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

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26

27 **Abstract**

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

36

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

39

40

41

42

## 43 **1. Introduction**

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

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

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

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

74

## 75 **2. Materials and methods**

76

### 77 *2.1. Preparation of micro-cracked and carbonated mortars*

78

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

83 After the cure at 23 °C and at 100% relative humidity for 28 days, annular mortar samples  
84 were cracked according to the controlled cracking device, the detailed procedure of which  
85 was described by Gagné and Argouges [13]. Cracking was achieved by applying a stress  
86 on the steel core with a press. The micro-crack obtained had an apparent opening greater  
87 than 150 µm on three sides of the annular mortar: openings on the top and bottom of the  
88 sample with 47.5 mm in length and opening on the side of the sample with 55 mm in  
89 length. In order to avoid any contribution of self-healing during the bioprecipitation  
90 treatment and to be representative of a concrete under *in situ* conditions, the micro-  
91 cracked specimens were subjected to accelerated carbonation at 23 °C under CO<sub>2</sub> (4%)  
92 with 40% relative humidity for one month. The carbonation makes it possible to obtain a  
93 surface pH of the mortar between 9 and 10 that is suitable for bacterial growth. The  
94 mortars were sterilized under an ultraviolet lamp before performing the bioprecipitation  
95 treatment.

96

### 97 *2.2. Bioprecipitation treatment of micro-cracked and carbonated mortars*

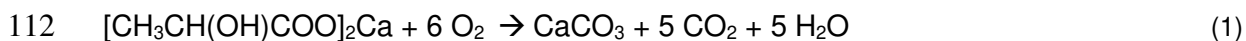
98

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<sup>9</sup> bacteria/mL. The consumption of calcium lactate was almost complete after 28  
124 days [6].

125

126 *2.2.2. Bioprecipitation treatment protocol applied in the laboratory*

127

128 The apparent opening of the micro-crack of the carbonated mortars was between 100 and  
129 250  $\mu\text{m}$  before the application of the bioprecipitation treatment to the surface of the  
130 mortar. The two samples reported in this study were chosen as the apparent opening of  
131 the micro-crack of the carbonated mortars ( $T_0$ ) was of different size (154  $\mu\text{m}$  and 92  $\mu\text{m}$   
132 for mortars A and B respectively). The size of these micro-cracks makes it possible to  
133 cover a range of micro-cracks compatible with that found in VeRCoRs.

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

145 The number of cycles realized during the biotreatment depends on the objective in relation  
146 to the thickness of the deposition of the composite calcium carbonate - biofilm on the  
147 surface of the mortar and in the micro-cracks. A significant reduction in the apparent  
148 opening of a micro-crack between 150 and 250  $\mu\text{m}$  was generally observed after 3 cycles  
149 [6]. Thus, 4 cycles were carried out to have a greater reliability of the results, leading to  
150 duration of 56 days for the biotreatment.

151

152 *2.2.3. Determination the apparent opening of the micro-crack after bioprecipitation*  
153 *treatment*

154

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

158 The effectiveness of the bioprecipitation treatment was evaluated by the decrease of the  
159 apparent opening of the micro-crack,  $\Delta Wt$  (Eq. (2)). The latter can also be used to  
160 calculate an apparent percentage of decrease of the micro-crack opening induced by  
161 bioprecipitation,  $r_w$  (Eq. (3)).

162

$$163 \quad \Delta Wt = T_0 - T_{bio} \quad (2)$$

$$164 \quad r_w (\%) = (T_0 - T_{bio}) \times 100 / T_0 \quad (3)$$

165 with  $T_0$ , size of the apparent opening of the micro-crack of the carbonated mortar ( $\mu\text{m}$ )  
166 and  $T_{bio}$ , size of the apparent opening of the micro-crack of the carbonated mortar after  
167 treatment with bioprecipitation ( $\mu\text{m}$ ).

