Construction and Building Materials 88 (2015) 196–203

Contents lists available at ScienceDirect

Construction and Building Materials

journal homepage: www.elsevier.com/locate/conbuildmat

# Screening of bacteria and concrete compatible protection materials

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## HIGHLIGHTS

- Screening of protection materials for possible biological self-healing concrete.
- The influence on setting and strength properties of mortar was evaluated.
- Self-immobilized bacterial cultures had no influence on mortar properties.
- Granular activated carbon is the most promising protection material available.

## ARTICLE INFO

*Article history:*
Received 27 February 2015
Received in revised form 10 April 2015
Accepted 11 April 2015
Available online 29 April 2015

*Keywords:*
Self-encapsulation
MICP
Setting
Strength
Self-healing

## ABSTRACT

Bacteria that can induce calcium carbonate precipitation have been studied for self-healing concrete applications. Due to the harsh environment of concrete, i.e. very high pH, small pore size and dry conditions, protection methods/materials have been used to preserve the bacterial agents. A wide screening of commercially available materials is thus required to evaluate them as alternatives. This study describes the influence of six commercially available possible protection approaches (diatomaceous earth, metakaolin, expanded clay, granular activated carbon, zeolite and air entrainment) on mortar setting and compressive strength when combined with either *Bacillus sphaericus* spores or *Diaphorobacter nitroreducens* and their respective nutrients. The influence of two novel, self-protected, bacterial agents was also investigated within the same scope. The most severe effect on setting time was observed as an undesirable delay of 340 min in all samples containing nutrients for ureolytic bacteria. Samples containing *B. sphaericus* spores showed the most significant decreases in compressive strength up to 68%. Yet, the addition of either *D. nitroreducens* or its respective nutrients did not cause major impact on both the setting times and the compressive strengths of the mortar specimens. The latter thus appears to be a suitable bacterial agent for further research on self-healing concrete. Likewise, the use of the novel self-protected bacterial agents did not affect the setting and the compressive strength of mortar. These results pave the way to replace protection materials with self-protection techniques. The latter should be further investigated for development of microbial self-healing concrete.



## 1. Introduction

The interactions between concrete structures and living organisms is becoming more and more important in relation to the durability of concrete structures. Bacteria able to precipitate $CaCO_3$ through different pathways are being investigated to develop self-healing concrete structures [1,2]. The main obstacle for the use of bacteria in concrete is its harsh environment, i.e. very high pH (pH ~ 13), relatively small pore sizes (<0.1 μm) and dry conditions [3]. Accordingly, unprotected axenic bacterial cultures could not remain viable in long term, when incorporated in mortar specimens [4]. Consequently, in microbial self-healing concrete studies, protective materials or encapsulation techniques have been tested [3,5–8].

Bacterial vegetative cells immobilized in silica gel and polyurethane revealed biological activity at considerable levels and they were able to heal concrete cracks up to 0.4 mm [5]. Moreover, it was shown that microencapsulated bacterial spores were able to induce $CaCO_3$ precipitation that was enough to close cracks up to 1 mm [7]. Hydrogels have also been used as encapsulation material to protect bacterial spores. The results showed that encapsulation

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http://dx.doi.org/10.1016/j.conbuildmat.2015.04.027
0950-0618/

with hydrogels allows bacteria to heal cracks varying between 0.2 and 0.5 mm [6]. However, all these encapsulation materials were reported to be expensive or inappropriate for concrete use [9]. In contrast, commercially available and less expensive materials such as diatomaceous earth and expanded clay were also examined as protection for the bacterial agents [3,8]. The immobilization of bacterial vegetative cells with diatomaceous earth proved to be effective regarding the maintenance of biological activity in cement paste [3]. The study showed that cracks up to 0.17 mm could be closed using diatomaceous earth immobilized bacteria. Bacterial spores encapsulated in expanded clay particles could close cracks up to 0.46 mm [8].

