Changes of color and water-absorption of Hungarian porous limestone due to biomineralization

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Abstract. Bacteria induced calcium carbonate precipitation nowadays is a widely examined process being a possible alternative for traditional stone conservation methods. While research has been mostly limited to laboratory measurements, application connected, further in situ experiments should be performed in order to evaluate the applicability of the method. In our experiment, several bio-based treating compounds were compared, which have already been analyzed in different laboratories. Method for the treatment was based on the treatment of a French research group, and the compounds were applied on Hungarian porous limestone slabs, in situ. For inoculation bacteria strains Bacillus cereus and Myxococcus xanthus were used, and non-inoculated compounds were also analyzed. After the treatment, specimens were analyzed by means of discoloration effect, water absorption and migration characteristics. Almost all the treating compounds gave favorable or acceptable results for the examined properties, comparing to the properties measured in the non-cured state. Measurements on the chromatic- and on the water absorption aspects gave significant results, while further measurements are running for the more exact evaluation of the migration characteristics, i.e. effective migration depth and wetted volume.

1. Introduction
Bacteria induced carbonate precipitation has been explored for the protection and consolidation of ornamental stone for more, than twenty years. The ability of certain strains of bacteria to produce calcium-carbonate crystals in adequate environment, i.e. biodeposition, has been investigated with the purpose of producing consolidating or waterproofing layer onto the porous stone surface. Until now large number of different biodeposition-inducing compounds has been examined by several research groups. Most of these researches were done under laboratory circumstances and so far only a few experiments were done in situ. While most of the researchers were engaged in the development of their own compounds, quite a few comparative studies were done in order to evaluate the differences in the efficiency of the existing compounds. So far these different treating compounds were analysed on different porous materials under different circumstances, which made the objective evaluation of their performance almost impossible.
However, objective comparison of different treatments and evaluation of life-like and life-size experiments are necessary for the effective development of the applied biomineralization [1].

In our experiment we attempted to compare the performance of different biomineralizing compounds. The treatment was carried out under environmental circumstances, in situ on slabs cut out from Hungarian porous limestone of Sóskút. Our aim was to determine the expectable changes of water-soaking properties, chromatic aspects and migration properties of the specimens due to the biodeposition.

2. Experimental

2.1. Carbonate stone

The origin of the porous limestone is Sóskút, Hungary. It has ooidic texture with fine grains. Its density is 1.55 ± 0.04 g/cm³ (n=10), and its apparent porosity is 29.4 ± 0.9 v/v % (n=10) (both values were measured on samples taken out of the stone slabs). From a chromatic point of view, the original color of the specimens is beige-burosso-yellowish, with L* = 81.98 to 87.20; a* = -1.60 to +1.49 and b* = 8.26 to 13.72 typical value ranges. 11 limestone slabs (10 specimens for the treatment and 1 control specimen) with dimensions of 38 x 12 x 6.5 cm were cut out of four limestone blocks. Treatment and measurements were carried out on the largest side of the slabs, on a marked surface with dimension of 15 x 15 cm. Thus the effect of the biodeposition treatment was analyzed on surface areas of 225 cm².

2.2. Microorganisms and growth conditions

Two types of microorganisms, *Bacillus cereus* and *Myxococcus xanthus*, five types of biomineralizing compounds (CB, CC, M3, M3-P and P1) and one conventional stone consolidating compound (T1) were used. *Bacillus cereus* was provided by the French Calcite Bioconcept company in lyophilized form. *Myxococcus xanthus* was obtained from the Hungarian Collection of Microorganisms, its strain number is NCAIM B01663. In order to evaluate the necessity of the microbial activity in the calcium carbonate precipitation, all the biodeposition treatments, except the P1 were performed both with bacteria-inoculated (marked with +) and non-inoculated compounds (marked with -).

The bacteria and components for the CB+ and CB- treating compounds were provided by the French company Calcite Bioconcept. The microorganism in this case is *Bacillus cereus*. Bacteria were inoculated into a liquid compound made of distilled water and other secret (due to industrial secret) components in form of a yellowish powder. Inoculation was performed 20 hours before the treatment, i.e. application onto the surface, in non-sterile conditions. During these 20 hours the liquid compound was stored in a closed plastic box at 20°C. The second three types of liquid compounds, CC, M3 and M3-P were produced according to the recipes of a Spanish research group. M3-P has already been used for in-situ bioconservation, and together with the other two compounds, CC and M3 has been tested under laboratory conditions, too [2]. Main components of the treating compounds can be seen in table 1. Detailed list of components, as well as the preparation of the precultures for inoculation can be read in the review article of De Muynck et al. [1]. Our experimental liquid compound P1 has the same ingredients, than M3-P, and was amended with further ingredients.
Table 1. Main components of the treating compounds

| Name of compound | source of CaCO$_3$ | Bacteria strain |
|------------------|-------------------|----------------|
| CC +/-           | CaCl$_2$, Ca(CH$_2$COO)$_2$ | NaHCO$_3$ | M. xanthus / - |
| M3 +/-           | Ca(CH$_2$COO)$_2$ | K$_2$CO$_3$ | M. xanthus / - |
| M3-P +/-         | Ca(CH$_2$COO)$_2$ | K$_2$CO$_3$ | M. xanthus / - |
| CB +/-           | n/a               | n/a           | Bacillus cereus / - |
| P1               | Ca(CH$_2$COO)$_2$ | K$_2$CO$_3$ | M. xanthus |
| T1               | no CaCO$_3$ (acrylate – polymer) | none |

