Biocatalytic Processes on Concrete: Bacterial Cleaning and Repair

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ABSTRACT

Biological techniques for cleaning and repair of concrete and stone surfaces can be an ecological alternative for traditional conservation techniques.

Concrete specimens weathered for over a decade in the Belgian moderate climate, showing a black organic outer layer, mainly consisting of lichens, were cleaned with a new biological technique. The weathered samples, made with Portland cement or with blastfurnace slag cement, were treated with *Thiobacillus* bacteria and an appropriate nutrient, by submersion or sprinkling. The *in situ* production of acid metabolites resulted in a cleaning effect, which was documented by the use of colorimetry and microscopy. Differences in the effectiveness were seen between concrete samples of a different cement type. The sprinkling treatment was quite effective for regaining the original appearance of the concrete surface. A side effect was the formation of a gypsum layer on some of the specimens.

For remediation of decayed stone and concrete, biomineralisation by *Bacillus sphaericus* was investigated. The best microbial strains were selected based on criteria such as the amount of the microbiologically produced calcium carbonate, adhesion of the created layer to the stone surface and change in capillary water absorption. Next to application of pure bacteria cultures, mortar samples of different porosity were also treated with ureolytic sludge. The most pronounced reduction in water absorption was reached for the most porous mortar samples. When urea, nutrient broth and an external calcium source were provided, the amount of water absorbed by the mortar samples after 200 hours was decreased by a factor 5 compared to untreated samples. This treatment also caused a significant reduction in total porosity. SEM and XRD analyses showed that a dense layer of calcite and vaterite crystals was deposited on the mortar surface.

KEYWORDS

Biological cleaning, biomineralisation, calcium carbonate precipitation, concrete, bacteria
1 INTRODUCTION

Concrete weathering is a complex process and may include physical, chemical and biological factors. Weathering can induce an increased porosity and structural degradation of the surface layer and can result in an unattractive appearance. A proper cleaning procedure should not only be regarded as an aesthetical operation, but can also increase the service life of building materials. The cleaning of building façades is a delicate operation that can bring about irreparable damage if not carefully performed using appropriate techniques. Aesthetical damage traditionally is repaired by a combination of physical and chemical cleaning. In forensic practice, damage cases such as excessive abrasion, staining, deposition of soluble salts, and biological growths, resulting from the inaccurate use of cleaning procedures, are frequently encountered [e.g. Maxwell 1992; Young & Urquhart 1992; Warscheid & Braams 2000]. With the use of traditional chemical products, there is a risk of pollution, especially when the formulation of the cleaning product is unknown, or when products and rinsing water are discharged directly in the sewer. Moreover, cleaning personnel working with organic solvents may suffer from irritation of the eyes and of upper respiratory tract [Anundi et al. 2000].

As an alternative for chemical and physical cleaning techniques, with their described disadvantages, new biological methods have been proposed. Hempel [1978] was one of the first to address the possibility of biological cleaning. He noted the effectiveness of a clay poultice containing urea and glycerol and proposed that microorganisms were at least partially responsible. Kouzeli [1992] has reported favourably on the technique in comparison with pastes based on EDTA or ammonium bicarbonate. Several authors focused on the application of Desulfovibrio in the reconversion of gypsum crusts into calcite [Heselmeyer et al. 1991; Gauri et al. 1992]. For the removal of sulphates, nitrates and organic matter from artistic stone works, carefully selected microbial cultures have been used [Ranalli et al. 2000]. A similar methodology has been applied for the elimination of insoluble calcium oxalate patinas from monuments [Tiano et al. 1996]. Enzymes, such as lipase, have been successfully used to remove aged acrylic resin coatings in paintings [Bellucci et al. 1999]. Besides the mentioned cleaning techniques, several research groups over the world are performing tests on a microbially induced protective calcium carbonate layer. Different species are being used for this purpose: Bacillus cereus by the French group, Bacillus pasteurii by the Americans, Myxococcus xanthus by the Spanish group, and Bacillus sphaericus by our Belgian research team [Tiano et al. 1999; Bang et al. 2001; Castanier et al. 1999; Rodriguez-Navarro et al. 2003; Hames et al. 2003]. However, since test conditions and parameters measured are not the same for different investigations, it is difficult to compare the capacities of the different species.

