Assessment of the functionality of bacteria-based repair system for concrete through ESEM analysis

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Abstract

Biodeposition, a method by which calcium carbonate (CaCO₃) precipitation is induced by bacteria, has been proposed as an interesting approach to protect building materials. The liquid-based system presented in this paper aims at the sealing of cracks and decrease of the porosity due to the production of a calcium-based biomineral. In this system, a silicate-based compound, which has a composition similar to concrete, is associated with Microbial Induced Precipitation (MIP), without involving urea hydrolysis pathway. Instead, the metabolic conversion of organic salts through bacterial respiration is used for MIP. The novelty of such system is to combine advantages of both, traditional repair system for concrete (fast reacting, and short term efficiency), and bio-based methods (more sustainable, slow process, and long-term efficiency).

This paper presents how environmental scanning electron microscope (ESEM) has been used in the development of the bacteria-based repair system. It is a good evaluation technique to assess the functionality of the bacteria-based repair system and to evidence the involvement of bacteria in the mineral production. The functionality of the bacteria-based repair system has first been assessed in the repair system alone, and then after injection into porous concrete. These results bring more insight regarding the formation of the biomineral. Though the bacteria are active after 1 day, it requires longer time to indeed observe mineral formation. Several days are needed to bacteria to actively convert the feed and produce substantial amount of CO₂, leading to favorable chemical environment for calcium carbonate precipitation. The results also showed how FTIR and ESEM analysis are complementary. Vaterite and calcite have been identified thank to FTIR while ESEM observation highlighted the unique features of the biomineral in size, shape and texture.

Keywords: bacteria-based system, repair, concrete, biomineral, bacteria imprints

I. INTRODUCTION

The development of bacteria-based systems for the protection of concrete structures has gained lot of attention over the past few years. These systems, engineered for self-healing concrete or concrete repair (Dhami et al., 2012), are based on Microbial Induced Precipitation (MIP), a method by which calcium carbonate precipitation is induced by bacteria.

The system presented in this study is a liquid-based system for concrete repair aiming at the sealing of the cracks and decrease of the porosity due to the production of calcium-based biomineral. In this system, a silicate-based compound, which has a composition similar to concrete, is associated with MIP using metabolic conversion of organic salts through bacterial respiration. The system is composed of two solutions, named A and B, which form a gel when mixed together. This gel is strong enough to allow a rapid sealing of the crack and is also providing a suitable environment for the bacteria to precipitate calcium carbonate (CaCO₃). By the time the gel becomes too weak, a substantial amount of CaCO₃ has been precipitated to seal the crack. And while assessing the crack sealing efficiency of the system can easily be done through water permeability test, the investigation and proof of the bacterial involvement in the mineralization process is not an easy task.

Indeed, the basic principle behind the proposed system is that organics (feed for bacteria) are efficiently converted by the bacteria into CO₂. Due to the alkaline environment in the system, the CO₂ is in turn converted into carbonate ions CO₃²⁻, which precipitate with the calcium present in the feed to form CaCO₃ (biomineral). Therefore, the performance of the proposed system is directly linked
to the efficiency of microbial induced precipitation and so to the biomineral production. However, after the crack has been successfully sealed, it is very delicate to determine whether the bacteria facilitated the CaCO$_3$ formation or if it is simply a result of physico-chemical conditions in the crack micro-environment as for instance it can also result from natural carbonation of concrete. In that area, environmental scanning electron microscopy is a precious tool as it may enable the observation of the biomineral which is characterized by a unique feature: the presence of bacteria imprints.

This paper presents how environmental scanning electron microscope (ESEM) has been used in the development of the bacteria-based repair system. It is a good evaluation technique to assess the functionality of the bacteria-based repair system and to evidence the involvement of bacteria in the mineral production. The functionality of the bacteria-based repair system has first been assessed in the repair system alone, and then after injection into porous concrete.

II. Materials and methods

Bacteria-based repair system

The bacteria-based repair system is a liquid-based system which transports a bio-based agent into concrete. The bio-based agent is composed of concrete compatible bacteria and feed which produces calcite-based minerals resulting in decreased porosity and sealing of the crack. The bacteria are from the genus Bacillus. They are added as endospores, dormant bacteria cells with characteristic compact round shape, typically in the size range of 0.8-1µm. When the environmental conditions are favorable (presence of water, nutrients and oxygen) these endospores germinate and grow into vegetative bacterial cells. Vegetative cells of Bacillus are rod-shapes and micrometer size (Buczynski and Chafetz, 1993).