2.3. Biodeposition treatment procedure

Performance of the treatment procedure was identical to the biomineralization technique of the Calcite Bioconcept Company [3]. The treatment itself took five days. Day 0 is for the preparation of the inoculum. On Day 1 the treatment was applied onto the stone surface, and Days 2 to 4 were for the application of the non-inoculated treating compounds onto the treated area, i.e. further feeding the bacteria. On Day 1 portions of 200 ml of the different liquid compounds were applied onto the stone slabs, respectively, by pouring them onto the surface from a glass beaker. On Day 2, treatment was repeated two times with 200 and 200 milliliters of the treating compounds, 24 and 36 hours after the first treatment (Day 1). On Days 3 and 4 (60$^{th}$ and 84$^{th}$ hours), the stone slabs were treated with 150 milliliters of treating compounds, respectively. All the 10, thus even the conventional, acrylate-polimer based consolidant were applied five times onto the surfaces. While required inoculation times of the bacteria differ, inoculation of Myxococcus xanthus started two days before the Day 1 (-48$^{th}$ hour), in order to be ready for application on Day 1. Stone slabs were washed three times by rainwater after the treatment and before the analysis of the changes.

2.4. Conventional consolidant

The conventional stone consolidant is a water-borne synthetic resin based primer. It is made of acrylate-polymer dispersed in water, and has a high penetration characteristic into porous materials. It was applied onto the surface without dilution, and in high, 35.6 l / m$^2$ (0.8 l / 225 cm$^2$) amount.

2.5. Evaluation of the biodeposition treatment

During and after the treatment limestone slabs were stored outdoor. During the three-week-long storage period, slabs were washed by rainwater three times, and were shaded by wooden sheets in order to protect them from intense sunlight (surfaces were oriented to south-west). The measurements were carried out three weeks after the treatment.

2.5.1. Colour measurement

Colour measurements were performed in order to determine the changes of shade and color due to the biodeposition treatment, using an ELCOM Comcolor Spectrophotometer. Four measurements were carried out before and after the treatment on the same four points of the surface on each slab, respectively. Values of the CIEM-LAB system, namely $a^*$, $b^*$ and $L^*$ were measured. $\Delta E$ was calculated as follows [4]:

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2}$$ (1)
ΔE is considered to be perceptible to the human eye, when its value is higher, than value 5 [5]. Changes were measured also on a non-treated control slab, and differences in color and in shade were compared to the differences measured on the control one.

2.5.2. Measurement of water absorption
Differing from the conventional pipe-test, water absorption was measured as a function of water absorbed in time from a glass pipette pressed against the vertical stone surface in a horizontal position with its pointed end. The total amount of water soaked by the stone was 5 cm$^3$. Changes in the water absorption properties were evaluated as the difference of time required for the absorption of the same amount of water before and after the treatment (see figure 1).

![Figure 1. Setup of the horizontal sorption test](image)

2.5.3. Analysis of the migration characteristics
While the calcium-carbonate crystals formed due to the biodeposition process bound to the inner surface of the pores, they reduce the apparent porosity. For the estimation of the changes in porosity, we used a “water-staining” test, where we expected a larger wetted surface and volume due to the decreased porosity. After the water absorption test, wetted area, i.e. the dimensions of the water stain on the surface were measured. The wetted volume i.e. the volume of the limestone percolated by the water was estimated using the dimensions of the water stain. Furthermore, using the vertical and horizontal dimensions of the wetted area, we could estimate the shape of the migration profile, and the migration depth. Results obtained before and after the treatment were compared, and the extents of the differences were evaluated. The expectable migration profile is be a half sphere in the equilibrium state.

3. Results and evaluation

3.1. Evaluation of the biodeposition treatment

3.1.1. Colorimetric measurements
The biodeposition treatment resulted in a change of the shade and the chromatic aspect of the limestone surfaces. We measured differences also on the non-treated control slab, suggesting, that exposure to outdoor circumstances has a primary effect on the appearance of the stone. However, total color difference of the control slab is not perceptible with its $\Delta E_{\text{average}} = 2.44$ (standard deviation = 0.56), but while we considered this alteration inevitable, $\Delta E$ values of the other slabs were reduced with this value (resulting the corrected $\Delta E$ values). While values of shade alteration ($\Delta L^*$) changed in a similar way, than values for total color difference, they were evaluated similarly to $\Delta E$. Medians and errors of the corrected $\Delta E$ values can be seen in figure 2.
Figure 2. Corrected \( \Delta E \) values generated by the different treating compounds

Only the application of one treating compound, CC-, showed lower color difference, than that of the control slab’s. The highest difference belongs to the CB+ treating compound, which was a yellowish liquid, therefore the change in the chromatic aspect was not unexpected. The total color difference resulted in the application of the compound CB+ is significantly higher, than \( \Delta E \) values measured on the slabs, and it is perceptible, too, while its mean value is higher, than 5 (5.67). Color changes belonging to the other compounds are not perceptible.

