

# Evidence of a bacterial carbonate coating on plaster samples subjected to the Calcite Bioconcept biomineralization technique

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## Abstract

Degradation of historical buildings is mainly due to the intrusion of water which is the main vector of pollutants. Different types of surface treatment have been proposed to avoid or limit this effect. One alternative to chemical treatments is the use of the carbonatogenesis property of some bacteria. This bacterial production has been evidenced on concrete and on limestone samples in an aqueous environment. However, the carbonate production was measured indirectly and the experimental protocol was far from real conditions of use. In this paper, with the same protocol as an industrial one, and using a surface selective investigation method, grazing incidence X-ray diffraction, we show the structural and morphological evolution of the carbonate coating produced on model plaster samples. This substrate was chosen in order to unambiguously detect the bacterial carbonate production.

**Keywords:** Coating; bioremediation; carbonate production; plaster; grazing incidence; X-ray diffraction; bacterial surface treatment; weathering; SEM; microprobe mapping.

## 1 Introduction

Weathering of buildings is a widespread problem encountered in most countries around the world, and concerns buildings made of concrete as well as historic buildings made of stone or brick. These porous materials are subjected to deterioration due to the action of external environmental agents (physical, chemical and biological) [1, 2]. In all cases, water transfer within the whole volume of the porous media is the common point to weathering. To prevent this transfer, several surface treatments have been proposed in the past. The conventional ones involved water repellents that were also used for the chemical reinforcement of stone [3]. In the short term, they give rather good results but can prove harmful in the long term due to their irreversible features. Indeed, these resins polymerize, resulting in a film on the pore surface, but in some cases, depending on the stone, the resin polymerizes inside the pores, completely filling up the porosity [4]. In the latter case, although water transfer

from outside toward the inside of the stones treated by water repellent is totally stopped, water (in its gaseous form) inside the pores can no longer escape and remains near the polymerized resin. And finally, water repellent induced desquamations [5, 3].

Moreover, it is clear that while it is necessary to prevent the intrusion of water into the stone, it is also important to maintain a gaseous exchange between the stone and its environment [6]. To sum up, as complete stone waterproofing is too radical, human action should be confined to limiting water penetration from the exterior, while allowing gaseous transfer in both directions. Furthermore, the current trend is to preserve the original stone without any chemical additives and with a minimum human action. To meet these various criteria, a bio-mineralisation treatment was developed. The initial method, developed by Aldolphe, Castanier and others used the ability of bacteria to induce calcite precipitation [7, 8, 9, 10]. This process has been improved and used industrially by the Calcite Bioconcept company. Other ways to produce calcite have also been investigated. The crystallisation of struvite and calcite by dead cells and cellular fraction of myxococcus xanthus were studied by Rodriguez-Navarro *et al.* [11]. The Biobrush consortium selected bacteria for their ability to firstly remove black crusts and then to precipitate calcite [15], while the Bioreinforce Consortium recommends the application of organic matrix molecules to reinforce the stones. Recently Tiano applied the latter method *in situ* on marble [17]. A review of these bio-techniques can be found in Webster and more recently in De Muynck [18, 19].

The possibility of using this capacity *in situ*, in building conservation practice, was first studied by Levrel *et al.* [20]. The bio-coating was successfully used for the first time on a surface area of 50 m<sup>2</sup> on the south-east tower of the Thouars church (France) in 1993 [21]. Since then, this treatment has been applied on numerous buildings, monuments and houses in Paris and all around France. The properties of the coating were determined by water absorption measurements (Karsten pipe), colorimetry, roughness measurement, macroscopic and microscopic observations of the stone surface and counting of the bacterial population [20]. Two sets of measurements were done on Thouars church, six months and one year after the treatment. Stone powdering and roughness were decreased, no color change was observed and water absorption was decreased by a factor varying between four to eight times. The colorimetry test is important as a prerequisite for the restoration of historic buildings is that a treatment must not alter the color of the building [20].

Furthermore, carbonate crystallization has been highlighted by other indirect methods, using different bacteria colonies [22, 23]. Levrel sprayed limestone cubes with a bacteria solution and daily fed them (during five days) by spraying a nutrient solution onto the stones. The amount of carbonate produced was estimated by weighing the cubes before and after treatment. However, as pointed out by Levrel, the bacteria production was weighed with other crystallized salts present in the nutrient solution, making the absolute calcite quantification rather difficult. De Muynck *et al.* immersed mortar cubes and concrete cylinders for 24 hours in a stock culture of bacteria or covered them with ureolytic culture sludge prior to submersion in different compositions of culture media. The crystallographic form of the crystallized calcium carbonate by X-ray diffraction (XRD) revealed the presence of calcite and also small quantities of vaterite, which is strongly dependent on the composition of the culture media. Similar to many papers on biomineralization on limestone (see Rodriguez-Navarro *et al.*

[11], Jimenez-Lopez *et al.* [24]) De Muynck *et al.* performed biomineralization experiments under optimal conditions in the lab (i.e. in aqueous environment) as proof-of-concept. Furthermore, their procedure improves the amount of produced carbonate rather than the bacterial colony growth and needs to be adapted before it can be applied in practice. Castanier *et al.* used a bacterial sludge on plaster and pulverized a nutrient solution daily during 5 days. They observed a coating using optical microscopy and scanning electron microscopy, and showed that a modification of the surface was obtained [25].

The goal of this paper is to characterize and analyze the phase mineralogy produced by bio-treatment using the recommended industrial process already applied to Thouars church. In this process, *Bacillus cereus* was mainly selected for its rapid growth and important carbonatogenesis properties, as well as for its non-hazardous character for human health and for the environment. In order to limit the development of fungi present on the stone, a fungicide (Natamycine) was added to the nutrient solution (0.125g/l). Laboratory experiments showed that *Bacillus cereus* was resistant to this amount of fungicide and was still able to produce carbonates while a larger quantity affected the bacteria development. Moreover, experimental tests showed that *Bacillus cereus* was resistant to heavy metals [26]. In addition, the precipitate does not produce any stone color change [23].

