Microbial Self-Healing Concrete: Denitrification as an Enhanced and Environment-Friendly Approach

Yusuf Cagatay Ersan[1,2], Nico Boon[2], Nele De Belie[1]

[1] *Magnel Laboratory for Concrete Research, Ghent University, Technologiepark Zwijnaarde 904, B-9052 Ghent, Belgium – e-mail: yusufcagatay.ersan@ugent.be; nele.debelie@ugent.be*
[2] *Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium – e-mail: nico.boon@ugent.be*

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ABSTRACT
Concrete tends to crack due to its relatively low tensile strength which jeopardizes the good condition of steel reinforcement bars. Concrete cracks start at micro level and facilitate ingression of corrosive substances such as SO_4^{2-}, Cl^-, O_2. Corrosion of the rebar may result in failure of the structure. Self-healing concrete can provide crack repair immediately after crack initiation. One of the self-healing mechanisms is the use of bacteria to induce $CaCO_3$ precipitation in the crack environment. So far two different bio-chemical pathways, aerobic oxidation of lactate and ureolysis were used for microbial self-healing concrete. Despite the reported successful results, there are critical drawbacks of these two metabolic pathways to be improved such as oxygen limited performances, toxic byproducts, odor and negative effects on mechanical properties of concrete. To overcome these issues, we proposed an alternative pathway based on microbial NO_3^- reduction (denitrification) which also leads to $CaCO_3$ precipitation.

We tested two axenic NO_3^- reducing cultures, *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens* for microbial self-healing through denitrification. Bacterial agents were encapsulated within expanded clay particles (0.5-2mm) and tested for multiple crack closure (crack range 100-500 µm) in mortar specimens under wet and wet/humid conditions.

Under wet conditions, specimens with bacteria could completely close the cracks up to 250 µm in 2 weeks and 350 µm in 4 weeks. After 4 weeks, for 235 ± 35 µm original crack width, mortar prisms containing *P. aeruginosa* and *D. nitroreducens* absorbed 47% and 51% less water than reference specimen, respectively. Overall, the denitrification pathway was found to be as effective as existing methods while it is more environment-friendly.

1. INTRODUCTION

Denitrification (NO_3^- reduction) is microbial oxidation of organic matter by using NO_3^- as electron acceptor in the absence of O_2 (Eqn 1) and induces $CaCO_3$ precipitation.

$$CH_3COO^- + 2.6H^+ + 1.6\ NO_3^- \rightarrow 2CO_2 + 0.8N_2 + 2.8H_2O \qquad (1)$$

Furthermore it produces 2 times more carbonate per mole electron than ureolysis [1]. In order to overcome the major issues of existing microbial self-healing pathways, we propose NO_3^- reduction as an alternative microbial self-healing mechanism. This study presents self-healing properties of mortar specimens incorporated with NO_3^- reducing bacteria.

2. MATERIALS AND METHODS

2.1. Bacterial strains and encapsulation in expanded clay particles

Pseudomonas aeruginosa and *Diaphorobacter nitroreducens* were used as bacterial agents. Axenic bacterial cultures were isolated through an isolation and selection procedure by applying heat, starvation and dehydration stress as described in our previous studies [2-3]. Bacteria were incorporated within ARGEX expanded clay (EC) particles (0.5 – 2mm) by using a vacuum saturation technique. Bacterial solution containing 2.25 g bacteria was incorporated in 22.5 g sterilized EC particles under vacuum (-0.85 bar). Afterwards the closed system was over pressurized (1.2 bar) to keep bacterial solution inside the pores of EC particles and promote bacterial attachment.

2.2. Mortar specimens and crack creation

Series of mortar specimens (30 × 30 × 360 mm) with steel reinforcement (Φr = 6 mm, Lr = 660 mm) were prepared by using CEM I 52.5 N, tap water and standard sand accordingly to the norm EN 196–1 and further cured at 20^oC and RH > 90% for 28 days prior to crack creation. As nutrient supply for bacteria $Ca(NO_3)_2$ 3% w/w and $Ca(COOH)_2$ 2% w/w cement were used in relevant batches. In total, four different batches: (1) reference (0.5 w/c), (2) reference + EC + nutrients, (3) reference + nutrients + EC incorporated *D. nitroreducens*, (4) reference + nutrients + EC incorporated *Pseudomonas aeruginosa* were prepared and tested. Multiple cracks were created on specimens by applying uniaxial tensile load to the rebar as described in [4].

2.3. Treatment conditions for healing and evaluation

Both continuous immersion (4 weeks) and wet-humid cycles treatment (1 week immersion and 1 week curing at >90% humidity, 4 weeks in total) were tested. Specimens were immersed in tap water and crack closure was quantified under light microscope. After 28 days of treatment, a capillary water absorption test was conducted for each specimen.

