Bacteria-based agent for self-healing marine concrete

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ABSTRACT

Concrete can crack reducing its functional water tightness. If this water contains harmful chemicals such as those found in sea-water deterioration can further ensue until a concrete structures possible demise. A novel approach to self-healing is a bio-inspired technique, whereby bacteria immobilized in concrete are able to form a mineral healing precipitate hindering the ingress of aggressive chemicals. Calcium alginate is presented as a method for encapsulating both bacterial spores and magnesium acetate for the production of bio-based self-healing agent. Calcium alginate effectively encapsulated magnesium acetate, leaching the acetate within 24 hours when submerged in pure water. Capsules housed in cement paste expanded by 10 % and only in the first hour. Specific bacteria were able to respire on magnesium acetate and calcium alginate. These results provide key information on the way towards developing bacteria based self-healing concrete for application in the marine environment.

1. INTRODUCTION

Jonkers *et al*. first incorporated bacteria within cement paste for the development of autonomous bacteria-based self-healing concrete [1]. It was found that bacteria when directly added to the paste remained viable for only 4 months. A later study saw Jonkers use expanded clay particles to protect the bacterial spores in the concrete [2]. Other strategies have since been employed to protect bacteria in cementitious materials; Van Tittelboom et al. used glass tubes [3]; Wang *et al.* used diatomaceous earth [4]; and in a later Wang et al. used super absorbent polymers [5]. Alginate is a low cost non-toxic material used in the food industry to encapsulate and immobilize bacterial cells [6]. If alginate is to be used as an encapsulation method for the development of autonomous self-healing concrete, then it should be able to effectively house bacteria and also food and nutrients for the bacteria during the concrete mixing phase, later releasing in the event of cracking. Alginate may promote a two-phase healing process whereby cracks are initially blocked due to swelling. This swollen alginate could then provide a quiet environment for the bacteria, facilitating the partial replacement of the alginate by mineral precipitates. Alginates have been used to protect bacteria exposed to highly alkaline mortar surfaces [7], however, alginates are yet to be used to protect bacteria incorporated in cementitious materials. Here we present calcium alginate as a method for encapsulating both bacterial spores and magnesium acetate for the production of a bio-based healing agent for the development of self-healing marine concrete.

2. MATERIALS AND METHODS

2.1 Capsule composition and production

Based on a previous study magnesium acetate was selected as a suitable organic carbon source for bacteria isolated to thrive in marine concrete [8]. 2% (w/v) sodium alginate was prepared and mixed in a 7:3 ratio with magnesium acetate. The sodium alginate-magnesium acetate mixture was then syringed drop wise into a solution of calcium acetate. On entering the calcium acetate solution the droplets cross polymerize forming calcium alginate-magnesium acetate capsules. The capsules were removed from the solution after 30 min, washed 3 times in demineralised water and dried for 24 hours at 36ºC.

2.2 Leaching test

It is important that the capsules effectively lock up the acetate during concrete mixing making it available in the event of cracking. To assess this a leaching test was performed whereby both ground and whole capsules were placed in pure water (worst case scenario) and high-pressure liquid chromatography (HPLC) employed to measure increases in acetate of the water over time.

2.3 Expansion in paste

Alginate expands when hydrated. It is then important to know to what extent the capsules expand when incorporated in concrete, as excessive expansion will severely affect the strength of the concrete. Capsules were added to blast furnace slag cement (CEM III/B 42.5 N LH)(ENCI, The Netherlands) paste with a water to cement ratio of 0.5. Computer tomography (CT) was employed to assess expansion of the capsules in the paste over time. Images were taken every 10 seconds for 180 min. The resultant CT images were analysed with image-analysis software (Fiji, Is just ImageJ, http://fi ji.sc/) to measure capsule expansion.

2.4 Respiration on capsule components

Oxygen measurements were made to assess the ability of the bacteria to respire on the capsule components: magnesium acetate; and calcium alginate. A bacterial culture was grown and harvested during the exponential growth phase. The subsequent culture was washed twice and suspended in a basic media containing sea-salts (3 %) and sodium sesquicarbonate buffer (pH 10). Six 15 mL bottles containing oxygen sensors were prepared: (1) control containing basic media; (2) control with 2 mL of the bacterial suspension; (3) basic media plus 0.05 g of magnesium acetate; (4) basic media plus 0.05 g of magnesium acetate and 2 mL of the bacterial suspension; (5) basic media with 0.05 g of calcium alginate; and (6) basic media plus 0.05 g of calcium alginate and 2 mL of the bacterial suspension. Bottles were sealed with rubber stoppers making sure not to have trapped air bubbles. Bottles were then placed in a fridge at 8ºC and dissolved oxygen concentrations monitored (Planar Oxygen Sensor Spot, PreSens GmbH; Fibox 3 Oxygen Meter, PreSenes GmbH, Regensburg, Germany) over time.

3. RESULTS AND DISCUSSION

Both ground and whole capsules were placed in pure water. HPLC analysis was conducted on the water to measure leaching over time. Ground capsules leached their acetate payload immediately, while whole capsules leached it within 24 hours (Table 1). The capsules therefore effectively house the acetate before being submerged, locking it up at least initially, before later releasing it.