168

169 *2.3. Application of a pressure cycle to mortars after bioprecipitation treatment*

170

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

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



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

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

186

187 *2.4. Description of micro-cracked zones of internal concrete walls of VeRCoRs before*  
188 *bioprecipitation treatment*

189

190 VeRCoRs is a replica at 1/3 scale of a 1300 MW nuclear reactor enclosure, which was  
191 built at EDF Lab - Les Renardières (France) (Fig. 2). This double-walled containment,  
192 approximately 30 m high and 16 m diameter, has internal prestressed concrete walls 40 to  
193 60 cm thick depending on the location. The prestressed reinforced concrete structure of  
194 VeRCoRs is strongly reinforced to withstand mechanical stresses, especially during  
195 pressurization. It is a research tool used to simulate the behavior of prestressed concretes  
196 over time. In addition to the monitoring of the deformations of the building, VeRCoRs is  
197 equipped with 700 sensors and 2 km of optical fibers. The evolution of its leak tightness is  
198 quantified each year by the measurement of leaking during pressurization tests at 500  
199 kPa (absolute pressure).

200 Two zones with different types of micro-cracks were selected in interior concrete walls of  
201 VeRCoRs: a vertical zone in the middle of a vertical wall (named zone 1) and a horizontal  
202 zone corresponding to a vertical interface between two concrete batches (named zone 2)  
203 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  
204 respectively). These zones are instrumented to measure the leakage rate induced by the

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

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

212

### 213 *2.5. Bacterial growth tests to define a bioprecipitation treatment protocol adapted to* 214 *VeRCoRs environmental conditions*

215

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

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

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

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

259 0.45  $\mu\text{m}$  at the end of the bacterial growth after 35 days was evaluated by  
260 thermogravimetric analysis (TGA) (NETZSH STA 449 F3). The solid residue was dried at  
261 40  $^{\circ}\text{C}$  until constant mass prior TGA. The mass loss at 750 - 800  $^{\circ}\text{C}$  corresponds to the  
262 mass of carbon dioxide ( $\text{CO}_2$ ) released from the degradation of crystalline calcium  
263 carbonate. The percentage of calcium carbonate was determined by taking into account  
264 the molecular weight of these two constituents.

265 The last case was conducted to assess the kinetics of nutrient consumption by a mature  
266 bacterial population. In this case, a supply of fresh growth medium was performed when  
267 the initial growth medium was exhausted. A first bacterial growth was carried out in  
268 erlenmeyer flasks (250 mL) containing 100 mL of M1 medium modified with respect to  
269 calcium lactate concentration that was equal to 25 g/L. This medium was inoculated with  
270 250  $\mu\text{L}$  of the pre-culture containing approximately  $10^9$  bacteria/mL and was placed at 30  
271  $^{\circ}\text{C}$  with stirring under aerobic conditions. At the end of this first bacterial growth when  
272 lactate is completely consumed, 33.3 mL of the bacterial suspension were sampled and  
273 replaced by 33.3 mL of a new medium containing 75 g/L of calcium lactate. This allowed a  
274 second growth to take place starting with a mature bacterial suspension (2/3 of the  
275 bacterial population present at the end of the first growth) in the presence of 25 g/L  
276 calcium lactate (1/3 of 75 g/L). This initial lactate concentration was thus identical as the  
277 one used in the first growth starting with a bacterial inoculum. This test was done in  
278 triplicate. As in the case of bacterial growth tests at different temperatures, periodic  
279 sampling of the bacterial suspension (4 mL) was carried out over time and then filtered  
280 with a disposable filter at 0.2  $\mu\text{m}$ . Lactate concentration of filtrates was determined by ion  
281 chromatography. The kinetics of the bacterial growth was evaluated by monitoring the  
282 lactate concentration over time.

