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Bioprecipitation of a calcium carbonate – biofilm composite on the surface of
concrete for the maintenance of nuclear reactor enclosures

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Abstract

Bioprecipitation of calcium carbonate - biofilm composite on the concrete surface by spraying a bacterial suspension can reduce the permeability of concrete especially in the presence of micro-cracks. This process was applied to a replica at 1/3 scale of a nuclear reactor enclosure that was then subjected to pressurization at 500 kPa. The effectiveness of bioprecipitation treatment was demonstrated in areas where deformations of the concrete during pressurization remained minimal. Additional laboratory tests confirmed that the calcium carbonate - biofilm composite bioprecipitated in a micro-crack can withstand an air flow with a pressure of 450 kPa.

Keywords: bioprecipitation, calcium carbonate, bacteria, concrete, nuclear reactors, micro-cracks, pressurization, durability, maintenance

1. Introduction

The use of bacteria to bioprecipitate a composite composed of calcium carbonate and biofilm, also defined as inorganic biohybrids [1], at the surface, in the open porosity and in micro-cracks of granular mineral materials used in civil engineering, such as soils, stones or concretes; improves the durability of these materials [2,3,4,5]. Indeed, the layer of the calcium carbonate - biofilm composite that has a very low permeability reduces the overall permeability and diffusivity of the material [6,7,8,9]. Additionally, calcium carbonate is very resistant while being thermodynamically compatible with the minerals contained in the civil engineering materials, but also with most of the environments [10,11]. Thus, treatment of the concrete surface by bioprecipitation is a potentially very interesting process for the preventive or curative maintenance of concrete structures. Moreover, this process has a good ecological footprint while having a moderate cost thanks to its ease of implementation. Indeed, the latter can be achieved by simply spraying the surface of the concrete in cases that do not require deep bioprecipitation in the porosity and micro-cracks.

However, laboratory experiments are most often performed to demonstrate the effectiveness of bioprecipitation in reducing the permeability of micro-cracked mortars. Indeed, *in situ* experiments are more challenging to perform as the environmental conditions are less controlled than in the laboratory [12]. Consequently, this study aims at evaluating the effectiveness of bioprecipitation on micro-cracked concrete walls of an instrumented structure (named VeRCoRs), that was also subjected to pressurization cycles. Some of the VeRCoRs areas have been made available to apply bioprecipitation treatment and to evaluate its effectiveness during leak tests. Thus, the calcium carbonate - biofilm composite must not only reduce the permeability of concrete, but also withstand pressurization cycle of the structure.

This work reports on attempts (1) to evaluate whether bioprecipitation treatment of a micro-cracked mortar made under optimal conditions in the laboratory remains effective

after being subjected to a pressure cycle of 450 kPa, (2) to adapt the protocol of the bioprecipitation treatment under environmental conditions of a concrete interior wall of VeRCoRs, and (3) to evaluate the effectiveness of the biotreatment after the enclosure being subjected to a pressurization cycle.

2. Materials and methods

2.1. Preparation of micro-cracked and carbonated mortars

This part details the method of preparation of micro-cracked and then carbonated mortars, that were subsequently treated by bioprecipitation in laboratory. These mortars were used to estimate the resistance of the calcium carbonate - biofilm composite to a controlled pressure cycle.

After the cure at 23 °C and at 100% relative humidity for 28 days, annular mortar samples were cracked according to the controlled cracking device, the detailed procedure of which was described by Gagné and Argouges [13]. Cracking was achieved by applying a stress on the steel core with a press. The micro-crack obtained had an apparent opening greater than 150 µm on three sides of the annular mortar: openings on the top and bottom of the sample with 47.5 mm in length and opening on the side of the sample with 55 mm in length. In order to avoid any contribution of self-healing during the bioprecipitation treatment and to be representative of a concrete under *in situ* conditions, the micro-cracked specimens were subjected to accelerated carbonation at 23 °C under CO₂ (4%) with 40% relative humidity for one month. The carbonation makes it possible to obtain a surface pH of the mortar between 9 and 10 that is suitable for bacterial growth. The mortars were sterilized under an ultraviolet lamp before performing the bioprecipitation treatment.

2.2. Bioprecipitation treatment of micro-cracked and carbonated mortars

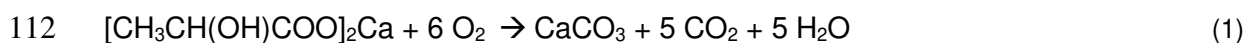
98

99 *2.2.1. Suspension of bioprecipitation*

100

101 The bacterial strain, *Bacillus pseudofirmus* (collection of DSMZ microorganisms,
 102 numbered DSM 2516), was used to bioprecipitate calcium carbonate. This bacterial strain
 103 was chosen because it was already used in previous studies for its ability to grow in
 104 alkaline environment and to generate calcium carbonate in the presence of a calcium
 105 source [6,14,15]. Moreover, this bacteria is an alkaliphilic strain able to survive to basic
 106 pHs up to 11 and thus at the surface of concrete walls [16]. It is aerobic and heterotrophic.
 107 So, in the presence of oxygen, this bacteria use organic compounds as source of carbon
 108 and energy. The biodegradation of lactate calcium by this bacteria through an
 109 heterotrophic pathway [17] leads to the formation of one mole of calcium carbonate per
 110 mole of consumed calcium lactate according to Eq. (1) [15,17]:

111



113

114 The bioprecipitation suspension was carried out by inoculating *Bacillus pseudofirmus* in a
 115 medium consisting of calcium lactate (75 g/L corresponding to 0.2 mol/L) and yeast
 116 extract (3 g/L) (named M1 medium). Yeast extract and calcium lactate provide the
 117 nutrients needed for the bacterial growth. Calcium lactate is a source of carbon for the
 118 bacterial growth and a source of calcium necessary for the bioprecipitation of calcium
 119 carbonate.

