CONSERVATION OF CARBONATE STONE BY MEANS OF BACTERIAL CARBONATOGENESIS: EVALUATION OF IN SITU TREATMENTS

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ABSTRACT

The carved crestings at the Royal Chappell (Capilla Real) in Granada, Spain, which are made up of a highly porous, friable calcarenite stone, are characterized by their complex surface morphology as well as their level of exposure to weathering agents. These factors contribute to their extensive deterioration and have prompted the implementation of conservation treatments, including the application of a conventional consolidant (ethyl silicate). Here, we evaluate the effects of a novel biological and bio-inspired bioconsolidation treatment, which was also applied on these decayed carved elements. We present the results obtained five months after the treatments and compare/discuss the efficacy of both the bioconsolidation treatment and the conventional ethyl silicate treatment. In particular, we have observed that the level of consolidation obtained by bacterial carbonatogenesis is similar to that achieved with ethyl silicate. However, 5 month after treatment application, the silica gel formed on the stone shows extensive cracking which jeopardizes its long term effectiveness as a consolidant. Conversely, neither detrimental effects, nor any reduction in the level of consolidation is observed in the case of the bioconsolidation treatment.

INTRODUCTION

The deterioration of monumental stone is the consequence of the interaction between the material and environmental factors such as moisture (water), heat, atmospheric pollutants and living organisms [1, 2, 3, 4]. These interactions lead to the gradual increase of stone porosity and result in the alteration of the mechanical characteristics of the stone. In the last decades, attention has been drawn to find out remedies against such a degradation and the associated loss of our cultural heritage. The conservation of monuments involves, in most cases, the application of consolidating products in order to strengthen the weathered stones and to avoid the complete deterioration of the material [5]. Numerous kinds of products, both inorganic and organic, have been used to this aim, but almost all have shown, in time, different negative results [6, 7]. The long-term incompatibilities of the substrate and the new cementing material used for consolidation as well as the plugging of pores induced by the consolidant have encouraged the search and development of new conservation treatments, especially suitable for carbonate stone. In this sense, bioconservation by means of bacterial carbonatogenesis has been developed to be an environmentally-friendly alternative approach, with which it is possible to induce the new formation of natural cementing products, like calcite [8, 9, 10, 11, 12, 13, 14]. Bacterially induced calcium carbonate precipitation can occur because bacteria are able to change the chemistry of their close environment as a result of their metabolic activity or by providing cell structures and cell debris as nuclei for heterogeneous crystallization, all in all leading to mineral precipitation [15, 16]. A number of
researchers are studying this biomineralization processes and their application for stone conservation [17]. We have developed a novel method based on the selective activation of the carbonatogenic microbiota that inhabits the stone by the application of a suitable nutritional solution [10]. Both the air-borne bacteria as well as the stone-inhabiting carbonatogenic microorganisms are thus stimulated to grow and induce the \textit{in situ} formation of new calcium carbonate cement. This new cement consolidates decayed carbonate stone (limestone, dolostone and marble). When using this novel conservation treatment, it is of paramount importance to shed light on how the microbial community develops upon the application of the nutritional broth. With this finality, attention was focused on the characterization of culturable bacteria isolated from deteriorated carbonate stone (calcarenite) during the \textit{in situ} bio-consolidation treatment performed in the apse of the church of San Jeronimo Monastery in Granada, Spain. The identification of these bacteria was carried out using REP-PCR and 16S rRNA gene sequencing to determine the taxonomic position of culturable population of bacteria activated on the stone when the nutritional solution was added. The results indicated that all of the microbiota activated was carbonatogenic bacteria and no detrimental side effects were observed [13].