283

### 284 **3. RESULTS AND DISCUSSION**

285

286 *3.1. Effect of bioprecipitation treatment on the micro-crack of mortars*

287

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

292 The micro-cracks were not completely sealed after 56 days. The reduction in the size of  
293 the apparent opening of the micro-crack was 62 and 132  $\mu\text{m}$  respectively for samples A  
294 and B, leading to an apparent percentage of decrease of opening of the micro-crack of 86  
295 and 67 % respectively (Table 1). Nevertheless, the size of the apparent opening of the  
296 micro-crack after the bioprecipitation treatment was similar for the two samples: 22 and 30  
297  $\mu\text{m}$  respectively.

298

299 *3.2. Effect of the pressure cycle applied after bioprecipitation treatment of micro-cracked*  
300 *mortars*

301

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

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

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

313 decreasing of the pressure in accordance with Darcy's law. Thus, the increase in pressure  
314 did not seem to modify the apparent opening of the micro-crack since the slope of the  
315 curve remained constant for both rising and decreasing parts of the pressure cycle (Figs  
316 6A and 6B). Moreover, this slope was identical for the rise and the descent in pressure for  
317 the mortar A. Thus, the pressure bearing at 450 kPa did not modify the apparent opening  
318 of the micro-crack of the mortar A. The mortar B behaved differently since the air flow  
319 increased slightly from 9.99 to 10.94 L/min in relation to a probable increase in the  
320 apparent opening of the micro-crack. However, the estimate of the apparent opening of  
321 the micro-crack using the air permeability cell specifically made to accommodate the  
322 mortar rings (Section 2.2.3) indicated a decrease in the apparent opening of 2 and 5  $\mu\text{m}$   
323 after pressurization for both mortars A and B respectively:  $T_p$  compared to  $T_{sc}$  (Table 2).  
324 The difference in apparent opening was consistent with the measurement error for mortar  
325 A, but slightly higher for mortar B. An explanation could result from the presence of small  
326 solid debris entrained by the air flow through the micro-crack during permeability test  
327 especially for mortar B. These solid debris could reorganize differently from one test to  
328 another, in particular as a function of the pressure used which was different between the  
329 CEMBURO type permeabilimeter and the permeabilimeter used to estimate the apparent  
330 opening of micro-crack. Nevertheless, these results demonstrated that the calcium  
331 carbonate - biofilm composite was not or almost not damaged after having been subjected  
332 for several hours to an air flow with a pressure of 450 kPa. The cohesion of the  
333 bioprecipitated calcium carbonate associated with the biofilm and the adhesion of this  
334 composite to the walls of the micro-crack were certainly at the origin of this behavior.  
335 Thus, the bioprecipitation of the composite calcium carbonate - biofilm on the surface of  
336 the concrete is a possible treatment for the maintenance of nuclear reactor enclosures.

337

338 *3.3. Adaptation of the bioprecipitation treatment on concrete from interior walls to*339 *VeRCoRs*

340

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

353

### 354 *3.3.1. Effect of temperature on the bacterial growth*

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

358 Lactate concentration decreased over time during bacterial growth tests performed at the  
359 three selected temperatures (Fig. 7). Lactate consumption, which is representative of the  
360 bacterial activity, was all the greater as the duration of the test was longer and the growth  
361 temperature was higher. At 7 days, 30% of the lactate was consumed at 20 °C against  
362 59% at 30 °C. This phenomenon was amplified at 10 °C with only 17% of the lactate  
363 consumed. After 35 days of culture, 40, 52 and 86 % of the lactate were consumed at 10,  
364 20 and 30 °C respectively.

365 These tests showed that a drop in temperature relative to the optimum temperature of 30  
366 °C greatly reduced the rate of bacterial growth and therefore the precipitation rate of

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

369

### 370 *3.3.2. Effect of the duration of a dry period on the survival of the bacterial population*

371

372 The survival of the bacterial population on the surface of a mortar initially sprayed with the  
373 bioprecipitation suspension and then dried in the presence of a relative humidity between  
374 45 and 60 % was evaluated by the weight percentage of calcium carbonate contained in  
375 the solid residue after 35 days of culturing a sample taken at the surface of the mortar  
376 after different drying times.