Several materials have already been described to protect bacteria from harsh environmental conditions. Among them, concrete compatible materials can be pointed out such as metakaolin, zeolite and granular activated carbon [10–15]. However, to our knowledge, these materials have never been tested for bacterial protection in concrete environment. So far, reported protection methods are study-specific and their influence on concrete properties are not clearly defined. Therefore, a wide screening of commercially available protection methods is needed. In this study the effects of diatomaceous earth, metakaolin, granular activated carbon, expanded clay and zeolite were tested and compared. In addition to the protection materials, three protection methods were considered namely, the use of air voids to house bacteria and the use of bacteria capable of either self-immobilization or salt encapsulation. It is known that extra air voids can be created in the concrete matrix by the addition of air entrainment products. Hence, the use of these admixtures could facilitate the housing of the bacterial agents inside the air voids. Besides, self-immobilization [16,17] and salt encapsulation [18] can be achieved during the production of non-axenic cultures if the appropriate conditions are provided. These bacteria are able to protect themselves from harsh conditions which avoids the need for additional protection material. It was also reported that the use of axenic cultures is 40 times more expensive than the use of non-axenic ones in concrete [9]. Not only the self-protection capabilities but the economic feasibility is also the advantage of these non-axenic cultures over the reported axenic cultures. The non-axenic cultures were then considered as promising options for concrete application.

An appropriate choice of the protection method is of critical importance for industrial application of self-healing concrete since it determines an important part of the product cost [9]. This study presents the effects of different, commercially available and, concrete and bacteria compatible protection approaches on mortar properties, particularly on setting time and compressive strength.

## 2. Materials and methods

### 2.1. Bacterial strains and nutrients

In this study, two types of axenic cultures were used i.e. (1) a ureolytic and sporulating strain, *Bacillus sphaericus* LMG 22557 (2) a vegetative $NO_3^-$ reducing strain, *Diaphorobacter nitroreducens*.

*B. sphaericus* was grown in MSB medium [2] in a 5 L bio-reactor. Bacterial spores were harvested for tests by centrifuging the 7 days old grown culture at 6300*g* for 7 min. Collected pellets were re-suspended in saline solution (8.5 g NaCl/L) and further pasteurized at 80 °C for 30 min.

*D. nitroreducens* which was previously isolated and characterized [19] was grown in 500 mL of nutrient media (NM) for 4 days and 10 L of NM was further inoculated with the grown culture. After 4 days of growth, bacterial cells were harvested (12.5 g cell dry weight) by following the aforementioned centrifugation and re-suspension procedure.

Apart from axenic cultures, two non-axenic bacterial agents were tested i.e. (1) Cyclic EnRiched Ureolytic Powder (CERUP), and (2) the Activated Compact Denitrifying Core (ACDC). CERUP is a ureolytic community protected by its high salt content and obtained from the further processing of side streams from vegetable industry. ACDC is a denitrifying microbial community protected by various bacterial partners and obtained in a sequential batch reactor by applying selective stress conditions.

*B. sphaericus* and CERUP are intended for microbial crack repair through ureolysis, while *D. nitroreducens* and ACDC are intended for microbial crack repair through denitrification. Therefore, *B. sphaericus* was supplied with 18 g urea + 4.5 g yeast extract and CERUP with 22.5 g urea, while both *D. nitroreducens* and ACDC were supplied with 13.5 g $Ca(NO_3)_2$ + 9 g $Ca(HCOO)_2$ and tested in relevant experiments described in "Section 2.4". Throughout the text, *B. sphaericus* + urea + yeast extract is termed as the ureolytic pack, while *D. nitroreducens* + $Ca(NO_3)_2$ + $Ca(HCOO)_2$ is termed as the denitrifying pack. Only the axenic strains were encapsulated. The nutrients were not incorporated with protection materials and directly added to the mixture during mortar preparation.