The paper is organized as follows. In section 2, the choice of the substrate is discussed and then described as well as the bio-treatment process and the experimental techniques which were used to analyze the coating. The results are given in section 3. Discussion and conclusion follow in section 4.

## 2 Material and Methods

The bio-treatment used in this study was applied on plaster. This material is commonly used in building construction and is composed of gypsum crystals. These are tabular and prismatic with a typical size close to 10 micrometers. The plaster came from Soisy-sous-Montmorency and was marketed as “neige n°2”. XRD analysis indicates only the presence of gypsum without any additive. This material was chosen so as to enable the calcite production of the bacteria to be detected unambiguously. As the bio-treatment is expected to produce calcite, it would be difficult to distinguish deposited calcite from a substrate made of calcite such as limestone.

The bio-treatment used in this work, under patent by the Calcite Bioconcept firm, involved *Bacillus cereus*, a bacterium particularly suitable for limestones [23]. Since one of the aims of this technique is to produce a coating of the same nature as the stone substrate, this technique has often been applied on limestones such as tuffeau [27, 28].

For practical reasons, the bacteria were lyophilized in order to be easily used on a building site. The freeze-dried bacteria were first re-hydrated with the nutritive solution recommended by the Calcite Bioconcept firm. Fifteen hours later, the culture medium was sprayed onto the building facade (about 1 liter per m<sup>2</sup>). The bacteria were fed with a nutritive solution 24, 32, 48 and 72 hours after spraying

Table 1: Sample treatments and labels.

| Samples | Bio-treatment          | Elapsed days before analysis | Label |
|---------|------------------------|------------------------------|-------|
| 1       | none                   | –                            | S1    |
|         | treated once           | 50                           | S1t   |
| 2       | treated once           | 110                          | S2t   |
|         | S2t treated once again | 40                           | S2tt  |
|         | S2t washed             | 10                           | S2tw  |
| 3       | treated once           | 7                            | S3t   |
|         | treated once           | 10                           | S3tb  |

of the culture medium. During these three days the bacteria colony exponentially increased. This bio-mineralisation treatment was optimized on limestone so as to be completed within one week, which is one of the restorers' requirements.

Surface samples were firstly observed by using scanning electron microscopy (SEM); in addition a fresh fracture was also scanned in order to obtain depth information. All the specimens were coated with gold prior to observation using secondary electron emission.

X-ray diffraction is a physico-chemical analysis method commonly used to determine the reticular plane distance and the crystalline structure of samples. More particularly, Grazing Incidence X-ray Diffraction (GIXD) is well adapted to the structural analysis of coatings or surfaces of bulk samples [29]. The GIXD system used was configured with a standard Cu-K $\alpha$  laboratory ( $\lambda = 0.15418$  secondary parallel plate collimator followed by a flat graphite secondary monochromatic and proportional detector [30]. Diffraction spectra were obtained at fixed angles  $\Omega$  (incidence angles through  $2\theta$  scan). The experiments presented in this paper were performed with a grazing incidence angle of  $\theta = 0.3^\circ$  and  $\theta = 1^\circ$  and a glancing incidence of  $\theta = 15^\circ$  to modulate the analysis depth from the sample surface. For this experiment on massive samples, the plaster samples were moulded in order to obtain pellets (diameter: 20 mm thickness: 5 mm)

The following chemical elements were mapped with a micro-probe Camebax SX 50 on the surface samples: Calcium (Ca), Sulphur (S), Sodium (Na), Potassium (K), Phosphorus (P), Magnesium (Mg) and Chlorine (Cl). Each map consists of points regularly spaced on 512 rows and columns; for each point, the counting time was 100 ms, for a voltage of 15 kV and a current intensity of 50 nA.

The following 7 samples were treated (1) and then analyzed:

- a reference sample (untreated: S1) that was treated after measurement (S1t),
- a treated sample (S2t), that was broken into two parts after measurement. The first part was treated a second time (S2tt) and the second part washed (S2tw),
- a treated sample for which the centre of the surface sample (S3t) and the border of the same surface sample (S3tb) were analyzed.

## 3 Results

### 3.1 SEM analysis

SEM micrographs for untreated plaster are given on figures 1(a) and (b) for magnifications of 1 000 and 5 000 respectively. They showed tangled gypsum needles giving a rather rough surface. After bio-treatment this surface was strongly modified and far less rough, with crusts covering the gypsum needles, producing a smooth surface plaster 2(a). Furthermore, ovoid bacteria bodies ( $2\ \mu\text{m}$  long and  $0.5\ \mu\text{m}$  wide) due to the mineralization of bacteria membranes (specifically carbonate mineralization [31]) created reliefs on this smooth surface. Moreover, crystal growth was observed after bio-treatment as well as numerous cracks (Fig. 2(b)). These cracks are a common feature of biofilms observed by SEM, and have also been observed on limestone samples. However, this feature is probably an artifact due to the high vacuum needed in the SEM chamber.

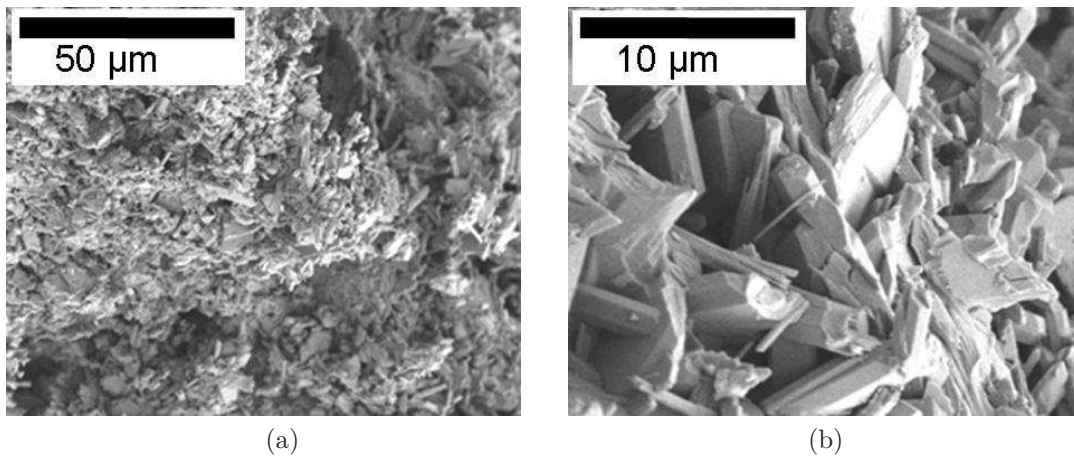


Figure 1: SEM image (secondary electron) of a plaster surface before bio-treatment (a) 1000 $\times$ magnification, (b) 5000 $\times$ magnification.

