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## Bio-removal of black Crust from marble surface: comparison with traditional methodologies and application on a sculpture from the Florence's English Cemetery

Eleonora Gioventù<sup>a\*</sup>, Paola Lorenzi<sup>b</sup>

<sup>a</sup>Free-lance conservator, Via XXV Aprile 24, 16039 Sestri Levante (Ge), Italy

<sup>b</sup>Opificio delle Pietre Dure, Via degli Alfani 78, 50121 Firenze, Italy

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### Abstract

In the present study we investigate the use of sulphate-reducing bacteria during the black crust's bio-removal process. The aim of our work is to compare microbial cleaning with more traditional cleaning methods (chemical and laser cleaning) and to discuss the pros and cons of this new technique. The research builds in two steps: a first experimental phase examining the process on stone materials coming from the Cathedral of Florence, on a Carrara marble column taken from the Baccio d'Agnolo Balcony under the Dome by Brunelleschi (treated in our laboratory) and on an external pilaster (treated in situ) on the left side of the Cathedral made out three different stone typologies: green serpentinite, red marlstone and white Carrara marble; a second application phase on a marble sculpture from 19th century representing the Allegory of Death from Florence's English Cemetery. The effects of the different procedures on the original surfaces were evaluated by a scanning electron microscopy coupled with energy dispersive X-ray (SEM/EDS) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and colour measurements. Results prove that microbial cleaning is the most controllable process and it has a great efficiency in sulphate removal. These techniques have also a great ecological importance as bacteria reproduce the same biological process achieved in natural contexts.

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\* Corresponding author. Tel. +39 3494466854  
E-mail address: [eleonora.gioventu@gmail.com](mailto:eleonora.gioventu@gmail.com).

## 1. Introduction

The recent developments of biotechnology and its use in many areas have attracted keen interest in its microbiological applications in the field of the cultural heritage. The application of sulphate-reducing bacteria to the artwork surfaces to be restored allows the complete removal of sulphate-based crusts, such as black crusts, by exploiting their specific metabolic processes. The studies of these microorganisms and their metabolism, beginning in 1989 (the earliest researchers were Gauri and Gwinn) right through to the present day, with recent applications developed by Ranalli et al. 1997 and Cappitelli et al. 2006 and 2007, allowed the optimization of the procedural protocol and the definition of an effective application methodology.

In this paper, we report on the use of sulphate-reducing bacteria for the cleaning of black crusts on stone materials exposed to the elements, and we draw a comparison between microbiological treatment and chemical and laser cleaning.

## 2. Materials and methods

The objective of this research was the further study of the effectiveness of sulphate-reducing bacteria used for the removal of black crusts, the assessment of their degree of non-invasiveness on the artwork surfaces, and the development of any considerations and improvements in the field of practical applications.

The research project was therefore divided in two main phases:

a first experimental phase, performed by means of a series of tests on architectural surfaces of Florence Cathedral, accompanied by the appropriate scientific analyses, to determine the non-invasiveness of the bacteria, crucial in minimizing detrimental effects on the artwork surface (Fig. 1);

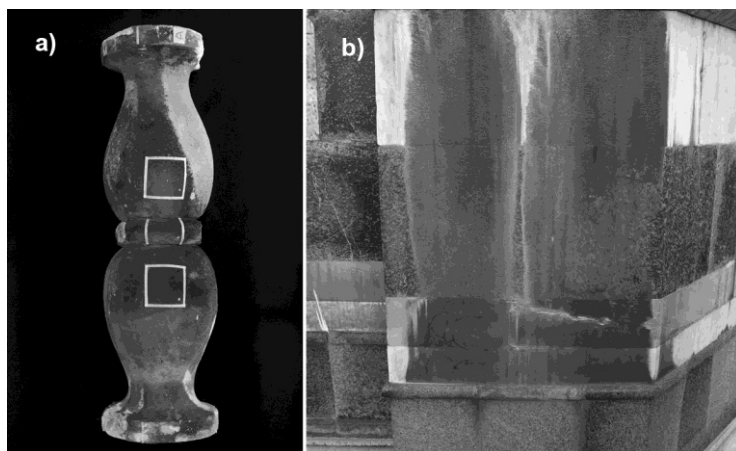


Fig. 1. Selected areas for the different cleaning methodologies. a) Column and b) External pilaster of the Florence's Cathedral showing three different lithotypes (from top: green serpentine, red limestone, Carrara marble) uniformly covered by black alteration.

and a subsequent application stage on the sculpture *Allegoria della Morte* by Giuseppe Lazzarini 1870 (Fig. 2) from the English Cemetery in Florence, characterized by a strong surface alteration and by the presence of large, thick black crusts.



Fig. 2. Selected area for the microbial cleaning application on the cloak enveloping the skull of the sculpture, before and after treatment

The methods of analysis selected for an evaluation, as objective as possible, of the results obtained, were: Optical Microscope observation with stratigraphy, Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM/EDS) Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy and colour measurements.

The cleaning treatments for black crust removal that were compared were microbial cleaning (poultice embedding sulphate-reducing bacteria), chemical cleaning (ammonium carbonate poultice) and laser cleaning (1064 nm, Nd:YAG laser).