The preparation and composition of the two solutions forming the repair system, solution A and solution B, is as described by Wiktor and Jonkers (2012). Briefly, it is composed of:

(i) Solution A – Sodium-silicate (alkaline buffer), Sodium-gluconate (Carbon source for bacteria growth), alkaliphilic bacteria.

(ii) Solution B – Calcium-nitrate (nitrate source for denitrification when O$_2$ is depleted and calcium for CaCO$_3$ precipitation), alkaliphilic bacteria.

Biomineral formation in the bacteria-based system

Previous results (Wiktor et al., 2013) have shown that the bacteria are active within 24h after the mixing of the two solutions and that efficient sealing of a crack is obtain after 6 weeks. However very little is known on the kinetic of the biomineral formation over these 6 weeks and whether the crack sealing is indeed due to MIP. The goal here is to follow in time the formation of the biomineral in the repair system. For this purpose, solution A and B are mixed together in small flasks and left in the lab at room temperature for 1, 2, 4, 6, and 8 weeks. At each time, the precipitate and gel are separated by filtration on a sintered-glass filter (pore size 10-16µm), washed with demi-water, dried at 35°C, grinded, and further analysed with
Figure 2: ESEM pictures of the precipitate obtained after filtration from the bacteria-based repair solution at (a) 1 week, (b) 2 weeks, (c) 4 weeks, (d) 6 weeks and (e) 8 weeks. (f) detail of picture (e). The arrows indicate bacteria imprints.

Fourier-Transformed Infra-Red spectroscopy (FTIR) for mineral identification and analysed with ESEM (Philips XL30 Series) equipped with an Energy Dispersive X-ray element analyzing system (EDS) to possibly observe bacteria imprints. The samples are not coated prior ESEM observation.

FTIR spectra were collected on a Perkin–Elmer Spectrum 100 Series spectrometer equipped with universal Attenuated Total Reflexion (ATR) unit. The spectra were recorded in the range of 4000–600 cm\(^{-1}\) with 2 cm\(^{-1}\) resolution, and 32 scans were collected each time. The ATR analyses require very small amount of sample (<5 mg), and furthermore no preparation or dilution of the sample is needed. The FTIR was first calibrated for background signal scanning, and then the experimental sample scanning was conducted. The spectra were normalized in order to compare them.

Biomineral formation in concrete-based material

In order to assess the functionality of the bacteria-based repair system in the concrete environment the repair system has been injected into porous concrete. By using porous concrete, large volume of repair solution can be injected in the material and therefore results in many spots within one specimen where the biomineral can be formed. The injection of the bacteria-based repair solution and preparation of the porous concrete is as described by Sangadji and Schlangen (2012).

Three weeks after the injection, the specimens are dried, and fully impregnated with low viscosity epoxy mixed with 1% by weight of hudson yellow pigment prior to sawing along the longitudinal cross-section. After epoxy impregnation of the new exposed surface, polished sections were then prepared from each sample, by grinding with water.
the specimen surface with paper grade P320, P500, P800, P1200 for 10 min each and P4000 for 20 min. The polished sections were observed with ESEM and elemental mapping was acquired with the ESEM/EDS. The polished sections are not coated prior ESEM observation.

III. Results and discussion

Biomineral formation in the bacteria-based repair system solution

The FTIR spectra (Figure 1) are indicative of the presence of calcium carbonate. The FTIR spectra of crystalline calcium carbonate polymorphs (calcite, aragonite and vaterite) have been extensively reported in the literature (Ghosh, 2001). Because of their different crystal structure, they can be discriminated using FTIR, a different spectrum is observed for each of the structural forms. The occurrence of the major vibrational bands specific to calcite are observed: the C-O asymmetric stretching vibration ($\nu_3$), and the C-O out of plane bending vibration ($\nu_2$) of carbonate centered at 1420 and 865 cm$^{-1}$ respectively. The C-O planar bending vibration ($\nu_4$) centered at 712 cm$^{-1}$, which is also the least intense of the three, has not been distinctly observed as it was probably overlapped by the peak present at 700 cm$^{-1}$ and therefore not shown on figure 1. Peaks at 835 and 872 cm$^{-1}$ are also observed at 1, 2 and 4 weeks. They decrease in time while the peak centered at 865 cm$^{-1}$ is increasing. These peaks likely correspond to the C-O out of plane bending vibration ($\nu_2$) of vaterite (Sato and Matsuda, 1968).