### 3.2 GIXD analysis

As shown by SEM micrographs (Fig. 3), the treatment produced a coating. Consequently GIXD was used in order to obtain information about the structure modifications of the plaster material surface and especially the crystalline nature of the deposit layer. The samples were mounted on the sample holder in exactly the same position and orientation before and after bio-treatment. This precaution made it possible to obtain an identical substrate diffraction contribution before and after bio-treatment and thus to shed new light on the coating composition. This precaution was taken for all successive analyses on the same sample.

Figure 4 shows the GIXD spectra of an untreated (S1) and bio-treated (S1t) sample. The GIXD spectrum of the untreated plaster (S1) showed only typical gypsum peaks without any calcite contribution, whereas the treated sample also shows calcium carbonate peaks (here vaterite), halite and

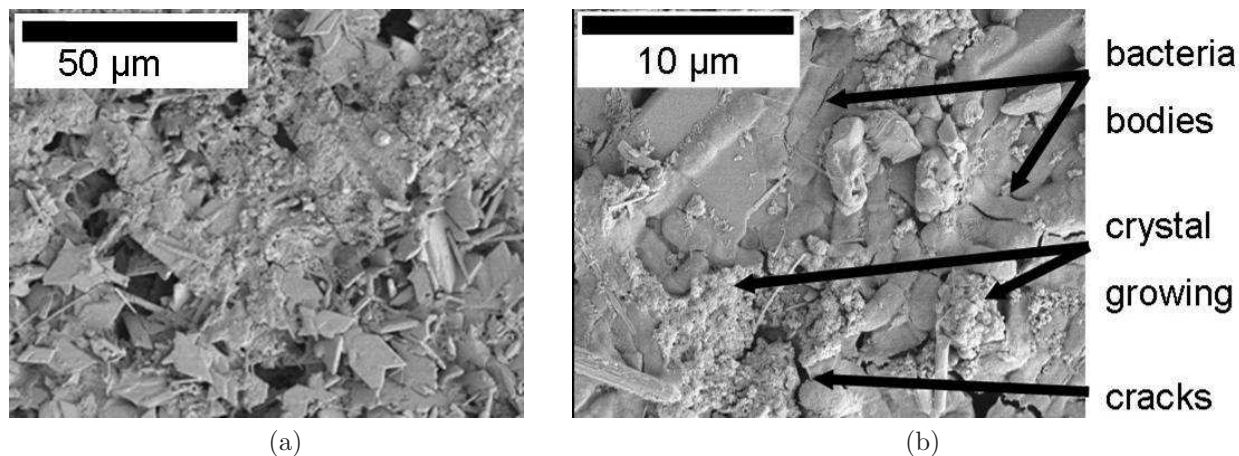


Figure 2: SEM image (secondary electron) of a plaster surface after bio-treatment (a) 1000×magnification, (b) 5000×magnification.

sylvite peaks. In addition, figure 4 exhibits the decrease in the gypsum surface contributions with respect to the untreated sample as a consequence of the formation of a coating by the treatment. Similar results were obtained for all the other analyzed samples, except that calcite was obtained instead of vaterite which was no longer detected.

To emphasize the effect of the treatment, it was applied on sample S2t a second time. The GIXD spectrum of this twice treated sample (S2tt) shows an increase in the amplitude of the calcite peaks, compared to the sample treated once only (S2t) (Fig. 5(a)). In addition the amplitude of the halite peaks also increases, those of the sylvite peaks remains almost the same, while the response of the substrate decreases as already observed for samples S1 and S1t.

High proportions of salts within the porous lattice of a building material can lead to severe damage due to the local force of the crystallization pressure (*e.g.* [32, 33, 34]). As rain is thought to discharge the salts from the surface, the coating was therefore washed in order to simulate the effect of rain. Sample S2t was immersed in distilled water for 30 seconds. The water was changed and the sample immersed again for 30 seconds. The sample was then dried in an oven during 12 days at 35°C and then analyzed. Its GIXD spectra (Fig. 5(b)) showed lower halite peaks, in comparison with S2t spectra while sylvite peaks were completely removed. This can obviate the salt problems due to bio-treatment. Furthermore, calcite peaks remained, indicating a rather good durability of the coating.

In order to have information about both the surface homogeneity of the treatment and its time evolution, a sample was analyzed only 7 days after bio-treatment on two distinct places: at the centre of the surface sample (S3t) and at its border (S3tb) (Fig. 6). We observed that the peak intensity of the salts is higher at the border than in the center. This is in agreement with a drying of the sample from the border to the center, implying both a preferential salt crystallization on the border and diffusion of the concentration of the saline solution from the center to the border. This effect does not

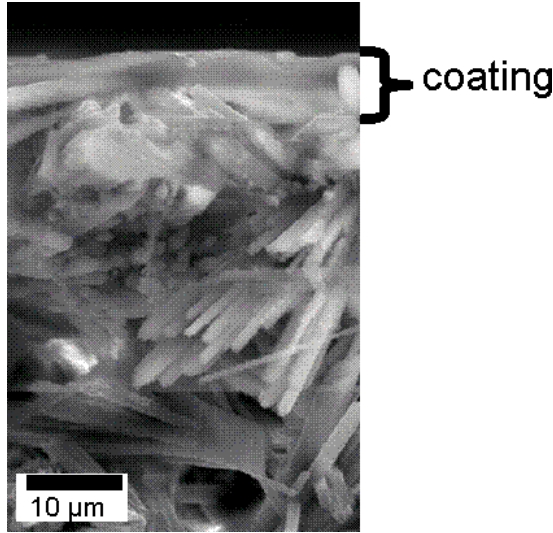


Figure 3: SEM image of a plaster sample section. Under the surface, needles of gypsum appear clearly while the coating is visible (on the top of the image).

appear for the calcite for which the peak sizes are almost the same for the two places, showing that the calcite deposit is constant. It is also interesting to note that even after 7 days, we observed the same peaks (sample S3t) as for an older treated sample (S2t), indicating that the whole mineralogy is established at the very beginning of the process. Other samples, such as sample S4t, 17 days aging, yielded the same results.

