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Nitrate reducing $CaCO_3$ precipitating bacteria survive in mortar and inhibit steel corrosion

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ABSTRACT

Microbial healing of concrete cracks is a relatively slow process, and meanwhile the steel rebar is exposed to corrosive substances. Nitrate reducing bacteria can inhibit corrosion and provide crack healing, by simultaneously producing NO_2^- and inducing $CaCO_3$ precipitation. In this study, the functionality of one non-axenic and two axenic NO_3^- reducing cultures for the development of corrosion resistant self-healing concrete was investigated. Both axenic cultures survived in mortar when incorporated in protective carriers and became active 3 days after the pH dropped below 10. The non-axenic culture named "activated compact denitrifying core" (ACDC) revealed comparable resuscitation performance without any additional protection. Moreover, ACDC induced passivation of the steel in corrosive electrolyte solution (0.05 M NaCl) by producing 57 mM NO_2^- in 1 week. The axenic cultures produced NO_2^- up to 26.8 mM, and passivation breakdown and pitting corrosion were observed. Overall, ACDC appears suitable for corrosion resistant microbial self-healing concrete.

1. Introduction

Steel reinforcement plays an important role for durability of concrete structures, particularly under tensile loads. Yet, concrete is prone to cracking due to its heterogeneous matrix and brittle nature. Early age cracks in concrete mostly occur few days after casting and facilitate the migration of aggressive substances all the way to the steel rebar. Repair of these cracks, therefore, is essential to increase the durability of the concrete structures. In practice, extrinsic maintenance methods such as injection of various repair agents into concrete cracks are used [1]. Meanwhile researchers have been trying to implement self-healing technology into concrete to avoid manual labor and to repair the cracks immediately after crack initiation, i.e. starting from the micro-level [2]. Self-healing in concrete is the intrinsic repair of the cracks with the aid of rupture triggered chemical or biochemical processes and their reaction products. In order to achieve biochemical self-healing concrete, several types of axenic bacteria have been investigated [3,4]. The main principle has been to close cracks by means of microbial induced $CaCO_3$ precipitation. Previous results indicated that cracks up to 1 mm could be healed in 3 to 14 weeks depending on the type of the bacteria, the biochemical processes and the crack width [4–6]. Such self-healing processes however lack the preventive action to avoid exposure of the steel surface to corrosive substances during the healing period. It is possible to achieve simultaneous corrosion inhibition and crack healing by using NO_3^- reducing bacteria.

Biological NO_3^- reduction takes place during the microbial oxidation of organic matter by use of NO_3^- as an electron acceptor instead of O_2 (Reactions 1–4). In the presence of calcium ions (Ca^{2+}) NO_3^- reduction induces $CaCO_3$ precipitation (Reaction 5). Our works have revealed that through NO_3^- reduction, even enhanced $CaCO_3$ precipitation performances could be achieved in nutrient-poor environments which makes the mechanism feasible for self-healing concrete [7]. Nitrate (NO_3^-) reduction is not only advantageous by inducing enhanced $CaCO_3$ precipitation; it can also lead to the production of NO_2^- (Reaction 1) which is known as corrosion inhibitor [8,9].

$$2HCOO^- + 2NO_3^- + 2H^+ \rightarrow 2CO_2 + 2H_2O + 2NO_2^- \quad \text{(Reaction 1)}$$

$$HCOO^- + 2NO_2^- + 3\,H^+ \rightarrow CO_2 + 2NO + 2H_2O \quad \text{(Reaction 2)}$$

$$HCOO^- + 2NO + H^+ \rightarrow CO_2 + N_2O + H_2O \quad \text{(Reaction 3)}$$

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$$HCOO^- + N_2O + H^+ \rightarrow CO_2 + N_2 + H_2O \quad \text{(Reaction 4)}$$

$$Ca^{2+} + CO_2 + H_2O \rightarrow CaCO_3 + 2H^+ \quad \text{(Reaction 5)}$$

Studies on NO_2^- for corrosion inhibition have revealed that the optimum corrosion inhibition could be achieved when the $[NO_2^-]$:$[Cl^-]$ ratio was in the range of 0.34–1 [10–12]. Nitrite (NO_2^-) is an intermediate product in biological NO_3^- reduction (Reaction 1). In alkaline conditions (pH ~ 9), microbial NO_2^- reduction is mostly suppressed by high rate NO_3^- reduction causing NO_2^- to accumulate, which is called partial/incomplete denitrification [8]. It is possible to achieve NO_2^- concentrations up to 0.065 M in a few hours through partial denitrification [8]. To our knowledge, despite its significant potential, biological NO_3^- reduction has never been investigated either for self-healing concrete or for corrosion inhibition.

It is known that to be used in concrete applications, bacteria should be able to withstand (1) high shear stress during mixing, (2) high temperatures during cement hydration, (3) starvation, (4) highly alkaline pH (pH ~13 in concrete and pH ~10 in cracks), (5) dehydration stress, (6) cement hydration related shrinkage and pore sizes <0.1 μm [3]. Previous studies revealed that regardless of their resilience, axenic cultures (spores or vegetative cells) require encapsulation or immobilization to withstand the harsh conditions, especially the high shear stress, the alkaline pH conditions and the crushing due to microstructure densification [5,13]. We previously reported two NO_3^- reducing axenic cultures namely, *Diaphorobacter nitroreducens* and *Pseudomonas aeruginosa* for their resilience to heat, dehydration and starvation [7]. Therefore, it is possible that with protective carriers these two axenic cultures could become functional in the context of self-healing of concrete.