120 The protocol for bacterial growth tests was detailed by Lors et al. (2017) [6]. After 7 days
 121 of culture under aerobic conditions at 30 °C with stirring, calcium lactate was rapidly
 122 consumed leading to increase the amount of bacterial cells by a factor 1000, leading to
 123 reach 10^9 bacteria/mL. The consumption of calcium lactate was almost complete after 28
 124 days [6].

2.2.2. Bioprecipitation treatment protocol applied in the laboratory

The apparent opening of the micro-crack of the carbonated mortars was between 100 and 250 μm before the application of the bioprecipitation treatment to the surface of the mortar. The two samples reported in this study were chosen as the apparent opening of the micro-crack of the carbonated mortars (T_0) was of different size (154 μm and 92 μm for mortars A and B respectively). The size of these micro-cracks makes it possible to cover a range of micro-cracks compatible with that found in VeRCoRs.

The bioprecipitation treatment was based on a 14-day cycle repeated four times. During a cycle, the upper surface of the annular mortar test specimen was sprayed at the beginning of the test (T_0) with a 3-day old bacterial suspension. Spraying the surface of the initially sterilized specimens was performed under biological safety cabinet to prevent external contamination. A second spray was carried out at 4 days with the same suspension of bioprecipitation, the latter being 7 days old. The cycle ended after 14 days. During the cycle, mortars were maintained at 20 °C in a bucket containing sterile distilled water at the bottom of the bucket. The water was not in contact with the mortar, but allowed the relative humidity to be maintained close to 100% HR inside the bucket. Indeed, the bucket was closed by a lid that contained a small hole with a foam stopper generating an air exchange to supply oxygen to the respiration of bacterial cells without contamination.

The number of cycles realized during the biotreatment depends on the objective in relation to the thickness of the deposition of the composite calcium carbonate - biofilm on the surface of the mortar and in the micro-cracks. A significant reduction in the apparent opening of a micro-crack between 150 and 250 μm was generally observed after 3 cycles [6]. Thus, 4 cycles were carried out to have a greater reliability of the results, leading to duration of 56 days for the biotreatment.

2.2.3. Determination the apparent opening of the micro-crack after bioprecipitation treatment

The apparent opening of the micro-crack was estimated by using an abacus from the measurement of the air flow carried out using an air permeability cell specifically designed to accommodate the annular mortar and its expansive steel core [13].

The effectiveness of the bioprecipitation treatment was evaluated by the decrease of the apparent opening of the micro-crack, ΔWt (Eq. (2)). The latter can also be used to calculate an apparent percentage of decrease of the micro-crack opening induced by bioprecipitation, r_w (Eq. (3)).

$$\Delta Wt = T_0 - T_{bio} \quad (2)$$

$$r_w (\%) = (T_0 - T_{bio}) \times 100 / T_0 \quad (3)$$

with T_0 , size of the apparent opening of the micro-crack of the carbonated mortar (μm) and T_{bio} , size of the apparent opening of the micro-crack of the carbonated mortar after treatment with bioprecipitation (μm).

2.3. Application of a pressure cycle to mortars after bioprecipitation treatment

Pressurization of the micro-crack treated by bioprecipitation was carried out using a CEMBURO permeabilimeter equipped with digital flowmeters (Fig. 1), since it was not possible to use the permeabilimeter specifically designed for the annular mortar fitted with their steel core to achieve pressures greater than 100 kPa.

The use of this type of equipment required first to remove the expansive core of the mortar sample after the bioprecipitation treatment and then to replace it with a polymer cylindrical plug of the same height as the mortar. A measurement of air permeability was

therefore performed using the methodology presented in section 2.2.3, to ensure that removal of the expansive core did not alter the opening of the micro-crack.

The mortar sample was then subjected to a pressure cycle with a gradual increase in pressure with steps of 50 kPa up to 450 kPa in 70 minutes. The pressure was maintained at 450 kPa for 18 hours. Then, a progressive decrease of pressure was carried out in steps of 50 kPa in 70 minutes. Air flow measurement was performed at each pressure level. At the end of the pressure cycle, the size of the apparent opening of the micro-crack of mortar (T_{pressure}) was measured with the protocol indicated in section 2.2.3.

2.4. Description of micro-cracked zones of internal concrete walls of VeRCoRs before bioprecipitation treatment

VeRCoRs is a replica at 1/3 scale of a 1300 MW nuclear reactor enclosure, which was built at EDF Lab - Les Renardières (France) (Fig. 2). This double-walled containment, approximately 30 m high and 16 m diameter, has internal prestressed concrete walls 40 to 60 cm thick depending on the location. The prestressed reinforced concrete structure of VeRCoRs is strongly reinforced to withstand mechanical stresses, especially during pressurization. It is a research tool used to simulate the behavior of prestressed concretes over time. In addition to the monitoring of the deformations of the building, VeRCoRs is equipped with 700 sensors and 2 km of optical fibers. The evolution of its leak tightness is quantified each year by the measurement of leaking during pressurization tests at 500 kPa (absolute pressure).

Two zones with different types of micro-cracks were selected in interior concrete walls of VeRCoRs: a vertical zone in the middle of a vertical wall (named zone 1) and a horizontal zone corresponding to a vertical interface between two concrete batches (named zone 2) of respective dimensions equal to $1.2 \times 0.5 \text{ m}^2$ and $0.8 \times 0.25 \text{ m}^2$ (Fig. 3A and Fig. 3B respectively). These zones are instrumented to measure the leakage rate induced by the

micro-cracks during the pressurization test. The method of measuring the leakage flow consists in applying a collecting box around the leakage zone, ensuring the leak tightness of the device for measuring the leakage flow and thus comparing the evolution of this zone between two cycles of pressurization (Fig. 4).

The surface of the interior concrete walls has undergone natural carbonation during the construction of VeRCoRs. Thus, the pH on the surface of the concrete is less than 10, which makes the concrete surface compatible with bioprecipitation treatment.