In the present article, a new cleaning procedure will be proposed, using bacteria of the genus Thiobacillus with an appropriate nutrient, in order to clean fouled concrete surfaces. Furthermore, concrete repair through biomineralisation by Bacillus sphaericus will be discussed.

2 MICROBIOLOGICAL CLEANING OF CONCRETE SURFACES

2.1 Principle

A new biological cleaning technique for concrete, using a biological sulphur solution called Thio-S is under development in our laboratories. Thio-S is a mixture of sulphur oxidising bacteria of the genus Thiobacillus with appropriate microbial nutrients. Thiobacilli are able to produce energy out of the oxidation of elementary sulphur and reduced inorganic sulphur bonds. Thiobacilli are acidophilic, or acid tolerant bacteria and are able to fix CO₂. The end product of the oxidation executed in their metabolism is sulphuric acid [Vincke et al. 1999]. The species T. thiooxidans can survive values below pH 1. The application of Thio-S on fouled concrete, results in acid production in situ, and in a local cleaning action. Since the organisms involved are very sensitive to desiccation and since further colonisation of building stones depends on the presence of reduced sulphur compounds, the application of Thio-S on façades can be adequately controlled.
2.2 Materials and methods

2.2.1 Concrete specimens

Two concrete cubes with sides of 200 mm, were weathered for over 10 years in a Belgian outside climate. One of the cubes was composed of blast furnace slag cement (BFS), the other was composed of ordinary Portland cement (OPC). The cubes were fouled with lichens and atmospheric pollution, forming a black patina. Using a reaction with potassium hydroxide and microscopic investigation, the fouling on the OPC cubes was characterized as *Lecanora albescens*, a white lichen common on mortars and calcareous stone; and *Candelariella aurella*, a dark grey crust with orange fruit bodies (apothecies). On the BFS cubes, only *Candelariella aurella* was found, but in slightly less dense crusts. Small concrete cubes with sides of approximately 4 cm, were sawed out of these cubes with a diamond cut saw. These small specimens had at least one fouled plane.

2.2.2 Biological sulphur solution

An active consortium of bacteria was selected by inoculation of biofilm material scraped off a corroded sewage pipe in mineral medium M35 (De Graef et al., 2003) and by repeated transfer of 10 ml of an acidified culture in the early stationary phase as inoculum to a new culture of 1 L at 28 °C. This procedure was repeated 4 times in triplicate until the fastest acidification rate was reached. The nutrient consisted of 10g/l powdered sulphur (S), 0.1 g/l NH₄Cl, 3.0 g/l KH₂PO₄, 0.1 g/l MgCl₂.6H₂O and 0.14 g/l CaCl₂.2H₂O. The dissolved oxygen amount of the medium was set at a minimum of 5 mg/l. In previous experiments, the Thio-S consortium had been applied to concrete samples as an acidic cell suspension, when the micro-organisms had reached late stationary phase, at an ambient temperature of 20 °C. The pH at application amounted to 1.0 – 1.2. In the current experiment, the aim was to quantify the biological effect, i.e. the production of metabolites *in situ*. The cell mass of a culture in the early exponential growth phase was harvested by centrifugation (10 min at 5000 x g) and put into fresh medium, with an initial pH of 7. During the test, which was run at 28 °C, the acidification was monitored.