### 2.2. Protection methods

In total eight protection methods, namely diatomaceous earth, expanded clay, granular activated carbon, metakaolin, zeolite, air entrainment, CERUP and ACDC were tested. Diatomaceous earth and metakaolin used in the experiments were 5–200 μm in size, while expanded clay, granular activated carbon, zeolite, CERUP and ACDC were 0.5–2 mm in size. MasterAir 100 from BASF was used as an air entrainment product.

### 2.3. Protection procedure

A concentrated bacterial suspension composed of only water and bacterial cells, either *B. sphaericus* or *D. nitroreducens*, was incorporated with protection materials by using a vacuum saturation technique. Initially, 22.5 g of each protection material was autoclaved (at 1 bar, 120 °C for 20 min). Afterwards, sterile protection material was vacuumed (−0.85 bar) inside a 100 mL bottle which was tightly closed with a rubber stopper and a plastic cap. Finally, 50 mL of a concentrated bacterial suspension (2.25 g CDW) was injected through the rubber stopper and the bottle was further over pressurized (+1 bar) with air to promote bacterial impregnation into pores. Bottles were kept in room temperature for 48 h prior to tests. The content was used during the mortar preparation after depressurization. The suspension not absorbed/adsorbed by the protection materials was also added to the mortar mixture. The amount of water in the bacterial suspension was deducted from the water content of the specimens to keep the water/cement ratio (0.5 w/w) constant. The former was calculated by using the given equation (Eq. (1)).

$$m_{\text{water}}\ (\text{g}) = m_{\text{final mixture}}\ (\text{g}) - m_{\text{dry materials}}\ (\text{g})$$

$m_{\text{water}}$ : the amount of water in the bacterial suspension

$m_{\text{final mixture}}$ : total sum of protection material and bacterial suspension

$m_{\text{dry materials}}$ : total sum of dry weight of bacterial cells and protection material

(1)

### 2.4. Experimental planning

The objective of this study was to evaluate the effect of promising protection materials/methods for so called microbial self-healing concrete. Thus, it was necessary to evaluate the influence of (1) the bacterial agent (ureolytic or denitrifying bacteria) (2) the strain-specific nutrients (3) the protection material, on mortar setting and strength properties.

Series of mortar specimens (40 × 40 × 160 mm) were prepared by using CEM I 52.5N, tap water and standard sand (Table 1) accordingly to the norm EN 196-1 and further cured at 20 °C and RH > 90% for 7 or 28 days prior to tests. During preparation of the samples with different combinations, aggregates were not replaced and thus sand:cement:water ratio was kept as 3:1:0.5. Further comparison of the densities indicated that the extra addition of protection material did not influence the mortar composition (see SI, Table S.1).

The influence of the type of bacteria was investigated by incorporating plain bacteria (either *B. sphaericus* or *D. nitroreducens*: 0.5% w/w cement) in mortar. The influence of the particular nutrients necessary for different self-healing mechanisms (ureolysis and denitrification) were tested by incorporating plain nutrients (5% w/w cement) (either 18 g urea + 4.5 g yeast extract or 13.5 g $Ca(NO_3)_2$ + 9 g $Ca(CHOO)_2$ in mortar. The influence of different protection materials was investigated by incorporating each protection material (5% w/w cement) in mortar. The amount of air entrainment used in this study (1% w/w cement) was determined according to the data sheet describing the effect of the product on the compressive strength. Finally, batches containing all the necessary components (protected bacteria + nutrients) were investigated. Prior to investigation of combined effects (protected bacteria + nutrients), protection materials were grouped in terms of their comparability. From each group one material was tested with one type of bacteria. For example, the effect of diatomaceous earth and the effect of metakaolin was considered as comparable and only the denitrifying pack was tested with diatomaceous earth while the ureolytic pack was tested with metakaolin. Based on the classification, metakaolin, zeolite and air entrainment were tested with the ureolytic pack