2.5. Bacterial growth tests to define a bioprecipitation treatment protocol adapted to VeRCoRs environmental conditions

Several bacterial growth tests were carried out to study the influence of environmental parameters in relation to the interior walls of VeRCoRs, on which the bioprecipitation treatment was carried out: (1) effect of low temperatures (10 and 20 °C) on bacterial growth, this temperature range was chosen because the temperature ranges between 15 and 20 °C at the surface of the interior concrete walls of VeRCoRs - (2) impact of a dry period on the survival of the bacterial population developed on the surface of a cementitious material - (3) effect of the supply of fresh growth medium to increase the duration of the bioprecipitation treatment.

In the first case, erlenmeyer flasks (250 mL) containing 100 mL of M1 medium were inoculated with 250 µL of the pre-culture containing approximately 10^9 bacteria/mL, then were placed at 10, 20 or 30 °C with stirring under aerobic conditions. As the solubility of calcium lactate decreases with temperature [18], the medium used in the laboratory tests contained 50 g/L of calcium lactate instead of 75 g/L, in order to avoid precipitation of calcium lactate after spraying the bacterial suspension at the surface of the concrete wall for temperatures above 10 °C. Each test was done in triplicate. Periodic samplings of the bacterial suspension (4 mL) were made over time. One mL of the bacterial suspension

previously diluted was used to perform a direct bacterial enumeration using an epifluorescence microscope (LEICA DMLP). The bacterial cells were harvested on a 0.2 μm filter and then stained with orange acridine (fluorochrome). The living bacteria can be quantified by microscopic counting using an ultraviolet light. The remainder of the bacterial suspension was filtered with a disposable filter made of acetate cellulose at 0.2 μm to remove bacterial cells and precipitates. The previously diluted filtrates were analyzed by ion chromatography (DIONEX ICS-3000, USA) for the determination of lactate concentration. This apparatus was equipped with a column (AS11HC, DIONEX, USA) and the elution was carried out with a mixture of 80% ultrapure water and 20% NaOH at 5 mmol/L. A volume of injection of 20 μL was used. Lactate concentrations were determined from a 5-point calibration curve at concentrations of 5, 10, 20, 40 and 80 mg/L. The kinetics of the bacterial growth was evaluated by monitoring the lactate concentration over time according to Eq. (1).

In the second case, the mortar sample received a single treatment cycle with a mature bacterial suspension (bacterial concentration greater than 10^9 bacteria/mL) resulting from a culture realized in M1 medium during 7 days. The storage of mortar sample was carried out in a bucket without water added in its bottom. Thus, the surface of mortars gradually dried over time. After 4 hours, 7, 14, 21 and 28 days, a part of the treated surface (surface area of about 25.5 cm^2) was sampled using a cotton swab soaked in sterile water. These samples were cultured in 100 mL of M1 medium under aerobic conditions at 30 °C with stirring. The bacterial growth was assessed with the visual observation of the medium turbidity, that is the result of both the bacterial growth and the subsequent calcium carbonate precipitation. As calcium carbonate precipitation does not occur without the bacterial growth, turbidity can be related to the bacterial growth. Additionally, the determination of the amount of calcium carbonate generated at the end of the bacterial growth is an indirect method to evaluate more quantitatively the bacterial activity. The percentage of calcium carbonate contained in the solid residue recovered by filtration at

0.45 μm at the end of the bacterial growth after 35 days was evaluated by thermogravimetric analysis (TGA) (NETZSH STA 449 F3). The solid residue was dried at 40 $^{\circ}\text{C}$ until constant mass prior TGA. The mass loss at 750 - 800 $^{\circ}\text{C}$ corresponds to the mass of carbon dioxide (CO_2) released from the degradation of crystalline calcium carbonate. The percentage of calcium carbonate was determined by taking into account the molecular weight of these two constituents.

The last case was conducted to assess the kinetics of nutrient consumption by a mature bacterial population. In this case, a supply of fresh growth medium was performed when the initial growth medium was exhausted. A first bacterial growth was carried out in erlenmeyer flasks (250 mL) containing 100 mL of M1 medium modified with respect to calcium lactate concentration that was equal to 25 g/L. This medium was inoculated with 250 μL of the pre-culture containing approximately 10^9 bacteria/mL and was placed at 30 $^{\circ}\text{C}$ with stirring under aerobic conditions. At the end of this first bacterial growth when lactate is completely consumed, 33.3 mL of the bacterial suspension were sampled and replaced by 33.3 mL of a new medium containing 75 g/L of calcium lactate. This allowed a second growth to take place starting with a mature bacterial suspension (2/3 of the bacterial population present at the end of the first growth) in the presence of 25 g/L calcium lactate (1/3 of 75 g/L). This initial lactate concentration was thus identical as the one used in the first growth starting with a bacterial inoculum. This test was done in triplicate. As in the case of bacterial growth tests at different temperatures, periodic sampling of the bacterial suspension (4 mL) was carried out over time and then filtered with a disposable filter at 0.2 μm . Lactate concentration of filtrates was determined by ion chromatography. The kinetics of the bacterial growth was evaluated by monitoring the lactate concentration over time.

3. RESULTS AND DISCUSSION

3.1. Effect of bioprecipitation treatment on the micro-crack of mortars

After 4 cycles of bioprecipitation treatment, a light yellow-colored calcium carbonate - biofilm composite layer was clearly visible on the surface of the two samples used in this study (referred to as mortars A and B), but also in defects such as the air bubbles and at the micro-crack that looked to be sealed at that scale (Fig. 5).

The micro-cracks were not completely sealed after 56 days. The reduction in the size of the apparent opening of the micro-crack was 62 and 132 μm respectively for samples A and B, leading to an apparent percentage of decrease of opening of the micro-crack of 86 and 67 % respectively (Table 1). Nevertheless, the size of the apparent opening of the micro-crack after the bioprecipitation treatment was similar for the two samples: 22 and 30 μm respectively.