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

386

### 387 *3.3.3. Effect of calcium lactate intake during the bacterial growth*

388

389 The first bacterial growth with a bacterial inoculum in the presence of a medium containing  
390 25 g/L of calcium lactate demonstrated that lactate was completely consumed after 14  
391 days of culture (E1 curve on Fig. 8). During the second bacterial growth that started at the  
392 end of the first bacterial growth at 24 days, the addition of 25 g/L of calcium lactate to the  
393 mature bacterial suspension also indicated a complete consumption of lactate after 15



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

401

#### 402 *3.3.4. Bioprecipitation treatment procedure for the interior concrete walls of VeRCoRs*

403

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

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

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

421 tests, the duration of the period between two intakes of culture medium was equal to 7  
422 days. Growth medium containing 50 g/L of calcium lactate and 3 g/L of yeast extract was  
423 sprayed at  $T_0 + 14$  days,  $T_0 + 21$  days and  $T_0 + 30$  days. The reduction in calcium lactate  
424 concentration from 75 to 50 g/L is related to the decrease in the solubility of calcium  
425 lactate with temperature [19]. Humidification of the surface of the concrete walls with  
426 distilled water was also done on the second and fifth days after each spraying of the  
427 bacterial suspension or the growth medium, so at  $T_0 + 2$  days,  $T_0 + 5$  days,  $T_0 + 9$  days,  $T_0$   
428  $+ 12$  days,  $T_0 + 16$  days,  $T_0 + 19$  days,  $T_0 + 23$  days,  $T_0 + 26$  days,  $T_0 + 30$  days and  $T_0 +$   
429  $32$  days. The amount of water sprayed was kept as minimal, in order to avoid leaching of  
430 the surface of the concrete that could reduce the efficiency of the bioprecipitation process.  
431 The bioprecipitation treatment of concrete internal walls of VeRCoRs lasted 32 days as a  
432 single bioprecipitation cycle with several stages. This procedure is shorter and simpler  
433 than that initially developed for laboratory tests that lasted 48 days. Indeed, it requires the  
434 preparation of a single bacterial suspension instead of four during laboratory tests.  
435 Another notable difference is the temperature since the temperature of the concrete walls  
436 during the *in situ* test was between 17 and 19 °C instead of 30 °C for laboratory tests.

437

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

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

449

450 *3.4. Effect of bioprecipitation treatment on the leakage rate of micro-cracks in the interior*  
451 *concrete wall of VeRCoRs*

452

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

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

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

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

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

483

#### 484 **4. Conclusion**

485

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

495

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

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

505

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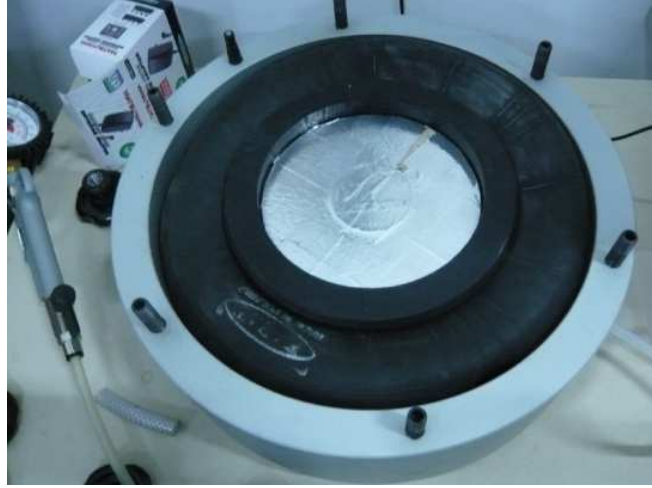
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585

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

588  
589  
590  
591  
592  
593

**A****B**

594  
595  
596  
597

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

598 with double walls.