With one exception, compound CC-, all the treating compounds darkened the original, beige-burrosso-yellowish color of the stone, but none of them is significant. Control value-reduced \( \Delta L^* \) values have negative sign except CC-, meaning, that the luminosity decreased in almost all the cases.

Significance of the mean value, as well as perceptibility of the color difference performed by the CB+ compound can be contributed to the yellowish color of the compound. Interestingly, compound CB- with similar chromatic characteristics behaved differently, suggesting, that microbial activity and its metabolic products have important role in the discoloring effect of bioconsolidating compounds. Other compounds were mainly whitish in color, and were transparent or opaque in appearance due to the solved components, therefore their discoloring effect was not significant.

The conventional, resin based compound altered the color in a non-significant way, but changes in the reflex-reflection properties of the stone should be seriously considered. Remarkable shiny appearance in our case is most possibly a side-effect of the “over-dosage” of the compound – as mentioned under 2.4. Nevertheless, while generally large stone surfaces, i.e. facades, walls etc. are affected by the stone consolidation, changes in the reflex-reflection properties should be avoided.

3.1.2. Water absorption

Time of the water absorption decreased in all the cases, indicating, that the surface porosity of the slabs changed in a favorable way. Therefore the time differences have positive signs in all the cases, and they are changing with the amount of soaked water around an average value – as it can be seen in figure 3. Highest differences (average value: +488.77 %) were achieved using the P1 treating compound, but the procession of the absorption and the values of the differences significantly differ from the results achieved with the other treating compounds. Therefore further measurements are running for a more exact evaluation of the P1’s performance. Application of compound M3-P+ achieved the highest difference (average: 149.4), and M3+ the lowest difference (average: 35.42). The traditional stone consolidant (T1) made the surface almost impermeable, thus water flown down the surface instead of being absorbed.
These preliminary results indicate, that inoculated compounds have a better performance, while three (CB+, M3-P+ and CC+) out of four mixtures gave better results for the capillary sorption-test. In case of the non-inoculated compounds, reduction of the sorption time can be imputed to the crystallization of the solved components on the stone surface and inside the pores.

3.1.3. Analysis of the migration characteristics

Water-stains with ellipsoidal and spherical shapes, and in one case (M3-P+) uneven pattern were observed. In case of the ellipse-patterns, diameters of the traverse- and conjugate axes were measured, and the semi-horizontal axis (minor or major, dependent on the orientation of the ellipse) was used as third (depth) dimension for the estimation of wetted volume. Hereby estimation was based on the volume of a half ellipsoid, of which two axes were measured, and the third one was based on the idea, that water propagates with the same velocity horizontally. The uneven wetted area and the belonging wetted volume was estimated with the average dimensions of the axes.

We measured enlargement of the wetted area, and consequently of the wetted volume in all the cases, except CB+ and M3-P+. In these cases the wetted volumes were estimated to be smaller with 5.38 and 2.53 %, respectively. The largest difference belonged to the M3- compound, with a difference of +100.47 %. However, dimensions of the migration profile were not measured, migration depth was approximated with the semi-horizontal axis of the wetted area, as it was mentioned before. According to this, compound CB- migrated the deepest with 34.25 mm, and CB+ the least, with 25.25 mm.

The analysis showed, that the orientation of the wetted areas (the elliptic stains) does not change before and after the treatment, indicating, that the precipitation occurred homogenously inside the stone. Nevertheless, neither the effect of inoculation, nor the measured decrease in sorption time was reflected by the results. Therefore further research is running for the determination of the role of these factors.

4. Conclusions

In our experiment five different microbial, and one conventional stone-consolidants were compared by means of discoloring effect, water absorption and migration characteristics.

Colorimetric measurements showed, that compound CB+ performed significantly higher total color difference, than the other compounds, and that was the only one causing perceptible (to the human eye) changes with a ΔE value of 5.67. This suggests, that the initial chromatic aspects of the treating compound should be considered, when components are being chosen for the treating of a certain stone surface.

Favourable changes in water absorption indicated, that the treatment was successful. Analysis of the differences in water absorption in time gave similar results, showing both the differences between the treating compounds, and the balanced changes of the differences in time.
Measurements on the migration properties also proved, that favourable changes occurred in the mass properties due to the treatment, and that there is difference between the precipitating properties of the different treating compounds. For more exact results and the determination of the expectable errors of the measurement further investigations are running.

5. Acknowledgements
We would like to thank Jean-Francois Loubière (Calcite Bioconcept) and Willem de Muynck (Ghent University) for his help and advices before and during the experiment. The presentation of the research has been supported in the framework of the project „Talent care and cultivation in the scientific workshops of BME” project by the grant TAMOP - 4.2.2.B-10/1 - 2010-0009.

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