It is also possible to obtain depth information by varying the grazing incidence. Increasing grazing incidence will also increase probe depth. Sample S2t was analyzed again with an incidence angle of  $\theta = 15^\circ$ . Taking into account the X-ray absorption of gypsum, an analytical calculation gives an estimation of the probe depth of about  $1\ \mu\text{m}$  for  $\theta = 1^\circ$  and  $10\ \mu\text{m}$  for  $\theta = 15^\circ$ . The diffraction lines shown on figure 7 correspond to the contributions of phases in the 1 micrometer layer and 10 micrometer layer from the surface. We estimated the depth evolution of the calcite and salts phases from the variations in integrated intensities. The (h,k,l) peaks of calcite (113), sylvite (200), halite (200) and gypsum (022) were chosen because they are isolated without overlapping with other phases. The intensity ratios  $I_{\text{calcite}}/I_{\text{gypsum}}$ ,  $I_{\text{sylvite}}/I_{\text{gypsum}}$  and  $I_{\text{halite}}/I_{\text{gypsum}}$  for both incidence angles were calculated. We obtained:

$$\begin{aligned} \left. \frac{I_{\text{calcite}}}{I_{\text{gypsum}}} \right|_{15^\circ} &= 0.3 & \left. \frac{I_{\text{calcite}}}{I_{\text{gypsum}}} \right|_{1^\circ} &= 1.1 \\ \left. \frac{I_{\text{sylvite}}}{I_{\text{gypsum}}} \right|_{15^\circ} &= 10 & \left. \frac{I_{\text{sylvite}}}{I_{\text{gypsum}}} \right|_{1^\circ} &= 16 \\ \left. \frac{I_{\text{halite}}}{I_{\text{gypsum}}} \right|_{15^\circ} &= 0.5 & \left. \frac{I_{\text{halite}}}{I_{\text{gypsum}}} \right|_{1^\circ} &= 3. \end{aligned}$$

The calcite/gypsum and salts/gypsum ratios decrease with depth, demonstrating that salt and calcite are present only in the superficial layer. As already mentioned, the validity of these ratios holds

only for the few first 10 micrometers from the surface. Moreover, the decrease is extremely strong in the halite salt case compared to the sylvite salt case. The results suggest that the halite salts were located on the carbonate coating and not within the porosity of the gypsum substrate, whereas the sylvite is rather in an intermediate position layer.

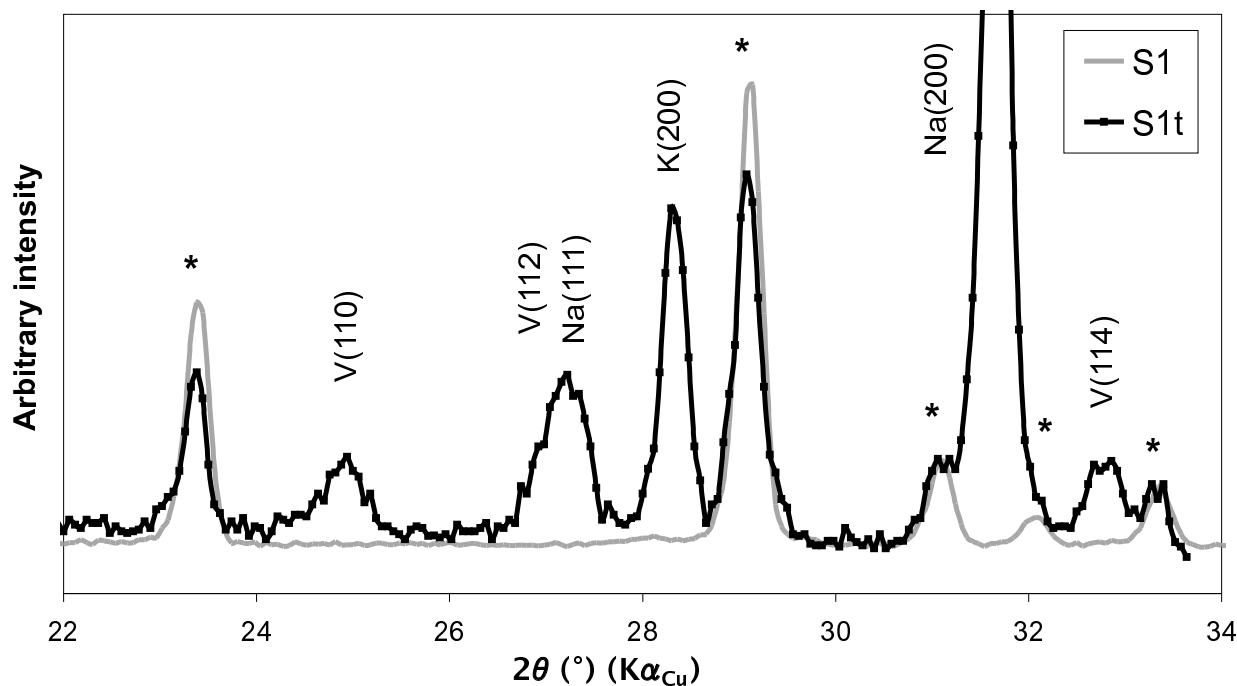


Figure 4: GIXD spectra for  $\theta = 0.3^\circ$  of sample 1. Continuous line untreated sample (S1) and dotted continuous line bio-treated (S1t). Gypsum, Vaterite, Halite and Sylvite peaks are labeled with stars (\*) for the former and capital letters V, Na and K respectively for the others. The associated numbers in parentheses correspond to diffraction  $(h, k, l)$  lines.