599  
600  
601

602

A



603  
604  
605

B



606  
607  
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611

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



612

613 **Fig. 4.** Collecting box around the leakage zone.

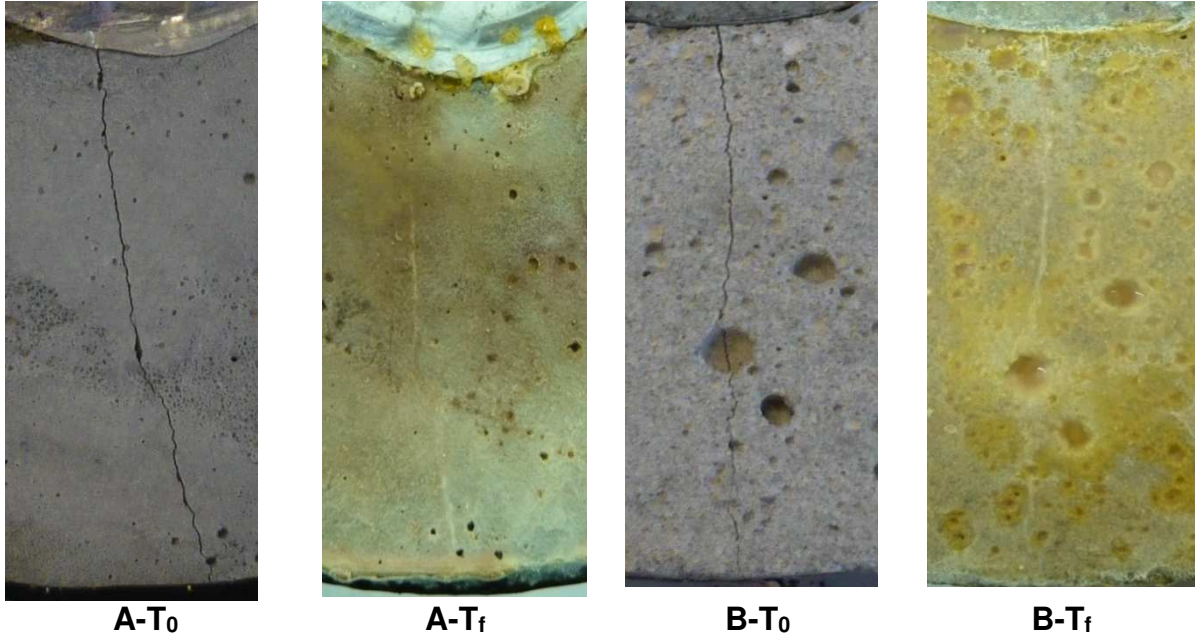
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**Fig. 5.** Observations of the surface of mortars A and B before ( $T_0$ ) and after ( $T_f$ ) treatment

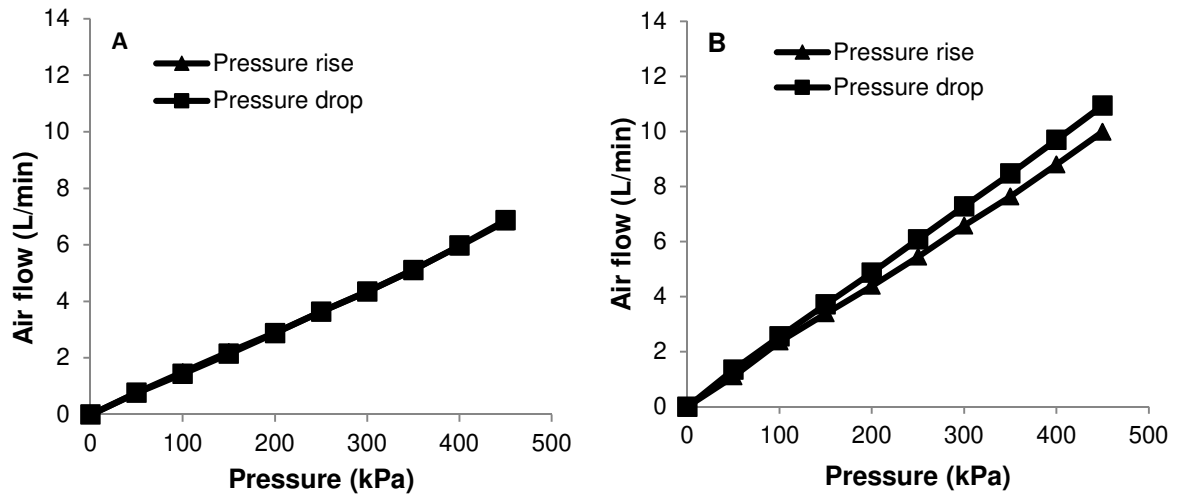
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by bioprecipitation.