2.2.3 Test procedure

In a previous experiment, 5 cubes from each set of concrete samples were immersed in an acidified Thio-S solution of pH 1.0-1.2. Another 5 cubes were immersed in water in such a way that only the fouled surface surmounted the water level with about 1 mm. Through capillarity, this surface remained continuously moist. On this surface, Thio-S was sprinkled with a brush four times a day. Simultaneous to this treatment, 3 cubes of each set were completely immersed in water as a control, and 3 cubes of each set were immersed in a sulphuric acid solution of the same initial pH as the Thio-S solution. These treatments all had a duration of three days per cycle. Three cycles were performed, in an atmosphere of 20 °C and 60 % relative humidity (RH). The pH of the Thio-S with the immersed concrete cubes rose during the test because of the high alkalinity of the concrete. The average concentration of sulphate after a cleaning cycle of three days in Thio-S fluid was 9.9 g SO₄²⁻/l. After each treatment cycle, the cubes were dried for 4 days in an atmosphere of 35 °C and a RH of 40%. These experiments indicated that the proposed technique was 30 to 100% more effective on concrete with ordinary Portland cement than on blast furnace slag cement samples. The sprinkling treatment was about 50% as effective as the submersion treatment (which had in turn a similar or higher effectiveness than submersion in sulphuric acid solution), but still had a good cleaning potential and had only an effect on the outer material surface. In the current test set-up it was the aim to apply a solution of neutral pH, being safer to work with for the cleaning personnel. Biomass at the early exponential growth phase was harvested, suspended in fresh medium of neutral pH, and applied at 28 °C, through immersion or through sprinkling as described above. The test cycle was stopped after nine days, since the exponential growth phase had ended at that time (end of active acidification). After this, a second cycle was carried out, where the centrifuged biomass in neutral medium was put
directly on the samples used for the sprinkling treatment, and where only the medium was sprinkled intermittently as described above.

2.2.4 Measurements to quantify the effect of cleaning

A X-rite SP60 colorimeter with a circular measurement area of 8 mm diameter was used to obtain spectral reflectance graphs of the fouled surfaces. The relative reflectance of light with wavelengths ranging from 400 nm to 700 nm was measured per 10 nm. The specular component of the reflected light was excluded. At the beginning of the test cycles, and after the drying period of 72 hours at 35 °C following each treatment cycle, three reflectance measurements were taken, evenly distributed over the fouled surface of each concrete cube. The mean reflectance curve of these three measurements was obtained, as an indication of the degree of fouling. In the case of concrete, the measured colours are all grey values, which results in a more or less horizontal reflectance graph. As an effect of the cleaning, the appearance of the concrete becomes lighter grey. Therefore the measured effect of the cleaning should be a higher curve, more or less parallel to the one of the fouled concrete and approaching the reflectance curve of clean concrete. An approximation of the values of clean concrete was made by measuring the saw planes in between the aggregates. The spectra can also be represented by tristimulus values, based on the spectral sensitivity of the human eye (CIE definition). In this case L* a*b* values under Standard Illuminant D65 (daylight standard) were chosen for the 10° standard observer (CIE 1964 supplementary colorimetric observer). The L* values range from 0 to +100 and respectively represent black and white. The negative and positive a* values represent green and red, respectively. The negative and positive b* values represent blue and yellow, respectively. These values can be plotted in a Cartesian co-ordinate system, called the L*a*b* colour space, with the a* and b* axes in the horizontal plane, and the L* axis perpendicular to that. In this colour space a colour difference can be expressed as the distance between the points of two colours

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}.$$  

In the case of concrete, the colour difference $\Delta E_{ab}^*$ is a distance more or less parallel to the L* axis. A greater colour difference between fouled and cleaned concrete means a lighter grey value, with more reflectance, and indirectly indicates the effectiveness of the cleaning of the concrete surface.

Viability staining was performed using commercial live/dead stain (L-13152, Molecular Probes, Leiden, The Netherlands). This stain allows fluorescence microscopy to distinguish between organisms with intact cell membranes (stained green and scored alive) and organisms with damaged cell membranes (stained red and scored dead). 25 µl stain was put directly on 1 cm² of mortar surface and was incubated for 10 min in the dark and examined by standard epifluorescence microscopy. The microscope was equipped with a Peltier cooled single chip digital colour CCD camera and connected to a PC to obtain digital images. For each treatment, two preparations were examined under fluorescent microscopy.

