated. The required intact cell count can be determined for the liquors by evaluating biomass with different viable cell amounts in terms of healing efficiency.
2. The addition of Yeast and HTN did not show any adverse effects on the 28th-day strength.
3. The addition of HTN required the adjustment of the initial mix design to keep the same flow and slump class. Further investigation on the effects of HTN on the flow should be conducted.
4. The addition of Yeast and HTN showed a visually robust crystal formation and a significant decrease in crack widths after healing. The underlying mechanism for both biomasses, being either direct or indirect MICP, should be further assessed.
5. The biomass-incorporated specimens showed similar performance to control samples in terms of permeability reduction.

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References