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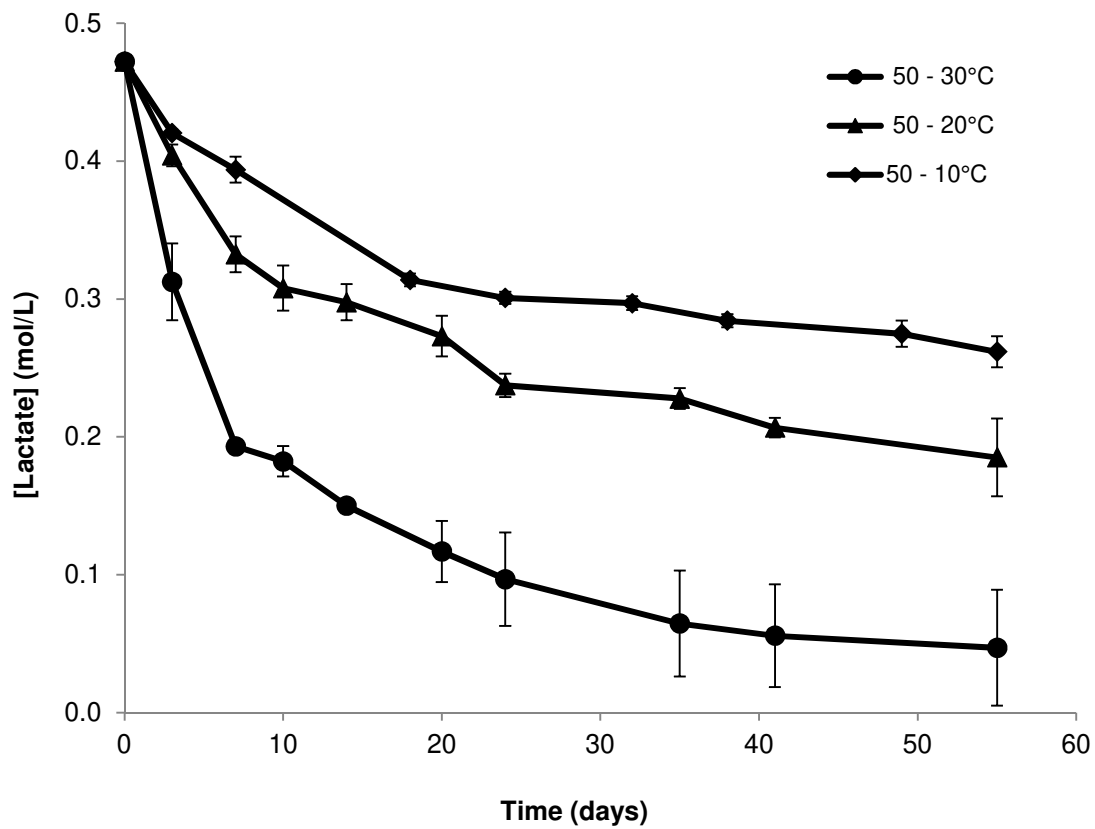


625 **Fig. 6.** Evolution of the air flow within the micro-crack of mortars A and B during  
626 the pressure cycle.