### 3.3 Microprobe analysis

Microprobe analysis was performed on treated and untreated samples on sulphur and calcium. Figures 8(a) and (b) give microprobe cartographies of calcium and sulphur respectively for a polished section of a treated sample. Figure 8(a) reveals the presence of a rather homogeneous calcium layer, clearly visible on the surface (right side of 8(a)), with a thickness of roughly  $10 \mu\text{m}$ . The comparison between figure 8(a) and (b) shows that this layer corresponds to an area in which almost no sulfur is present, meaning that this calcite layer was formed on the surface of the sample. However, this new calcite layer has a low intensity compared to values obtained inside the sample, and it is difficult to infer the depth penetration of the treatment from this information alone. The untreated sample does not present this feature and the distribution of both sulphur and calcium is homogeneous as expected. Although

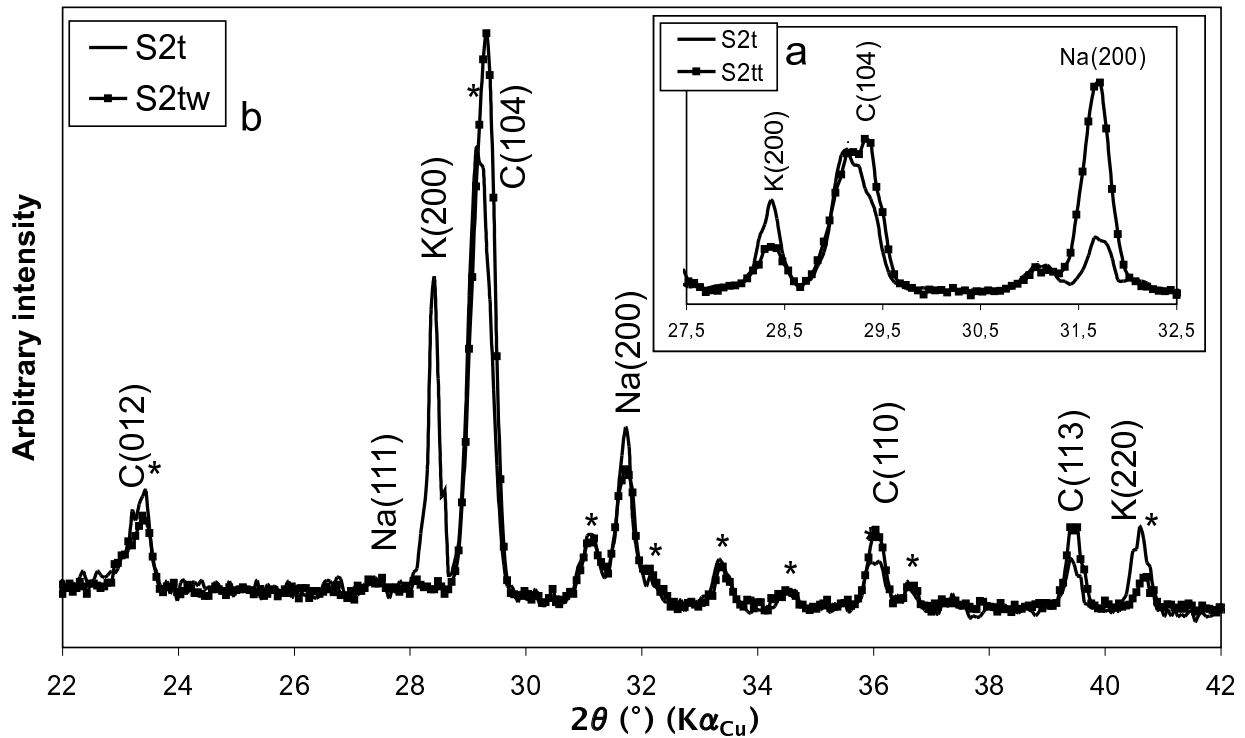


Figure 5: (a) GIXD spectra for  $\theta = 0.3^\circ$  of sample 2 treated once (S2t) and treated twice (S2tt). (b) GIXD spectra for  $\theta = 1^\circ$  of sample 2 treated once (S2t), then washed (S2tw). The peaks are labelled as in figure 4 and letter C stands for Calcite.

microprobe analysis does not give information of a mineralogical nature, it is a complementary tool to GIXD because it provides a depth description. However, the microprobe analysis confirms the surface results obtained by GIXD.

### 3.4 Water absorption measurements

Water absorption measurements were approached by measuring the absorption of a drop by the surface of a treated and untreated sample. 0.2ml of distilled water were placed on the surface of the stone and the time necessary for complete water absorption was measured. These experiments were done on five points for each sample and the average time is as follows: for the untreated stone, absorption was almost immediate (less than 2 s) while for the treated stone the drop kept its shape for about 20 s (like a water drop on a hydrophobic surface) and was completely absorbed after 30 s. It is clear that the water properties were modified by the treatment.

## 4 Discussion and conclusion

A bio-treatment was performed in the laboratory on plaster samples following an industrial process. The coating produced was observed by SEM, and analysed by microprobe X and GIXD. First of