3.2. Effect of the pressure cycle applied after bioprecipitation treatment of micro-cracked mortars

Table 2 shows the apparent opening of the micro-crack of mortars A and B subjected to treatment by bioprecipitation after the replacement of the expansive core by a cylindrical polymer plug (T_{sc}) and then after exposure to the pressure cycle (T_{p}).

Comparing T_{sc} with the value of the apparent opening of the micro-crack before the replacement of the expansive core (T_{bio} - Table 1), it can be assessed that the apparent opening was not modified by this operation since the differences observed were in the order of the experimental error.

For the two mortars tested, the air flow increased progressively when applying an increasing pressure until reaching 450 kPa. Oppositely, the air flow decreased with a decrease of the pressure (Figs 6A and 6B). A very good proportionality of the air flow as a function of the pressure was observed for the two samples during both rising and

decreasing of the pressure in accordance with Darcy's law. Thus, the increase in pressure did not seem to modify the apparent opening of the micro-crack since the slope of the curve remained constant for both rising and decreasing parts of the pressure cycle (Figs 6A and 6B). Moreover, this slope was identical for the rise and the descent in pressure for the mortar A. Thus, the pressure bearing at 450 kPa did not modify the apparent opening of the micro-crack of the mortar A. The mortar B behaved differently since the air flow increased slightly from 9.99 to 10.94 L/min in relation to a probable increase in the apparent opening of the micro-crack. However, the estimate of the apparent opening of the micro-crack using the air permeability cell specifically made to accommodate the mortar rings (Section 2.2.3) indicated a decrease in the apparent opening of 2 and 5 μm after pressurization for both mortars A and B respectively: T_p compared to T_{sc} (Table 2). The difference in apparent opening was consistent with the measurement error for mortar A, but slightly higher for mortar B. An explanation could result from the presence of small solid debris entrained by the air flow through the micro-crack during permeability test especially for mortar B. These solid debris could reorganize differently from one test to another, in particular as a function of the pressure used which was different between the CEMBURO type permeabilimeter and the permeabilimeter used to estimate the apparent opening of micro-crack. Nevertheless, these results demonstrated that the calcium carbonate - biofilm composite was not or almost not damaged after having been subjected for several hours to an air flow with a pressure of 450 kPa. The cohesion of the bioprecipitated calcium carbonate associated with the biofilm and the adhesion of this composite to the walls of the micro-crack were certainly at the origin of this behavior. Thus, the bioprecipitation of the composite calcium carbonate - biofilm on the surface of the concrete is a possible treatment for the maintenance of nuclear reactor enclosures.

3.3. Adaptation of the bioprecipitation treatment on concrete from interior walls to VeRCoRs

The method of treatment with bioprecipitation is based on the spraying of concrete walls by the suspension of bioprecipitation as this methodology is very easy to perform even in areas with a difficult access for the workers. Nevertheless, this methodology has been adapted for its application to VeRCoRs. Thus, growth tests of *Bacillus pseudofirmus* were performed at temperatures below 30 °C. Additionally, the relative humidity being lower than that in laboratory tests, it is likely that the surface of the concrete will dry during the period of time between two sprayings of the wall by the bioprecipitation suspension. Bacterial growth tests were thus performed to determine if the bacterial population would still develop after a dry period lasting from 7 to 28 days. Finally, for the sake of simplification of the bioprecipitation treatment, the possibility of spraying the nutrient medium instead of the bioprecipitation suspension was tested by adding nutrient medium at the end of a bacterial growth test for which the lactate was totally consumed.

3.3.1. Effect of temperature on the bacterial growth

Bacterial growth tests in the laboratory were carried out at different temperatures (10, 20 and 30 °C) to estimate the effect of temperature on the rate of consumption of calcium lactate and therefore on the rate of production of calcium carbonate.

Lactate concentration decreased over time during bacterial growth tests performed at the three selected temperatures (Fig. 7). Lactate consumption, which is representative of the bacterial activity, was all the greater as the duration of the test was longer and the growth temperature was higher. At 7 days, 30% of the lactate was consumed at 20 °C against 59% at 30 °C. This phenomenon was amplified at 10 °C with only 17% of the lactate consumed. After 35 days of culture, 40, 52 and 86 % of the lactate were consumed at 10, 20 and 30 °C respectively.

These tests showed that a drop in temperature relative to the optimum temperature of 30 °C greatly reduced the rate of bacterial growth and therefore the precipitation rate of

calcium carbonate. This reduction in the rate of bacterial growth is indeed not due to a lack of lactate since this nutrient remains available in a significant amount during all tests.

3.3.2. Effect of the duration of a dry period on the survival of the bacterial population

The survival of the bacterial population on the surface of a mortar initially sprayed with the bioprecipitation suspension and then dried in the presence of a relative humidity between 45 and 60 % was evaluated by the weight percentage of calcium carbonate contained in the solid residue after 35 days of culturing a sample taken at the surface of the mortar after different drying times.

A first sample taken after 4 hours on a still wet surface was used as a control to validate the method of collecting the bacterial population on the mortar surface using a cotton swab soaked in water sterile. The control sample taken in culture led to a notable bacterial growth and the percentage of calcium carbonate of the solid residue after 35 days of culture was close to 90% by weight (Table 3). Similar results were obtained for the samples taken on the surface after 7, 14, 21 and 28 days of drying. These results demonstrated a good survival capacity of *Bacillus pseudofirmus* on the dry surface of a mortar. This result may be related to the ability of *Bacillus pseudofirmus* to produce spores [18].

3.3.3. Effect of calcium lactate intake during the bacterial growth

The first bacterial growth with a bacterial inoculum in the presence of a medium containing 25 g/L of calcium lactate demonstrated that lactate was completely consumed after 14 days of culture (E1 curve on Fig. 8). During the second bacterial growth that started at the end of the first bacterial growth at 24 days, the addition of 25 g/L of calcium lactate to the mature bacterial suspension also indicated a complete consumption of lactate after 15

days of culture (E2 curve on Fig. 8). Thus, the rate of lactate consumption starting with a mature bacterial suspension (E2 curve on Fig. 8) is comparable than the one observed during the first bacterial growth starting with a bacterial inoculum (E1 curve on Fig. 8) containing 266 times fewer bacterial cells. This result validates an experimental protocol based on bioprecipitation cycles with sprays of a bacterial suspension at the beginning, and then sprays of nutrient medium at longer times once a bacterial colonization is well established on the support.