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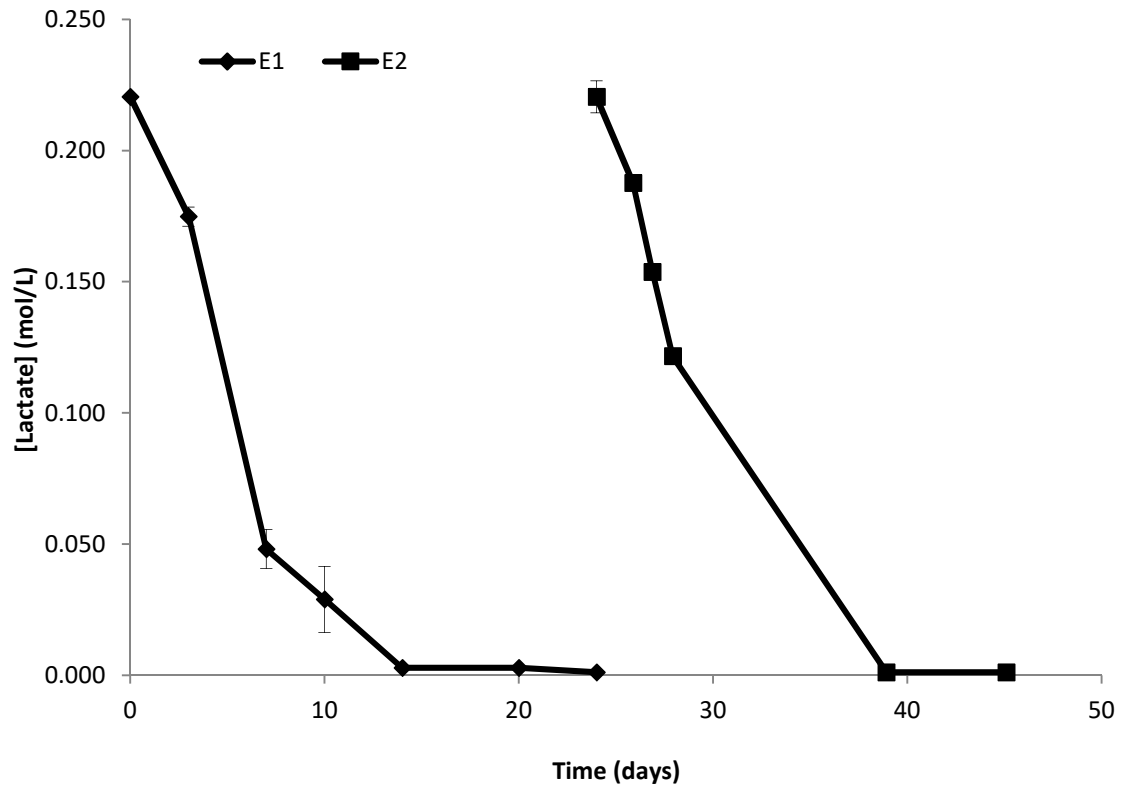
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631 **Fig. 7.** Evolution of lactate concentration in growth tests of *Bacillus pseudofirmus*

632 at different temperatures (10, 20 and 30 °C).