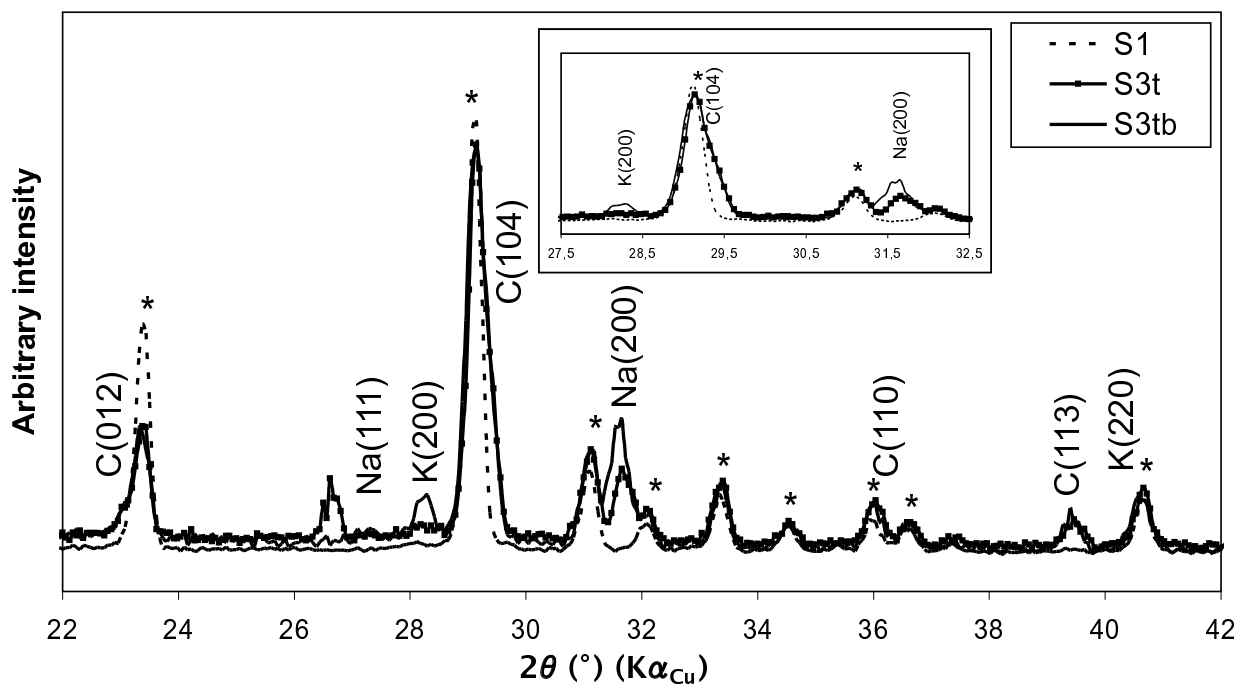


Figure 6: GIXD spectra for  $\theta = 0.3^\circ$  of sample S3t and S3tb analyzed on the center (dotted continuous line) and on the border (continuous line) respectively. The dotted line gives the GIXD spectra of the untreated sample S1 as a reference. The encapsulated picture is a zoom of the spectra. The peaks are labelled as in figure 5.

all, *Bacillus cereus* bacteria (and the established industrial protocol) produced calcite coatings on plaster surfaces. Compared to the sample treated once only (S2t), the sample treated twice (S2tt) presented more calcite coating, showing that calcite was created at each treatment. Moreover, the GIXD technique enabled the crystallographic composition of the coating to be clearly displayed, making it possible to analyze thin surface layers.

Despite the fact that the bio-treated samples were analyzed with different aging times, the crystal mineralogy remained the same. Indeed, except in one case, the only polymorphic state of carbonate calcium observed was calcite. No transitory state such as vaterite, which has been recognized to be a transient polymorph of calcite, was observed [35, 36, 37]. In one case, vaterite was found (aging: 53 days) but was not found again on other samples. A possible explanation for this could be that the environmental conditions of treatment were insufficiently controlled (humidity, temperature ...) in the laboratory, leading to vaterite formation. However these variations could also be expected to occur in an outdoor environment and consequently to produce different carbonate polymorphs on treated building frontages.

The main problem caused by the process was salt deposit (coming from the feeding solution) on and within porous materials. This study shows that simply washing the sample (rain in the real case of buildings) sufficed to remove these salts to a large extent. Nevertheless the calcite coating was still

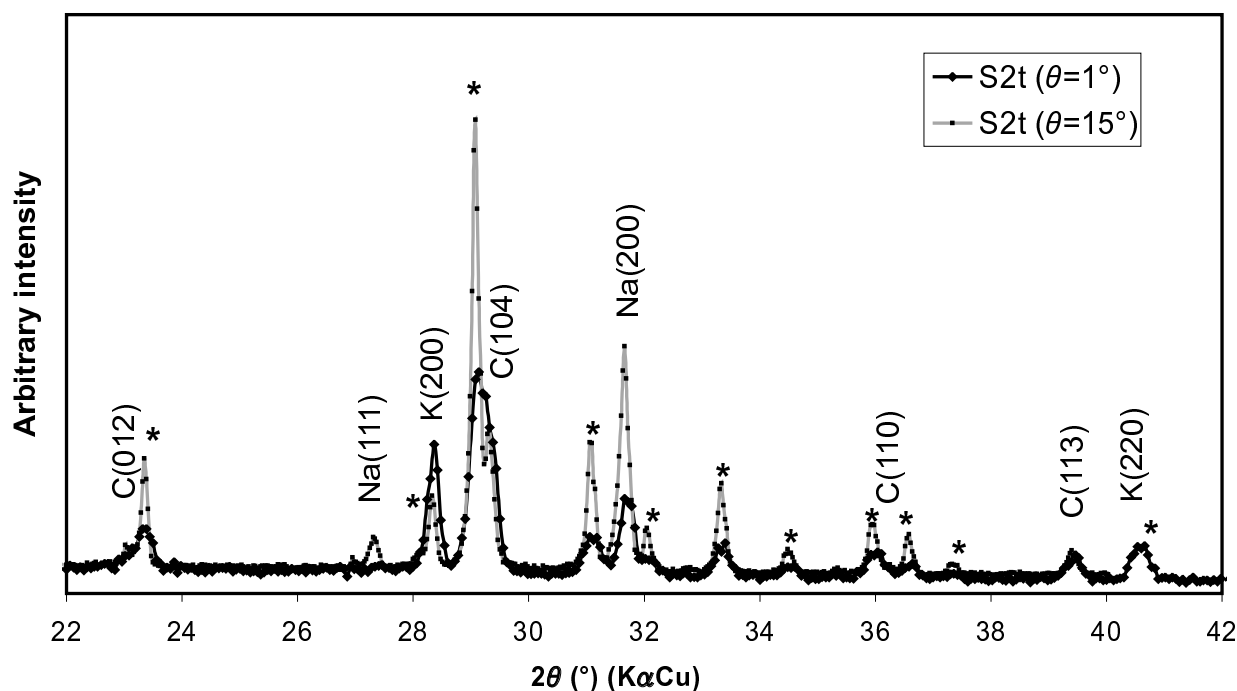


Figure 7: GIXD spectra for  $\theta = 1^\circ$  (continuous line) and  $\theta = 15^\circ$  (dotted continuous line) of sample 2 treated once (S2t). The peaks are labelled as in figure 5.

present and was not removed. Then, once washed, the bio-treated surface was almost salt-free and the surface porosity should be reduced due to the calcite deposit. However durability tests should be carried out in order to control the efficiency of the treatment over several years.