These positive results prompted us to carry out large-scale \textit{in situ} applications of this type of treatment on decayed calcarenite elements (carved cresting) at the Royal Chappell (Capilla Real) in Granada, Spain. Due to the complex surface morphology of these elements, as well as their level of exposure to weathering agents (rain, humidity, insolation, wind and pollution), damage in the form of granular disintegration, sanding, fracture development, case hardening, black crusts, and material loss is extensive. We aim at evaluating the effects of the bioconsolidation treatment on such an extreme damage situation in order to gauge the efficacy of this novel treatment. Currently, extensive conservation works are being undertaken in this building by the company TARMA using conventional consolidants such as ethyl silicate. Thus, this represents an unique opportunity to compare the short- and long-term effects and behavior of both the bioconsolidation treatment and the ethyl silicate treatment, which have been applied in similar decayed calcarenite elements. Here, we present, compare and discuss the effects of these treatments.

**EXPERIMENTAL**

**CALCARENITE STONE.** Conservation treatments were applied on the south-facing cresting at the roof of the Capilla Real (Figure 1a). These elements are carved on calcarenite, a porous (average porosity ca. 28%), buff colored limestone made up of different type of calcium carbonate bioclasts cemented by sparitic calcite. This stone, which has been thoroughly used in the area of Granada for building purposes, rapidly decays mainly due to the effects of rain (dissolution), urban pollution (sulfation and black crusts generation) and salt weathering (sanding and granular disintegration mainly due to salt crystallization). The areas selected for testing the treatments show extensive damage due to sanding, honeycomb weathering and scaling, as well as case hardening and black crust development. The carved cresting displays interconnected cracks which facilitate in-depth dissolution of carbonate cement, thus resulting in extensive damage, including the loss and crumbling of whole pieces (dm sized blocks sometimes fall off). Prior to treatment, the selected areas were cleaned by means of micro-sandblasting using aluminum silicate powder in order to eliminate black crusts. Biocides (Tributyltin-Naphthenate and Quaternary Ammonium Salts, [C.T.S.], standard biocides used in biodeterioration control) were also applied to eliminate lichens. **TREATMENTS AND THEIR EVALUATION.** Treatments were applied on two types of elements: (1) on elements that display a medium level of decay which enables in place application; and (2) on elements with a high to extreme level of damage. Such elements are being replaced with stone replicas. So, in this latter case, the elements were removed from their original location and placed
close-by the elements treated in place. For the bioconsolidation treatments, a sterile M3-P nutritional solution was prepared (1% Bacto Casitone, 1% Ca(CH\(_3\)COO)\(_2\) 4H\(_2\)O, 0.2% K\(_2\)CO\(_3\)·1/2H\(_2\)O in a 10 mM phosphate buffer, pH 8) [14] and readily applied on the stone surface by spraying (Figure 1b). The bioconsolidation treatment application was repeated twice every day during the 6 days of treatment to maintain a constant dampness. The temperature was kept nearly constant at 17-25 °C by convection of hot air. In order to avoid desiccation and direct impact of sunlight on the stone surface, the treated areas remained covered with an insulating foils (Figure 1c) (not directly in contact with the stone surface to enable air circulation) during the 6 days of treatment plus 3 more days until the stones became fully dry. Ethyl silicate was applied on elements displaying medium level of decay. No attempt was made to apply this treatment on stone elements that were removed from their original location.

To study the microbiota evolution with time: 1 g of stone sample was collected from each area before and after treatment (after 5 months) and different culture media were inoculated to test the carbonatogenic capacity of the activated bacteria (M3-P supplemented with 2 wt % purified agar), the total number of bacteria (TSA, Scharlau, Chemie SA, Barcelona, Spain), acid-producing bacteria (using a general culture medium supplemented with glucose [1%] as fermentable carbon source) and fungi (Sabouraud Agar; Scharlau Chemie SA, Barcelona, Spain).

Fig. 1  A) General photograph of the cresting at the Royal Chapel where the bioconsolidation treatments were applied; B) detail of spraying technique on a calcarenite element; C) treated element covered by an insulating foil; and D) detail of recollecting samples from treated and untreated areas.