3.3.4. Bioprecipitation treatment procedure for the interior concrete walls of VeRCoRs

The results reported in the previous three sections allow us to define a procedure of treatment by bioprecipitation of the interior concrete walls of VeRCoRs. This procedure is based on the following points:

First, a mature bacterial suspension (bacterial concentration greater than 10^9 bacteria/mL) can be used, in order to rapidly colonize the concrete wall surface despite temperatures lower than 20 °C. The first spraying at time T_0 was carried out with a bacterial suspension of *B. pseudofirmus* grown in the presence of 75 g/L calcium lactate and yeast extract (3 g/L) at 30 °C for 7 days. The unconsumed lactate concentration was close to 10.7 g/L, avoiding any calcium lactate precipitation due to concrete wall surface temperatures that were lower than 20 °C. For the second spraying, the remainder of the first bacterial suspension was mixed in a volume ratio of 1/3 with sterile M1 medium. This bacterial suspension was sprayed on the surface of the concrete after 7 days of the bacterial growth at 20 °C, so at $T_0 + 7$ days.

Secondly, humidification of the concrete wall surface was performed either with M1 medium or with water to reduce the duration of dry periods during which the bioprecipitation process was slowed down or even stopped. As lactate consumption remained rapid regardless of the temperature during the first days of laboratory growth

tests, the duration of the period between two intakes of culture medium was equal to 7 days. Growth medium containing 50 g/L of calcium lactate and 3 g/L of yeast extract was sprayed at $T_0 + 14$ days, $T_0 + 21$ days and $T_0 + 30$ days. The reduction in calcium lactate concentration from 75 to 50 g/L is related to the decrease in the solubility of calcium lactate with temperature [19]. Humidification of the surface of the concrete walls with distilled water was also done on the second and fifth days after each spraying of the bacterial suspension or the growth medium, so at $T_0 + 2$ days, $T_0 + 5$ days, $T_0 + 9$ days, $T_0 + 12$ days, $T_0 + 16$ days, $T_0 + 19$ days, $T_0 + 23$ days, $T_0 + 26$ days, $T_0 + 30$ days and $T_0 + 32$ days. The amount of water sprayed was kept as minimal, in order to avoid leaching of the surface of the concrete that could reduce the efficiency of the bioprecipitation process. The bioprecipitation treatment of concrete internal walls of VeRCoRs lasted 32 days as a single bioprecipitation cycle with several stages. This procedure is shorter and simpler than that initially developed for laboratory tests that lasted 48 days. Indeed, it requires the preparation of a single bacterial suspension instead of four during laboratory tests. Another notable difference is the temperature since the temperature of the concrete walls during the *in situ* test was between 17 and 19 °C instead of 30 °C for laboratory tests.

The two concrete surfaces, having been treated with the procedure previously described, had a pale yellow color, which is related to the formation of the calcium carbonate - biofilm composite (Fig. 9). Consequently, the bioprecipitation treatment procedure specifically defined for VeRCoRs was successful. The observed robustness may also be related to the involvement of bacterial populations present in the concrete walls in addition to *B. pseudofirmus* brought by bioprecipitation treatment. Indeed, some strains of the indigenous microflora, which were in a latent state on the concrete walls, may be activated during the bioprecipitation treatment that brings nutriment and water. Thus, after a first bioprecipitation treatment, the potential of bioprecipitation should remain and could be

reactivated when growing conditions become favorable again and especially by the spraying of the growth medium on the concrete walls.

3.4. Effect of bioprecipitation treatment on the leakage rate of micro-cracks in the interior concrete wall of VeRCoRs

VeRCoRs was put under a pressure of 500 kPa, 22 days after the last spraying of water of the bioprecipitation treatment, so at $T_0 + 54$ days. The leak rate was evaluated during this pressurization test at the two zones that had been treated by bioprecipitation (Table 4).

Zone 1, corresponding to the micro-cracked area in the middle of vertical wall, had a leakage rate after biotreatment of approximately 30 times greater than zone 2 located at the interface between two concrete batches. As deformations of 100-150 $\mu\text{m}/\text{m}$ were observed in zone 1 during pressurization of VeRCoRs whereas any deformation was recorded in zone 2, a greater leakage was expected for zone 1.

The effect of bioprecipitation treatment can be estimated by comparing the leakage rate when VeRCoRs is pressurized before and after the bioprecipitation treatment (Table 4).

Zone 1, with the highest leakage rate before biotreatment, showed a 26% increase in leakage rate. Thus, bioprecipitation treatment did not have a beneficial effect on this area that was further damaged by an additional pressurization cycle. As the bioprecipitation treatment was done by spraying on the concrete wall for reasons of simplicity of implementation, this method did not allow the calcium carbonate - biofilm composite to precipitate in the depth of the micro-crack. Thus, the mechanical properties of layer of composite calcium carbonate - biofilm formed on the concrete surface was not sufficient to oppose the deformation of the concrete. However, bioprecipitation suspensions, which have been optimized for deep injection in micro-cracks [20], could lead to better results in concrete areas subjected to deformations.

On the other hand, zone 2, that was not deformed during pressurization, had a leakage rate before bioprecipitation treatment that was approximately three times lower than zone 1. Moreover, this zone showed a decrease in the leakage rate of 88%. This result highlights the effectiveness of the bioprecipitation treatment and the resistance of the calcium carbonate - biofilm composite when subjected to an air pressure of 450 kPa. This result that is consistent to the reported laboratory results might be the consequence, on the one hand, of the very low deformation of zone 2, and on the other hand, to a crack that is easier to heal because having a lower initial leakage rate (253 L/h). In addition, the high roughness in this area that is at the interface of two concrete batches could be beneficial with respect to the adhesion of bacterial cells and the formation of the biofilm.