633

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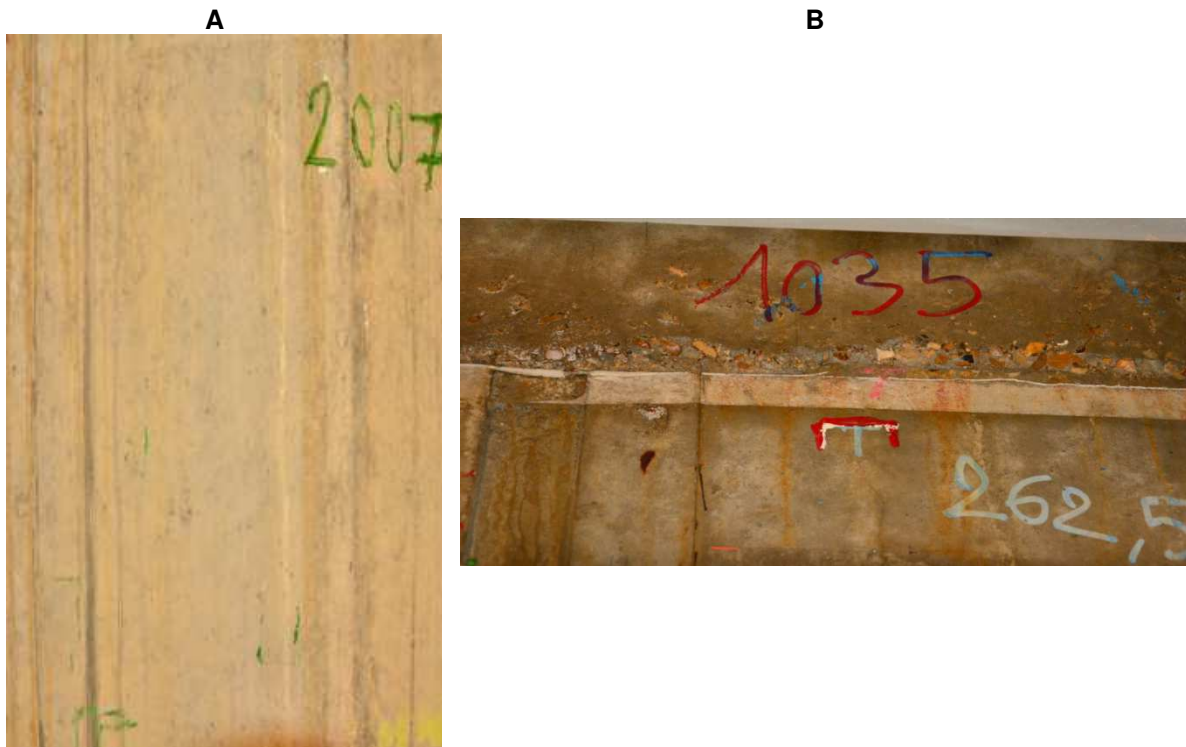


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637 **Fig. 8.** Evolution of lactate concentration during a first bacterial growth at 30 °C with a  
638 bacterial inoculum of *Bacillus pseudofirmus* in the presence of 25 g/L of calcium lactate  
639 (E1 curve) followed by a second bacterial growth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

642





643  
644  
645  
646

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

647

648 **Table 1.** Apparent openings ( $\mu\text{m}$ ) of the micro-cracks before ( $T_0$ ) and after ( $T_{\text{bio}}$ ) treatment649 by bioprecipitation, reduction of the size of the apparent opening of the micro-crack ( $\Delta Wt$ )650 and apparent percentage of decrease of opening of the micro-crack ( $r_w$ ) for the mortars A

651 and B.

652

<b>Mortar</b>	<b><math>T_0</math> (<math>\mu\text{m}</math>)</b>	<b><math>T_{\text{bio}}</math> (<math>\mu\text{m}</math>)</b>	<b><math>\Delta Wt</math> (<math>\mu\text{m}</math>)</b>	<b><math>r_w</math> (%)</b>
Mortar A	154	22	132	85.7
Mortar B	92	30	62	67.4

653

654

655

656 **Table 2.** Apparent openings ( $\mu\text{m}$ ) 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

<b>Mortar</b>	<b><math>T_{sc}</math> (<math>\mu\text{m}</math>)</b>	<b><math>T_p</math> (<math>\mu\text{m}</math>)</b>
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
<b>Turbidity of medium</b>	+	+	+	+	+
<b>CaCO<sub>3</sub> (%) of solid residue</b>	93.21	95.30	90.64	84.10	88.17

665

666

667

668

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

669

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

672

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

673

674

675