2.3 Results

In Fig. 1, the average colour difference $\Delta E_{ab}^*$ between fouled and cleaned concrete is shown for two Thio-S treatment cycles with acidification in situ. Ordinary Portland cement samples and blast furnace slag cement samples are portrayed with their respective reference values, i.e. the mean colour difference between the fouled surface and the colour of the saw planes measured in between the aggregates. After one cycle of nine days, an effect was noticeable on the specimens of the sprinkling treatment. No significant effect was apparent on the specimens that underwent the immersion treatment. This could be due to limited diffusion of CO₂ and O₂ to the submerged samples. Presumably, the sprinkling treatment provided a good environment for the organisms to settle and to perform their cleaning action, provided that the humidity was sufficient. This cleaning cycle had a duration of 9 days, because the acidification was monitored during the test (Table 1), and the test was stopped at the end of the exponential growth phase (end of active acidification). After this, a second
cycle was carried out, where the centrifuged biomass was put directly on the samples used for the sprinkling treatment, and where only the medium was sprinkled on intermittently as described above. For the immersed treatment, which was performed in the same way as the first cycle, this cycle was more effective than the first cycle, although the effect remained small. On the other hand, for the sprinkling treatment, the reference value, i.e. the mean colour difference between the fouled surface and the colour of the saw planes measured in between the aggregates, was quite well approached after this second cycle, for OPC as well as for BFS specimens. Table 1 shows that acidification was limited in the second cycle of the submersion treatment. The results suggest however that acidification did take place in the case of the second sprinkling cycle.

Clusters of live cells could be detected on the mortar cubes treated with the Thio-S culture, which were not seen on untreated specimens. These cells were present as groups of attached individual cells and also as organised in biofilm structures. It was proposed that these cells represented active *Thiobacillus* sp. cells from the Thio-S culture. The results were similar for Portland and blast furnace slag cement samples. These results suggest that the micro-organisms can use the concrete as a substratum and that they can locally produce sulphuric acid and as such exert a cleaning effect.

### 3 MICROBIOLOGICAL REPAIR OF CONCRETE SURFACES

#### 3.1 Principle

The general term biomineralisation refers to biologically induced mineralization in which an organism creates a local micro-environment, with conditions that allow optimal extracellular chemical precipitation of mineral phases [Hamilton 2003]. *Bacillus sphaericus* is able to precipitate CaCO₃ on its cell constituents and in the environment by degradation of urea into ammonia and carbon dioxide.
[Hammes et al. 2003]. The bacterial degradation of urea apparently increases the pH at the cell surface and this promotes the microbial deposition of carbon dioxide as calcium carbonate [Warren et al. 2001]. Through this process the bacterial cell is coated with a layer of calcium carbonate of increasing thickness, resulting in death of the microorganism. However, in the meantime a loose carrier material such as sand can be bound together, or a protecting layer can be deposited on damaged concrete or stone surfaces. Biomineralisation technology has been successfully applied on limestone monuments [Castanier et al. 1999; Tiano et al. 1999] and results show that the characteristics of this biological coating even improve with time. In our research groups, first the criteria for the selection of calcium precipitating Bacillus strains were established. Bacillus sphaericus strains capable of the remediation of Euville limestone, by precipitating a dense and coherent calcium carbonate layer and concomitantly inducing a reduction of capillary water absorption, were characterised by a high urease activity, abundant EPS-production, a good biofilm production and a very negative ζ-potential [Dick et al. 2004]. In the current research mortar samples of different porosity were treated with ureolytic sludge (which is cheaper than the use of pure bacteria cultures and allows fast biomass production) and the effect of CaCO₃ deposition on water absorption and porosity was determined.