GIXD spectra obtained on different areas of the same sample (S3 and S3b) show a higher concentration of halite and sylvite on the edges than in the center. This could be explained by the fact that the sample dried more quickly at the edges than in the center, creating favorable thermodynamic conditions for salt precipitation. This border effect, due to the shape of the building blocks could also appear *in situ*.

Initial measurements on water absorption indicate that water penetration was reduced for a treated sample, suggesting that the surface porosity was reduced, though this point needs to be controlled by porosity measurements. However, as the coating is  $10\ \mu\text{m}$  thick, intrusive porosity measurements (*e.g.* mercury porosimetry) would require extracting  $10\ \mu\text{m}$  thick slices in order to follow the depth porosity change. This is obviously not possible (without any resin induration) and an efficient way to measure this porosity can be obtained by image analysis. This requires access to X-ray tomography with high resolution ( $0.25\ \mu\text{m}$ ) only accessible nowadays on synchrotrons. This study will be the subject of future work and of a forthcoming publication.

Finally, as water absorption is reduced, this conservation technique appears to be perfectly efficient

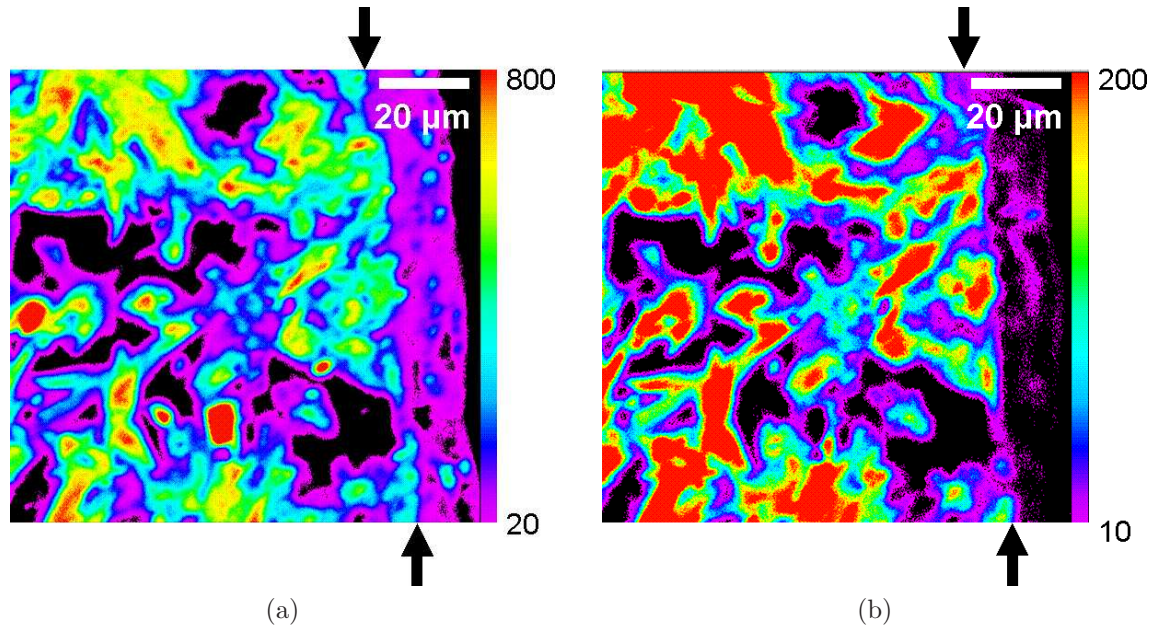


Figure 8: Microprobe cartographies of calcium (a) and sulphur (b) of a section of a treated plaster sample. The treated surface corresponds to the right side of the pictures, indicated by arrows. The microprobe measured intensity is given next to the color scale of the pictures.

on plaster and this study should be extended to other porous building materials. Indeed, as this industrial protocol was recommended for limestones, future studies will be carried out on these building materials, which will involve addressing the difficulty of distinguishing bio-calcite from calcite limestone. A preliminary SEM study on bio-treated tuffeau showed the same kind of surface coating as the plaster coating. In addition, this study proves that grazing incidence X-ray diffraction, commonly used for low roughness ( $< 10$  nm) material surfaces, is well adapted to highly porous and granular mineral material.

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## References

- [1] Camuffo D. Physical weathering of stones. *Science of The Total Environment* 1995;167(1-3):1–14.