4. Conclusion

The treatment of the concrete surface by bioprecipitation of a calcium carbonate - biofilm composite using a bacterial suspension of *B. pseudofirmus* can be used for the maintenance of structures. Nevertheless, the bioprecipitation protocol has to be optimized according to the environmental conditions of the structure. In the specific case of VeRCoRs, which leads to additional constraints, such as pressurization to 500 kPa, the bioprecipitation treatment led to efficient results in areas where deformations of the concrete during pressurization remained minimal. Additional laboratory tests confirmed that the calcium carbonate - biofilm composite bioprecipitated can withstand an air flow with a pressure of 450 kPa.

Treatment of concrete surface by bioprecipitation of a calcium carbonate - biofilm composite is a very promising method that could be applied to the preventive maintenance of concrete structures. However, several improvements could be made to further increase the performance of this process: first, take advantage of the persistence

of bacteria inoculated on the surface of the concrete during biotreatment and also of the potential participation of the indigenous microflora to perform bioprecipitation treatment periodically by simply spraying a growth medium on the surface of the concrete ; second, in the specific case of micro-cracked concrete areas subjected to deformation, using injection instead of spraying could improve the performance of biotreatment.

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582



Fig. 1. Permeability cell accommodating the mortar coated with adhesive aluminum foil except at the micro-crack (pressure arrival side), the other side having undergone treatment by bioprecipitation was not covered with adhesive aluminum.

**A****B**

Fig. 2. A- VeRCoRs: replica at 1/3 scale of a nuclear reactor enclosure; B- Sectional view with double walls.

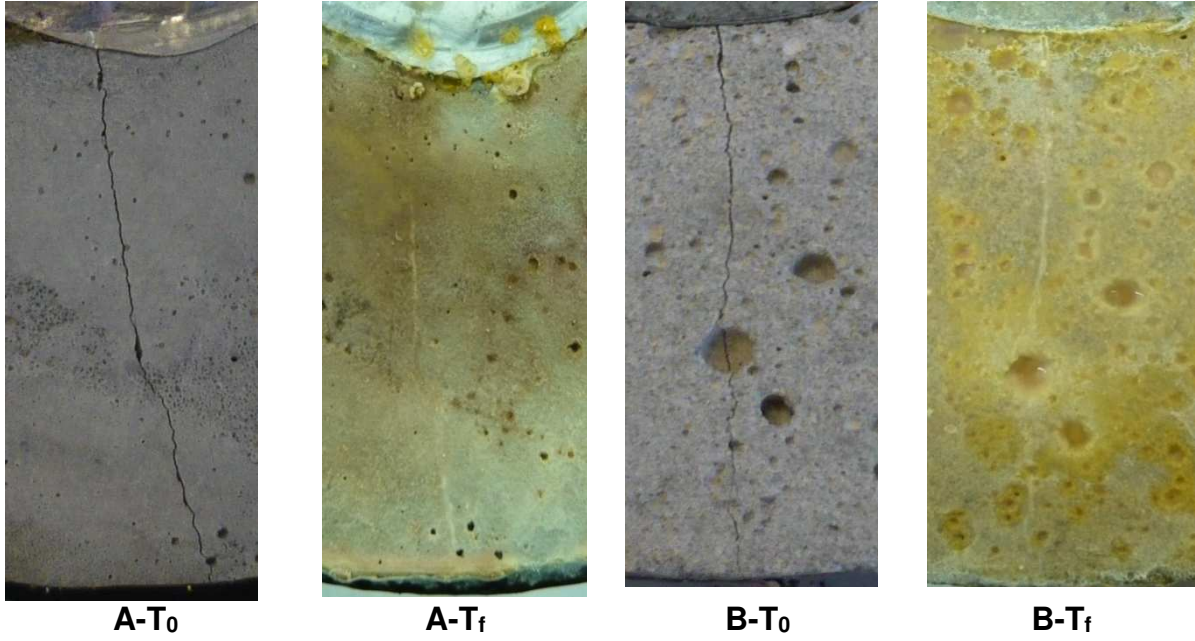
A**B**

Fig.3. Zone 1 (A) and zone 2 (B) selected to perform bioprecipitation treatment.



Fig. 4. Collecting box around the leakage zone.

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620

Fig. 5. Observations of the surface of mortars A and B before (T_0) and after (T_f) treatment

621

by bioprecipitation.

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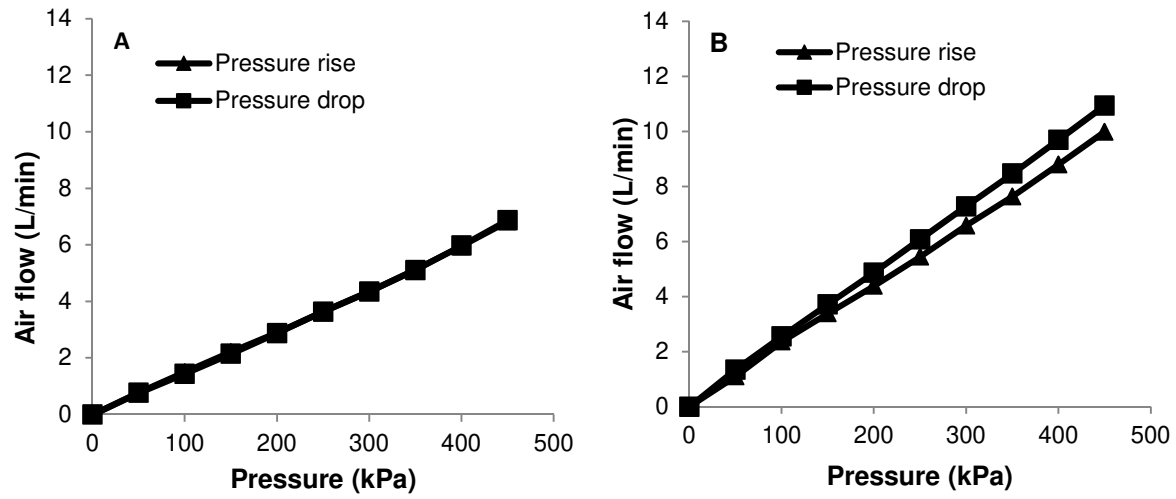


Fig. 6. Evolution of the air flow within the micro-crack of mortars A and B during the pressure cycle.