### 3.2. Materials and methods

Standardized mortar prisms of 40 x 40 x 160 mm were prepared with ordinary Portland cement (OPC) or blast furnace slag cement (BFS). Prisms were not only made with a water-to-cement ratio (w/c) of 0.5, but also with w/c ratios of 0.6 and 0.7, to obtain a more porous microstructure (simulating a degraded material). Cubes with sides of 40 mm were sawn out of these prisms. Ureolytic sludge was obtained through cultivation of active sludge, obtained from an aerobic sewage water treatment plant, in a semi continuous active sludge (SCAS) reactor. The SCAS reactors were filled with 1 litre activated sludge. After sedimentation in Imhoff cones, 300 ml of supernatant was replaced by the same volume of tap water, containing 1 g/l nutrient broth, 5 g/l urea and 10 g/l SLM 1228. One g/l of the SLM 1228 represents a chemical oxygen demand (COD) of 1135 mg/l, a phosphorus concentration of 50 mg/l and a Kjeldahl N concentration of 44 g/l. The reactors were continuously stirred at 150 rpm and 28 °C. Every second day, part of the reactor content was replaced, using the procedure described above. This procedure offered ureolytic bacteria a selective advantage and therefore stimulated their growth in the sludge microbial community. An adaptation period of 7 days was respected, to start the experiment with a system in equilibrium. The dry matter content and the concentration of volatile organic compounds were monitored continuously and amounted to 20.68 ± 1.14 g/l and 13.8 ± 1.01 g/l respectively. On three surfaces of the mortar cubes a paste of centrifuged ureolytic sludge (10 minutes at 8075 x g) of 0.5 – 1 mm thickness was applied. After 10 minutes settling, allowing the paste to attach itself more or less to the mortar surface, the mortar cubes were immersed in solutions of varying composition in order to investigate the effects of the provided nutrient and of an external calcium source (Table 2).

<table>
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<tr>
<th>Treatment</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<td>OPC</td>
<td>OPC</td>
<td>OPC</td>
<td>BSC</td>
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<td>Number of samples</td>
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<td></td>
<td></td>
<td></td>
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<td>1</td>
<td>3</td>
</tr>
<tr>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
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<td>2</td>
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</tr>
<tr>
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<td>yes</td>
<td>no</td>
<td>yes</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CaCl₂.2H₂O</td>
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<td>90</td>
<td>-</td>
<td>-</td>
<td>90</td>
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<tr>
<td>Urea</td>
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<td>25</td>
<td>25</td>
<td>-</td>
<td>25</td>
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<tr>
<td>Nutrient broth</td>
<td>-</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>26</td>
</tr>
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</table>

Table 2. Overview of the different test series according to cement type, presence of biomass and composition of the nutrient medium

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The concentration of the different medium components per treated surface area was the same in all experiments (e.g. 0.105 g CaCl$_2$.2H$_2$O per cm$^2$ of mortar surface). The cubes were removed from the solution after deposition of a crystalline layer on the surface, which was generally after 2 to 3 days.

To determine the increase in water penetration resistance obtained by depositing the CaCO$_3$ layer, a modified version of the sorptivity test, based on the Belgian standard NBN B 05-201, was carried out. The mortar specimens were coated at the four edges adjacent to the treated side, to ensure unidirectional absorption through the treated side. The coating consisted of two layers of polysiloxane and one layer of silicon paint. After coating, the test cubes were dried at 70 °C in a ventilated kiln, establishing a mass equilibrium of less than 0.1% between two measurements at 24 hour intervals, to ensure low uniform moisture content in the matrix. The specimens were then exposed, to 10 +/- 1 mm of water, with the treated side facing downwards. This takes place in an atmosphere of 20 °C and relative humidity of 60%. At regular time intervals, the specimens are removed from the water and weighed, after drying the surface with a wet towel. Immediately after the measurement the test cubes are submerged again. After the last measurement (200 h of capillary water absorption) the slope of the St versus time curve was determined. Then, a vacuum saturation was performed in order to establish a full saturation. After application of a vacuum (2.7 kPa for 2.5 hours), water was injected in such a way that the samples were completely submerged within 1 h. This submerged state was maintained for 24 h and the surface-dry weight of the samples was measured. The capillary water absorption $E_{c,t}$ during the first part of the experiment is expressed as