Color measurements were performed on several spots (at least three spots in each of the treated stones) using a Minolta Chroma Meter portable spectrophotometer equipped with Xenon lamp (illuminant C) and diffuse reflectance geometry. The CIE-\(L^*a^*b^*\) color space was selected. Color
variations due to the treatment were reported as $\Delta E$, which was calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a*)^2 + (\Delta b*)^2},$$  

(1)

where $\Delta L$, $\Delta a^*$ and $\Delta b^*$ are respectively the difference between the untreated and treated stone of $L$ (lightness: 0 being black and 100 being diffuse white), $a^*$ (negative values indicate green while positive values indicate magenta) and $b^*$ (negative values indicate blue and positive values indicate yellow) values.

In order to quantify the increase in superficial hardness of the stones subjected to the consolidation treatment we chose to use the so-called Peeling tape test [18]. Several 7 x 3.5 cm$^2$ adhesive tape pieces were stuck on the surface to be tested and removed rapidly. This operation was performed before the treatment started and after it was completed. Each tape was weighted before and after application to determine the amount of material removed. The consolidation efficacy was calculated as the weight reduction in material removed following treatment. Note that adhesive tapes remove loose or poorly cemented surface carbonate grains. Therefore, variations in the mass of grains removed by the peeling tape enable quantification of the real superficial consolidation achieved by the treatment. In addition, this test was applied to the elements treated with the ethyl silicate to verify the efficacy of this type of treatment in comparison with our biological and bio-inspired treatment applied in this study.

Stone samples were also analysed by scanning electron microscopy (SEM) before and after treatments.

RESULTS AND DISCUSSION

Results of the peeling tape test are presented in Table 1. The treatment with the nutritional solution shows a clear reduction of the weight loss in comparison with the control (untreated) stone. The treatment with the ethyl silicate also shows similar consolidation effect on the stone. Despite the fact that the calcarenite stone is very heterogeneous, these results show that a significant strengthening was achieved following treatments. During the evaluation of the effectiveness of consolidation treatment applied on heterogeneous porous stone a common fact is the high values of the deviation standards of the weight loss. The same observations were reported by many authors [19, 20, 21]. It follows that the results of tests designed to evaluate the efficacy of conservation treatments applied on heterogeneous stones, including the peeling tape test, have to be considered with caution. Nonetheless, the trend in the reduction of weight loss values we have observed suggests that a significant strengthening was achieved after the treatment of the calcarenite stone.

Table 1: Weight loss (g/m$^2$) following the peeling tape test of treated stone

<table>
<thead>
<tr>
<th></th>
<th>Control cresting (g/m$^2$)</th>
<th>M3-P treated cresting (g/m$^2$)</th>
<th>Ethyl silicate treated cresting (g/m$^2$)</th>
</tr>
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<tbody>
<tr>
<td>Peeling tape test</td>
<td>39 ± 22.5</td>
<td>5.72 ± 5</td>
<td>7.27 ± 2.77</td>
</tr>
</tbody>
</table>

Color measurements before and after treatment of the calcarenite stone blocks show no significant differences in $L$, $a^*$ and $b^*$ values (Table 2). These results confirm that the treatment does not alter the appearance of the calcarenite stone, thus complying with current conservation guidelines (International Conservation Charts).
Table 2: Color measurement before and after treatment of the carved cresting with the nutritional solution M3-P