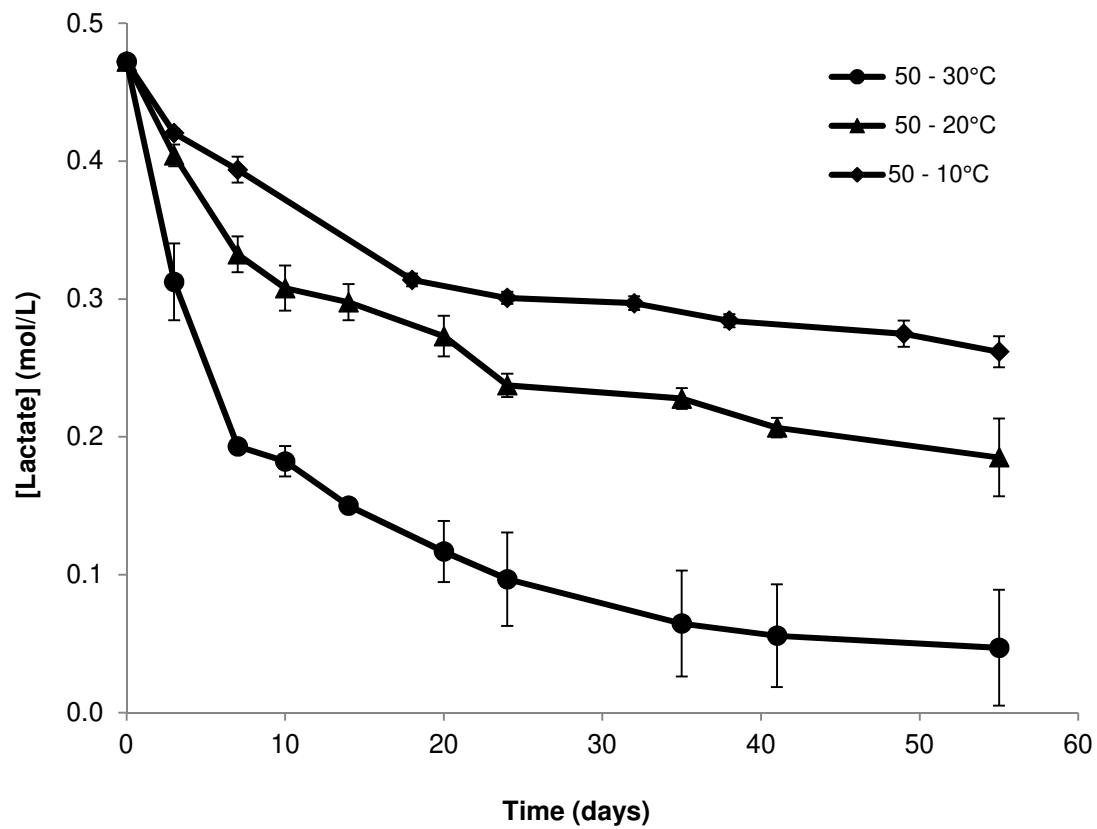


Fig. 7. Evolution of lactate concentration in growth tests of *Bacillus pseudofirmus* at different temperatures (10, 20 and 30 °C).