3. RESULTS AND DISCUSSION

Crack sizes measured under the light microscope were between 100-500 µm. It was found that, crack width, type of treatment and treatment duration are the most important parameters for self-healing performance of concrete. The variation on crack closure ratio increased with an increasing crack width (Figure 1). Moreover, longer treatment period resulted in closure of more cracks and wider crack widths. After 28 days immersion in tap water, for a reference specimen, crack closure up to

150 µm was observed (Figure 1a). For the same treatment and time period, 200 µm crack closure was observed with a second reference specimen containing only nutrients (Figure 1a). Specimens containing both nutrients and either one of the bacterial cultures showed crack closure up to 350 µm (Figure 1). Furthermore, 250 µm crack width was healed in 2 weeks.
After 4 weeks, for 235±35µm crack width, mortar prisms containing *P. aeruginosa* and *D. nitroreducens* absorbed 42 % and 47% less water than reference specimen, respectively, in the first 24 hours of the capillary water absorption test (Figure 2).

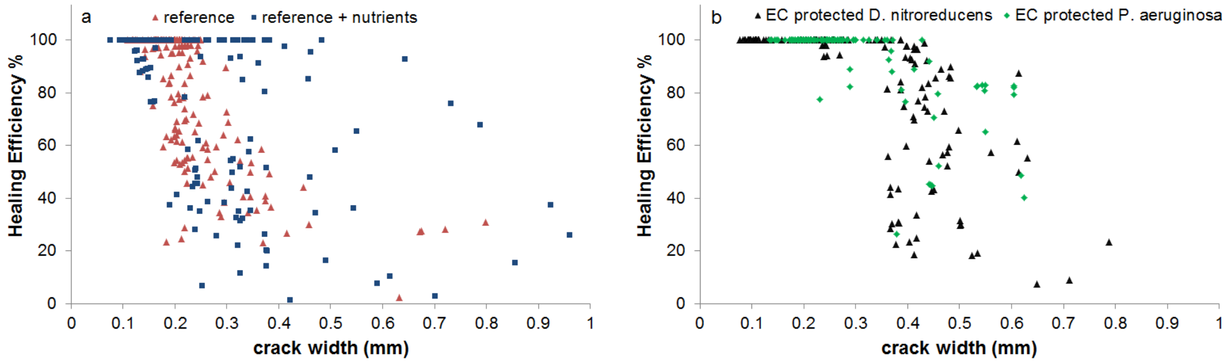

Figure 1. Bacteria improve crack healing capacity during immersion time of 28 days a: crack closure in with reference specimens; b: crack closure with bacteria incorporation

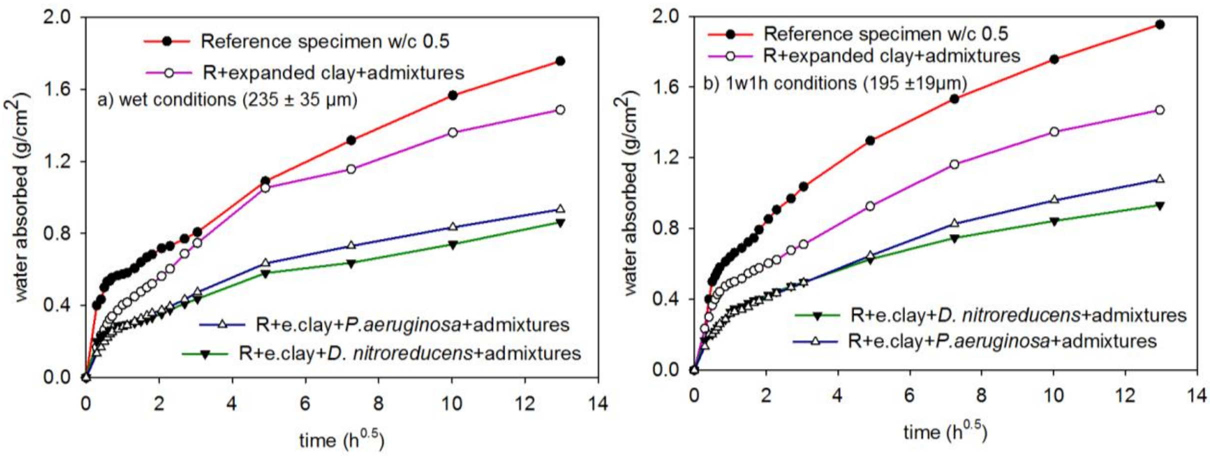

Figure 2. Microbial self-healing resulted in less water absorption than autogenous healing after 4 weeks wet or wet/humid treatment
a) 4 weeks wet treatment crack width 235±35 µm; b) 1 week wet 1 week RH >90 treatment crack width 195 ±19 µm

It was found that humidity (relative humidity >90 %) was not sufficient for either autogenous or bacterial crack healing. Reference specimen could completely heal up to 100 µm. Specimens containing different type of bacteria showed different performances. The specimens containing *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens* could close crack widths up to 200 µm and 250 µm, respectively.
Since this is the first study describing denitrification as a self-healing mechanism, results can only be compared with the self-healing results obtained through lactate oxidation or ureolysis. For the same time period with the same protective agent

(expanded clay), bacterial cultures used in this study revealed faster crack healing compared to those achieved by [4-5]. Effective crack healing around 350 µm was reported in at least 40 days by using lactate oxidizing bacteria [5], and similarly effective crack healing could not be achieved in 28 days by using ureolytic bacteria in EC.

4. CONCLUSIONS

Microbial NO_3^- reduction can induce self-healing of cracks as effective as existing microbial methods while it is more environment-friendly. Crack width, treatment duration and type of treatment have the highest influence on self-healing performance of concrete. Humidity is not enough to initiate microbial self-healing.

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