- [2] Gaspar PL, De Brito J. Quantifying environmental effects on cement-rendered facades: A comparison between different degradation indicators. *Building and Environment* 2008;43(11):1818–1828.
- [3] Amoroso GG, Fassina V. *Stone decay and conservation : Atmospheric Pollution, Cleaning, Protection*, Amsterdam:Elsevier, 1983, 453 p
- [4] Perrier R. *Les roches ornementales*, Pro Roc, 2004, 704 p
- [5] Camaiti M, Borselli G, Matteoli U. Prodotti consolidanti impiegati nelle operazioni di restauro. *Edilizia* 1988;10:125–134.
- [6] Černý R, Drchalová J, Hošková Š, Toman J. Methods for evaluation of water-proofness quality and diffusion properties of coating materials. *Construction and Building Materials* 1996;10(8):547–552.
- [7] Adolphe J-P, Billy C. Biosynthèse de calcite par association bactérienne aérobie. *C.R. Acad. Sc. Paris D* 1974;278:2873–2875.
- [8] Castanier S, Maurin A, Bianchi A. Participation bactérienne à la production du carbonate. *C.R. Acad. Sc.* 1984;299(19):1333–1336.
- [9] Billy C. Isolement des constituants d'une association bactérienne productrice de calcite. *C.R. Acad. Sc. Paris* 1975;281:621–623.
- [10] El Kahoui R., Adolphe J.-P., Daudon M., Identification of Early Bacillus-induced Crystals in vitro Using Fourier Transform Infrared Spectroscopy, *Microbes and Environments* 2000;15(3):161–171.
- [11] Rodriguez-Navarro C, Rodriguez-Gallego M, Ben Chekroun K, Gonzalez-Muñoz M T. Conservation of Ornamental Stone by Myxococcus xanthus-Induced Carbonate Biomineralization, *Appl. Environ. Microbiol.* 2003;69(4):218–2193.
- [12] De Muynck W, Debrouwer D, De Belie N, Verstraete W. Bacterial carbonate precipitation improves the durability of cementitious materials. *Cement and Concrete Research* 2008;38(7):1005–1014.
- [13] Adolphe JP *et al.* Etude expérimentale de la redistribution du  $^{45}\text{Ca}$  par intervention des microorganismes, *Spelunca Mémoires* 1985;14:106–108.
- [14] Rodriguez-Navarro C., Jimenez-Lopez C., Rodriguez-Navarro A., Gonzàlez-Muñoz, M.T, Rodriguez-Gallego M. Bacterially mediated mineralization of vaterite *Geochim. Cosmochim. Acta* 2007;71(5):1197–1213.
- [15] May E., *Biobrush research monograph : novel approaches to conserve our European heritage.* EVK4-CT-2001-00055 2002-2005
- [16] <http://www.ub.es/rpat/bioreinforce/bioreinforce.htm>

- [17] Tiano P., Cantisani E., Sutherland I., Paget J.M., Biomediated reinforcement of weathered calcareous stones, *J. Cult. Herit.* 2006;7:49–55.
- [18] Webster A. and E. May, Bioremediation of weathered-building stone surfaces, *Trends Biotechnol.* 2006;24(6):255–260.
- [19] De Muynck W., De Belie N., Verstraete W., Microbial carbonate precipitation in construction materials: A review, *Ecol. Eng.* 2009, doi:10.1016/j.ecoleng.2009.02.006.
- [20] Le Métayer-Levrel G., Castanier S., Oriol G., Loubière J.-F., Perthuisot J.-P., Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in building and historic patrimony, *Sedimentary geology* 1999;126(1-4):25–34.
- [21] Le Métayer-Levrel G, Castanier S, Oriol G, Loubière J-F, Perthuisot J-P. From Carbonatogenesis concepts to bacterial regeneration of limestones (microbial lifting). in: IAS-ASF-IGCP 380 International Workshop on “Microbial mediation in carbonate diagenesis”. Chichilianne 22-24/09/97, Abstract book, Publication ASF, Paris, 26, 1997.
- [22] De Muynck W, Cox K, De Belie N, Verstraete W. Bacterial carbonate precipitation as an alternative surface treatment for concrete. *Construction and Building Materials* 2008;22(5):875–885.
- [23] G. Le Métayer-Levrel, Microbiogéologie du carbonate de calcium: applications industrielles, implications géologiques, Ph.D. Thesis, discipline : Sciences de la Terre, Université de Nantes, 1996.
- [24] Jimenez-Lopez C, Rodriguez-Navarro C, Piarñar G, Carrillo-Rosúa FJ, Rodriguez-Gallego M, Gonzalez-Muñoz M T. Consolidation of degraded ornamental porous limestone stone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. *Chemosphere* 2007;68(10):1929–1936
- [25] Oriol G, Levrel G. Première approche de traitement de surface du plâtre par biominéralisation. In: *Le plâtre : l’art et la matière*, Ed. Georges Barthe, Creaphis 2002, pp 347–355, ISBN 2-913610-19-6
- [26] Loubière J-F, personal communication.
- [27] Dessandier D, Antonelli F, Rasplus, L. Relationships between mineralogy and porous medium of the craie tuffeau. *Bulletin de la Société Géologique de France* 1997;168(6):741–749.
- [28] Rozenbaum O, Le Trong E, Rouet J-L, Bruand A. 2-D image analysis: A complementary tool for characterizing quarry and weathered building limestone. *Journal of Cultural Heritage* 2007;8(2):151–159.
- [29] Andreatza P, De Barros M I, Andreatza-Vignolle C, Rats D, Vandebulcke L. In-depth structural X-ray investigation of PECVD grown diamond films on titanium alloys. *Thin Solid Films* 1998;319(1-2):62–66

- [30] Andreatza P, Andreatza-Vignolle C, Kante I, Devers T, Levesque A, Allam L. Buffer layer effect in nanostructured tin electrodeposition on insulating and conducting substrates. *Progress in Solid State Chemistry* 2005;33(2-4):299–308
- [31] Castanier S, Le Métayer-Levrel G, Perthuisot J-P. Ca-carbonates precipitation and limestone genesis – the microbiogeologist point of view. *Sedimentary Geology* 1999;126(1-4):9–23.
- [32] Benavente D, García del Cura M-A, Ordóñez S. Salt Influence on evaporation from porous building rocks. *Construction and Building Materials* 2003;17(2):113–122.
- [33] Scherer GW. Stress from crystallization of salt. *Cement and Concrete Research* 2004;34(9):1613–1624.
- [34] Cardell C, Benavente D, Rodríguez-Gordillo J. Weathering of limestone building material by mixed sulfate solutions. Characterization of stone microstructure, reaction products and decay forms. *Materials Characterization* 2008;59(10):1371–1385.
- [35] Spanos N, Koutsoukos PG, The transformation of vaterite to calcite: effect of the conditions of the solutions in contact with the mineral phase. *Journal of Crystal Growth* 1998;191(4):783–790.
- [36] Katsifaras A, Spanos N. Effect of inorganic phosphate ions on the spontaneous precipitation of vaterite and on the transformation of vaterite to calcite. *Journal of Crystal Growth* 1999;204(1-2):183–190.
- [37] Wolf G, Königsberger E, Schmidt H., Königsberger L-C, Gamsjäger H. Thermodynamic aspects of the vaterite-calcite phase transition. *Journal of Thermal Analysis and Calorimetry* 2000;60:463–472.