After the FTIR analysis, the powder has been observed with the ESEM and the corresponding pictures are presented on figure 2. A change in the morphology of the particles is noted as the time increases. Indeed, at 1 week (Figure 2a), the particles are arranged in small flakes with no distinctive structure neither well define edges. The presence of silicon and calcium as indicated by EDS analysis, and the presence the Si-O stretching vibration centered at 1000 cm$^{-1}$ on the FTIR spectra (data not shown) suggest that the powder obtained after 1 week is mainly composed by a form of calcium silicate. This calcium silicate results from the immediate reaction between the silicates present in the solution A and the calcium in solution B.

At 2 weeks (Figure 2b) globular morphology (calcium-based) with small rod-like imprints are observed. These globular shapes become bigger in size and more structured as the time increases (Figure 2c-e) suggesting crystallization. Taking into

Figure 3: Observation of polished section of porous concrete specimen prepared 3 weeks after injection of the bacteria-based repair system: (a) and (b) ESEM pictures, (c) and (d) Elemental mapping corresponding to (a) showing the silicon and calcium distribution respectively. Arrows indicate bacteria imprints. Agg.=aggregate, c.p.=cement paste, b=biomineral.
account that vaterite has been detected with FTIR at 1, 2 and 4 weeks and calcite has been detected at 4, 6 and 8 weeks, we can assume that the globular morphologies are calcium carbonate, mainly in the form of vaterite in the first weeks turning into calcite from week 4. The globular morphology observed in the first weeks are characteristic of vaterite. However, at week 4, this morphology is very different from the typical rhombohedral morphology usually observed for calcite. This can be explained by the presence of organics in the system which are known to have a profound influence on the crystal shape and size. Interestingly, the rod-like imprints are randomly distributed over the crystal surface and their occurrence also increase with time. The shape and size of these imprints are in good agreement with those of bacteria suggesting them to be bacteria imprints rather than default in the crystal structure. This is a strong indication of the involvement of the bacteria in the calcium carbonate formation. Indeed, vegetative cells are characterized by rod-shaped structure whereas endospores (the form in which bacteria have been added) are smaller compact round cells (Buczynski and Chafetz, 1993). Moreover, a closer look at the globular particle surface (Figure 2f) shows that this morphology indeed seems to be the results of the assembly of small calcite crystals grown around the bacteria cells.

**Biomineral formation in concrete-based material**

After showing that the bacteria-based repair system is functional in solution, it is of prime importance to assess its functionality and behavior in concrete-based material. The Figure 3 show a polished section of a cross section of porous concrete specimen 3 weeks after it has been impregnated with the bacteria-based repair system.

It can be seen from Figure 3a and b that the bonding between the epoxy and the specimen is not good as some epoxy seems to have scaled off from the surface. Considering that during the preparation of the polished sections the grinding was performed with water and that each specimen has been in contact with the grinding paper and water for 60 min, it can be concluded that the food dissolved resulting in holes what then appears as bad bonding between epoxy and the matrix. However, this “de-bonding” can serve as indicator of the presence of food, and help to locate CaCO₃ formed due conversion of food by bacteria.

Moreover, though morphological information is lost due to the polishing, the de-bonding around round particles (Figure 3b) reveals a globular morphology and bacteria imprints, similarly to the previous ESEM observation suggesting that it is actually the biomineral. The silicon and calcium mapping (Figure 3 c and d) of the specimen indicate that these globules are calcium-based and are primarily formed in the vicinity of silicon based material possibly cement paste and silicates from the repair system where the pH is alkaline. Therefore considering that the bacteria added to the system are alkaliphilic (grow at high pH) these observations support the assumption that the round calcium-based particles are probably calcium carbonate resulting from MIP.

**IV. Conclusion**

These results bring more insight regarding the formation of the biomineral. Though the bacteria are active after 1 day, it requires longer time to indeed observe mineral formation. Several days are needed to bacteria to actively convert the feed and produce substantial amount of CO₂, leading to favorable chemical environment for calcium carbonate precipitation. The results also showed how FTIR and ESEM analysis are complementary. Vaterite and calcite have been identified thanks to FTIR while ESEM observation highlighted the unique features of the biomineral in size, shape and texture. The presence of bacteria imprints gave strong indication on the bacterial involvement in the mineral formation underlying the functionality of the bacteria-based repair system in solution as well as in cement-based material.

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**References**