Besides, it is reported that bacteria are able to create self-organizing clusters, composed of compact self-immobilized cells to protect themselves from harsh conditions [14]. Moreover, our work revealed that such self-immobilized cultures are concrete compatible [15]. Therefore, a clustered microbial culture containing an activated compact denitrifying core (ACDC) could also be an option. The identified options (*Diaphorobacter nitroreducens*, *Pseudomonas aeruginosa* and ACDC) were tested for their potential to develop a corrosion resistant microbial self-healing concrete in three consecutive steps, (1) activity in alkaline pH environments (pH 9.5–10, pH ~ 13) (2) survival in mortar, (3) effect of biochemically produced NO_2^- on steel corrosion.

2. Materials and methods

2.1. Bacterial cultures

2.1.1. Production of axenic cultures

Axenic cultures were grown in nutrient media (NM) and harvested (1.25 g cell dry weight/L) by centrifuging at 6300 g for 7 min. Collected pellets were re-suspended in saline solution (0.15 M NaCl) prior to inoculation of test bottles.

2.1.2. Production of ACDC culture

The ACDC culture was cultivated in a cylindrical sequencing batch reactor (SBR) (effective h = 30 cm, Ø = 12.5 cm and 50% volume exchange ratio) by following a previously described procedure [16]. The SBR was operated with anoxic/aerobic period sequence (180 min anoxic and 155–168 min aerobic period). Different from the feed solution described in Ersan et al. [16], minimal nutrient solution (COD:N – 5:1) was used as feed (4 cycles/day) (Table 1). ACDC granules were achieved in 4 weeks and the reactor was operated for 7 months in total. ACDC culture was separated from the bulk liquid through sedimentation (5 min settling). Wet cultures were stored at 4 °C until the pH dependent activity tests. Additionally, a portion of the collected granules were dried for 48 h in a drying tunnel at 60 °C with ventilation and stored at room temperature inside closed containers until the survival tests (mortar incorporation).

Table 1
The feed composition for cultivation of ACDC.

Compounds	Concentrations (mM)
$NaNO_3$	20
NaHCOO	79
$Ca(HCOO)_2$	5
Na_2HPO_4	0.33
$MgSO_4.7H_2O$	0.37

2.2. Protection materials

During the two specific tests, the pH related activity of the cultures and the survival of the cultures in mortar; the axenic cultures were tested with and without protection. Performances were assessed by using three different protective carriers, namely diatomaceous earth, expanded clay and granular activated carbon. The diatomaceous earth powder used in the experiments was 5–200 μm in size, while expanded clay and granular activated carbon particles were 0.5–2 mm in size.

Dried ACDC culture was used for survival tests. To be consistent with the sizes of protective carriers, dried ACDC granules of 0.5–2 mm in size were used. Sieving technique was used to achieve the portion with a desired size range. Cultivated ACDC granules consist of bacteria (0.7 w/w) and inorganic matter (0.3 w/w). Inorganic matter inside the agglomerates were mainly $Ca_3(PO_4)_2$ and $CaCO_3$ formed during the production process. Throughout the manuscript, ACDC amounts were given as cell dry weight (CDW) which represents 70% of the total amount used.

2.3. Encapsulation procedure

A concentrated bacterial suspension containing either *Pseudomonas aeruginosa* or *Diaphorobacter nitroreducens* was incorporated in a protection material by using a vacuum saturation technique. Prior to tests, each protection material was autoclaved (at 1 bar, 120 °C for 20 min). Sterile materials were individually vacuumed (−0.85 bar) inside 100 mL bottles which were tightly closed with rubber stoppers and metal caps. Finally, a concentrated bacterial suspension was injected through the rubber stoppers and the bottles were further pressurized (+1 bar) with air to promote incorporation of bacteria in protective carriers. Pressurized bottles were kept at room temperature for 48 h to enable migration of bacteria from solution to the surface or pores of the protection materials. The pressure was released just before the inoculation of the buffered media and addition to the mortar mix in pH dependent activity tests and survival tests, respectively. The incorporated amounts of protective carriers and bacteria in different setups are given in Table 2. The protective carrier:bacteria ratio was 50:1 and 10:1 w/w during the pH related activity and the survival in mortar tests, respectively. The amount of water in the bacterial suspension was deducted from the water content of the mortar specimens to keep the water/cement ratio (0.5 w/w) constant.

2.4. Effect of pH on bacterial activity

Bacterial NO_3^- reduction activities were tested at pH 7 (neutral pH), pH 9.5 (expected pH in a concrete crack due to carbonation) and pH 13 (mortar mix pH) [17] with and without using protective carriers. Both protected and unprotected cultures were tested in 100 mL buffered minimal media by using a bacterial concentration of 2.5 g/L. Consistently, a non-axenic culture, ACDC, was also tested by using a bacterial concentration of 2.5 g CDW /L (total ACDC concentration 3.6 g/L = 2.5 g/L bacteria + 1.1 g/L inorganic matter). Since ACDC is self-immobilized microbial culture, the culture as such – without further protection – was