$$E_{c,t} = \frac{m_t - m_1}{m_1} \times 100 \text{ (%)},$$

while the water absorption under vacuum $E_v$ is expressed as

$$E_v = \frac{m_v - m_1}{m_1} \times 100 \text{ (%)},$$

with $m_1$ the initial mass of the test cube after drying in the oven at 70 °C; $m_t$ the mass at time $t$ after the start of the water absorption test and $m_v$ the mass after water absorption under vacuum. The results of the capillary absorption measurement can then be expressed as the relative impregnation rate ($S_t$) on a certain moment $t$

$$S_t = \frac{E_{c,t}}{E_v} \times 100 \text{ (%)}$$

The sorptivity of the cubes is calculated as the slopes of the functions representing the volume of absorbed water per surface area, versus the square root of time. The total porosity is estimated by calculating the volume of absorbed water after vacuum saturation.

The morphology and mineralogical composition of the deposited CaCO$_3$ crystals were investigated with scanning electron microscopy and X-ray diffraction.

### 3.3 Results

For the treated cubes the lowest water/cement ratio resulted in the fastest increase of relative impregnation rate $S_t$ at the beginning of the experiment (slope of the $S_t$ versus time curve) and also the highest final value at 200 hours (e.g. for treatment 2: $S_{t,final} = 20\%$ for w/c = 0.7 and $S_{t,final} = 40\%$ for w/c = 0.5), while the untreated cubes showed an opposite trend (for treatment 4: $S_{t,final} = 90\%$ for w/c = 0.7 and $S_{t,final} = 80\%$ for w/c = 0.5). All treatments resulted in a reduction of the slope of the $S_t$ versus time curves and final $S_t$ values in comparison with untreated cubes. The largest effect was seen for the cubes with w/c = 0.7 which underwent treatment 2. The treated cubes with blastfurnace slag cement (treatment 5) showed higher slopes of the $S_t$ versus time curves and higher final $S_t$ values (50-65%) when compared to the Portland cement cubes which underwent the same treatment. The slope of the curve provides information on the initial rate of water absorption, while the final impregnation rate allows one to judge the effectiveness of the treatment after prolonged exposure to water. For the cubes with lower w/c, which are normally less porous, the water absorption under vacuum $E_v$ will be lower, and therefore the $S_t$ value can be higher. This effect could be noticed for the treated cubes.

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The sorptivity curves of the untreated samples typically have a bilinear shape with a fast increase of sorptivity up to 5-6 hours, after which the curve levels off (Fig. 2). For the treated samples a linear change with a highly reduced slope, can be noticed within the measurement interval (Fig. 2). The final sorptivity values were somewhat higher for treatment 3 (0.15 – 0.19 cm³/cm²) than for treatments 1 and 2 (0.09 – 0.14 cm³/cm²), which indicates the effect of an externally supplied calcium source. The differences between cubes with different w/c ratio were not significant. The most pronounced reduction in water absorption compared to untreated samples was reached for the most porous mortar (w/c = 0.7) and when urea, nutrient broth and an external calcium source were provided (treatment 2): the amount of water absorbed by the mortar samples after 200 hours was then decreased by a factor 5.

![Sorptivity of untreated Portland cement mortar samples with different water/cement ratios (left); sorptivity of Portland cement mortar samples with water/cement ratio 0.7 treated with biomass, urea, CaCl₂·2H₂O and nutrient broth (right)](image)

All treatments also caused a reduction in total porosity from 14-18% for untreated Portland cement cubes to 6-9% for treated cubes. SEM and XRD analyses showed that a dense layer of calcite and vaterite crystals was deposited on the mortar surface. For Portland cement mortar samples, no effect of the treatment on the carbonation depth was observed.

4 ACKNOWLEDGEMENTS

The financial support of the Fund for Scientific Research – Flanders (project G.0054.02) is gratefully acknowledged.

5 REFERENCES


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