<table>
<thead>
<tr>
<th>Measurement spots</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>∆E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>1</td>
<td>70.7</td>
<td>3.1</td>
<td>14.7</td>
</tr>
<tr>
<td>2</td>
<td>70.6</td>
<td>3.0</td>
<td>12.7</td>
</tr>
<tr>
<td>3</td>
<td>66.9</td>
<td>2.6</td>
<td>12.6</td>
</tr>
<tr>
<td>4</td>
<td>67.2</td>
<td>1.9</td>
<td>11.2</td>
</tr>
<tr>
<td>Average</td>
<td>68.9</td>
<td>2.7</td>
<td>12.8</td>
</tr>
</tbody>
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The bacterial counting shows a negligible to slight increase after treatment (5 months) in comparison with the control stone. The bacterial load was ≈ 2.5 x 10^4 CFU/g and all bacteria were able to precipitate calcium carbonate in M3-P culture medium. These bacteria were also inoculated on a specific medium for acid-producing bacteria to find out if they were able to produce acids when a medium with glucose is used for a bioconsolidation treatment. A relatively high number (50 %) of bacteria, obtained in our study, were able to produce acids in a medium with glucose. This clearly demonstrates that the glucose-free medium used in our treatment is more suitable than the glucose-containing medium used by others (e.g., [8]). As expected, no variation of the number of fungi (≈ 1.5 x10^2 CFU/g) was observed following the bioconsolidation treatment. Our results show that the treatment does not alter the appearance of the treated stones; the opposite would be esthetically unacceptable when a restoration/consolidation treatment is performed.

SEM analyses show several textural differences in the stone before and after treatment. Before treatment stones showed several deterioration features including carbonate dissolution pits and corroded surfaces, gypsum crusts, and original stone biofilm as it is shown in Figure 2. Conversely 5 months after the bioconsolidation treatment we could observe the consolidation effect on the stone grains. It is well-known that micritic (size < 2 µm) calcite grains are more sensitive to both decay and consolidation than the sparitic ones (size > 2 µm). As a consequence, the former grains appear remarkably loose before treatment, while after the bioconsolidation treatment they were cemented all together and no more loose grains were observed (Figure 3). Interestingly, the pores did not appear plugged or clogged. This latter positive effect ensures that the vapor permeability of the stone is not affected by the treatment.

SEM analyses of stone treated with ethyl silicate showed the presence of a gel between grains of the stone, which confirm the consolidation effects observed previously by the peeling tape test. The shortcoming of this type of treatment is the development of fractures upon the drying and aging of the silicate gel which are already evident only 5 months after the treatment (Figure 4). The results obtained after consolidation treatments of the stones with the highest level of deterioration (i.e., those that were replaced) were very similar to those obtained for the cresting stones. These preliminary results show that the bioconsolidation treatment here presented, is highly effective for in situ application as a novel consolidation/protection treatment. This is so because the carbonatogenic bacteria present in the decayed stone are activated and prompted to generate a newly formed carbonate cement that strengthens and consolidates the stone in a highly effective way. In order to test the long term effectiveness of the bioconsolidation treatment, (a) measurements of surface strengthen (peeling test) and colorimetry, and (b) the evolution of the microbiota with time will be performed for up to two years after the treatment application.
Figure 2: Cresting stone before treatment showing extensive deterioration features. A) Carbonate dissolution; B) Details of dissolution pits; C) Gypsum crusts; D) Detail of an original biofilm on the control stone.

Figure 3: Bioconsolidation treatment effects on cresting stone. A) Before treatment; B) 5 months after treatment
CONCLUSIONS

The bioconsolidation treatment tested here showed a highly effective consolidation effect observed on decayed calcarenite stone elements with complex surface morphology and extensive damage. The consolidation level and efficacy of the treatment was evaluated by the peeling tape test, as well as by means of spectrophotometric (i.e., color measurements) and scanning electron microscopy (SEM) analyses. The efficacy of a conventional treatment with ethyl silicate was also evaluated and compared with that of the bioconsolidation treatment. It has been observed that the aging of the silicate gel formed after ethyl silicate application develops several fractures, thus jeopardizing the long term effectiveness of the treatment. Conversely, upon the bioconsolidating treatment, it was observed the formation of new carbonate cement due to the fact that all of the activated culturable microbiota was carbonatogenic. The development of no dangerous microbial communities was detected after treatment. These results confirm that activation with a suitable culture medium (without glucose) of the carbonatogenic microbiota inhabiting decayed ornamental carbonate stones is an effective conservation treatment for in situ application.

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