Table 1. Acetate concentration in pure water over time.

	Time [h]		
	0	**24**	**168**
Capsules		**Acetate [mmol]**	
Ground	21.96	21.37	19.63
Whole	3.43	22.99	21.33

Calcium alginate-magnesium acetate capsules were incorporated in to cement paste and analysed through CT to quantify any expansion. Analysis of images taken over a 3 hours period showed a 10 % expansion of the capsules only in the first hour. This is interesting, as excessive expansion during cement mixing would lead to weak concrete. It may, however, be beneficial for the capsules to expand in the event of cracking as this expansion may provide a two phase healing process where: (1) the crack is initially blocked by the expanding alginate; and (2) the alginate is taken over by a subsequent mineral phase.

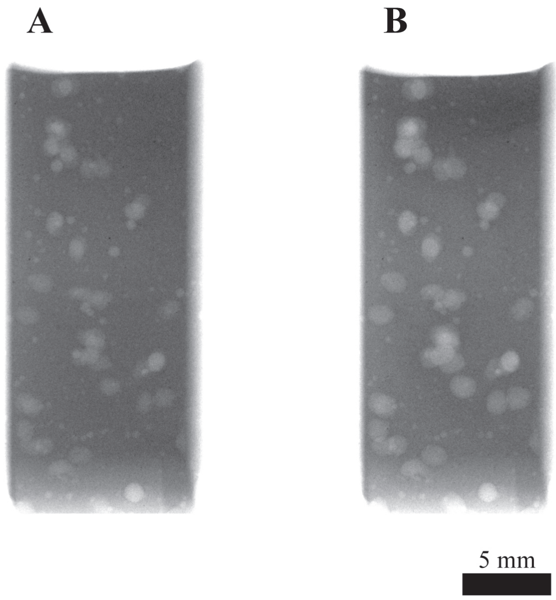

Figure 1. CT images taken of paste containing the capsules: (A) is an image taken after 4 min; and (B) taken after 180 min.

Facultative bacteria are able to respire and metabolize certain organic compounds according to the generalized reaction (Eq. (1)):

$$C_6H_{12}O_6 + 6H_2O + 6O_2 \longrightarrow 6CO_2 + 12H_2O + \text{energy} \quad (1)$$

In the presence of portlandite the produced carbon dioxide molecules can then react with calcium from the portlandite forming calcium carbonate (Eq. (2)):

$$CO_2 + \underset{\text{Portlandite}}{Ca(OH)_2} \longleftrightarrow CaCO_3 \quad (2)$$

Figure 2 shows dissolved oxygen consumption of solutions, theses solutions being: (1) control (salt and buffer); (2) control with bacteria; (3) magnesium acetate; (4) magnesium acetate and bacteria; (5) calcium alginate; and (6) calcium alginate with bacteria. Both controls and solutions without bacteria maintained constant dissolved oxygen content, while those with bacteria showed a linear decrease. Consumption of oxygen was quickest in the solution with bacteria and magnesium acetate. Surprising was the reduction in oxygen in the bottles containing bacteria and calcium alginate. Selected bacteria are then likely able to metabolize the acetate and perhaps the alginate or more likely some element of the solution containing alginate.

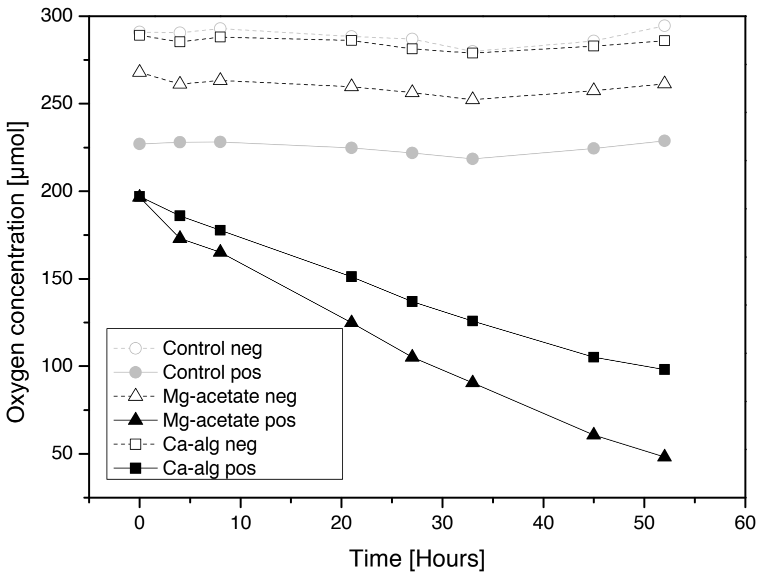

Figure 2. Shows dissolved oxygen measurements of the solutions in the bottles over time.

5. CONCLUSIONS

Calcium alginate effectively encapsulated magnesium acetate as an organic mineral precursor compound. Capsules submerged in pure water leached their acetate payload within 24 hours. Capsules incorporated in cement paste expand only in the first hour by 10 %. Specific bacteria respired in the solutions containing magnesium acetate and calcium alginate. Experiments are underway to tune the agent with the aim of incorporating it in cementitious materials for the development of self-healing concrete.

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