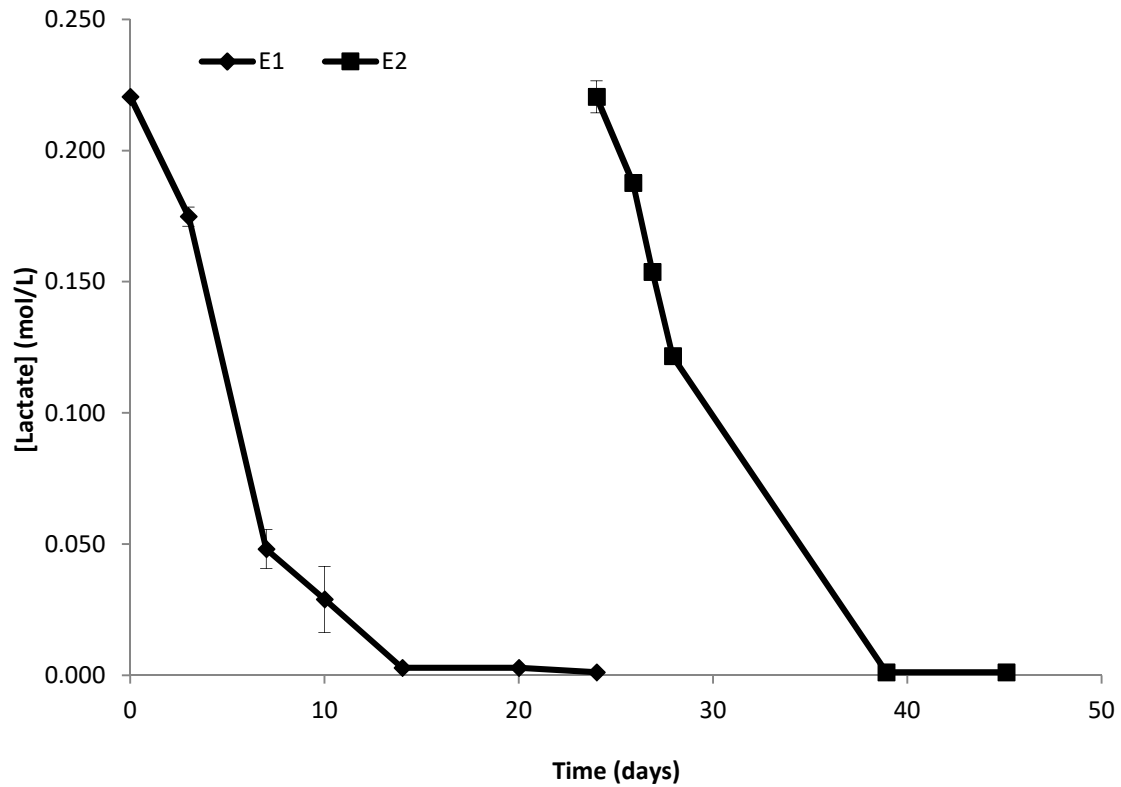


Fig. 8. Evolution of lactate concentration during a first bacterial growth at 30 °C with a bacterial inoculum of *Bacillus pseudofirmus* in the presence of 25 g/L of calcium lactate (E1 curve) followed by a second bacterial growth of the mature bacterial suspension (starting at 24 days) in the presence of 25 g/L of calcium lactate thanks to the addition of calcium lactate to the bacterial suspension (E2 curve).

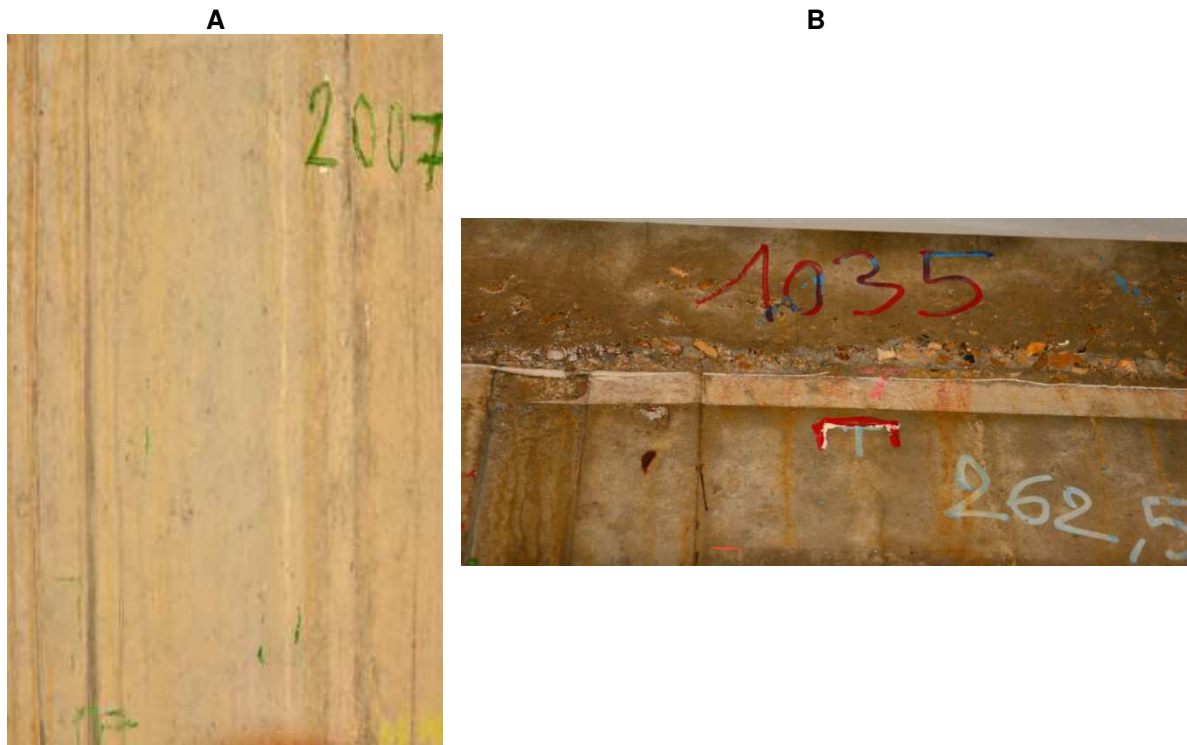


Fig. 9. Zone 1 (A) and zone 2 (B) after bioprecipitation treatment.

647

648 **Table 1.** Apparent openings (μm) of the micro-cracks before (T_0) and after (T_{bio}) treatment
 649 by bioprecipitation, reduction of the size of the apparent opening of the micro-crack (ΔWt)
 650 and apparent percentage of decrease of opening of the micro-crack (r_w) for the mortars A
 651 and B.

652

Mortar	T_0 (μm)	T_{bio} (μm)	ΔWt (μm)	r_w (%)
Mortar A	154	22	132	85.7
Mortar B	92	30	62	67.4

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655

656 **Table 2.** Apparent openings (μm) of the micro-cracks of mortars A and B after removal of
 657 the expansive core (T_{sc}) and after exposure to the pressure cycle (T_p).

658

Mortar	T_{sc} (μm)	T_p (μm)
Mortar A	24	22
Mortar B	29	24

659

660

661

662 **Table 3.** Weight percentage of calcium carbonate of the solid residue after 35 days of culture for
 663 samples recovered on the surface of mortar that was dried between 7 and 28 days.

664

	Sampling time after initial spraying of the bioprecipitation solution				
	0.16 d (Control)	7 d	14 d	21 d	28 d
Turbidity of medium	+	+	+	+	+
CaCO₃ (%) of solid residue	93.21	95.30	90.64	84.10	88.17

665

666 +: presence of turbidity in the inoculated medium, evidencing a bacterial growth

667

668

669

670 **Table 4.** Evolution of leakage rates during pressurization of VeRCoRs before and after the
671 bioprecipitation treatment.

672

Zone	Leakage rate when pressurized before bioprecipitation treatment (L/h)	Leakage rate when pressurized after bioprecipitation treatment (L/h)	Evolution of the leakage rate (%)
Zone 1	690	870	+ 26
Zone 2	253	30	- 88

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