### 2.1. Compared cleaning methodologies

The bacteria employed were *Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579 maintained in DSMZ 63 medium (Cappitelli et al. 2006), an aerotolerant strain that in its metabolic processes transforms sulphate ( $\text{SO}_4^{2-}$ ) into sulphide ( $\text{S}^{2-}$ ). This is why they have characteristic of high selectivity, non toxicity and eco-compatibility. Before using them in the treatment, the cells were grown in DSMZ 63 medium, modified by eliminating any iron source. After centrifugation the cell pellet was suspended in deaerated phosphate buffer supplemented with 0.599 g/l sodium lactate at pH 7.0. All the manipulations described above were done under anaerobic conditions in a glove box. The delivery system chosen for intervention was Carbogel (Borgioli et al. 2001), which produces a translucent gel that can trap the bacterial cells in suspension and keep them in humid conditions, as well as maintaining partially anaerobic conditions. Application is usually performed by positioning tissue paper, followed by a coating of the gel with cling paper. The poultice is left on for a time considered appropriate, and then the softened black crust is removed using a damp cotton swab.

For chemical cleaning the poultice was prepared by mixing 100 g of ammonium carbonate and 10 ml of Tween 20 (a non-ionic detergent) with 1000 ml of distilled water, and suspending Carbogel (42 g in 1000 ml) in the solution.

For laser cleaning the choice of the irradiation parameters was based on previous research, principally performed by the Opificio delle Pietre Dure and IFAC CNR, Florence (Lanterna and Matteini 2000; Giamello et al. 2004;

Siano et al. 2005). The laser used was *Smart Clean II* (El.En. Spa), a short free running Nd:YAG laser, 1064 nm. The laser spot, 4.5 mm, and the repetition rate, 5 hz, were maintained constant throughout the test, while the range of fluence varied according to the type of stone treated and the thickness of the crusts, from 5 J/cm<sup>2</sup> to 8.8 J/cm<sup>2</sup>. The ablation test was performed under water-assisted conditions (Bromblet et al. 2003).

## 2.2. Treated surface

The objective of this in-depth study was to compare microbial cleaning with more traditional cleaning methods, and to this end we treated various types of stone at Florence Cathedral that were covered by black crust. The tests were performed first on a Carrara marble column from Baccio D’Agnolo’s Balcony, and then on an external pilaster, both on Florence Cathedral (Fig. 1). The external pilaster consisted of three different kinds of stone: green serpentine, red marlstone and Carrara marble. Two are metamorphic rocks, and one is a calcareous stone (red marlstone), all quarried in the Tuscan Apennines.

On the surface of every stone type, three different areas were chosen in order to compare the three different methods applied.

After this study had confirmed the non-invasiveness of the microbiological technique and its efficacy, this method was applied to the sculpture *Allegoria della Morte* (1870) by Giuseppe Lazzarini, from the English Cemetery in Florence.

The bio-removal of black crust on the *Allegoria della Morte* was performed only on two areas of the surface: a small test area with a crust of medium thickness, and the area of the cloak enveloping the skull (Fig. 2) which had a crust with a high thickness, namely several millimetres, with a marked granoblastic structure on the surface, and well-developed stratification in the part closest to the marble.

## 3. Results

From the results of tests performed on the column (Fig. 3) it was possible to evaluate the effectiveness of the microbial poultice in removing the black crusts.

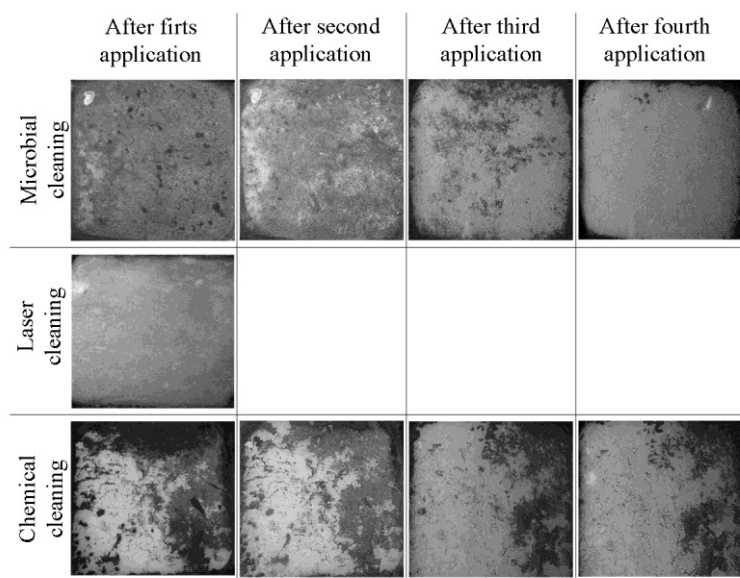


Fig. 3. Sequence images of cleaning applications on the Carrara marble column.

Stratigraphic analysis by SEM and FTIR spectra in fact confirmed the total removal of sulphates; colorimetric analysis and absorption analytical techniques also demonstrated the effectiveness of the cleaning procedure. In this instance, comparisons with other methods of cleaning were particularly favourable for microbial treatment. In fact, direct visual observation, as well as additional stratigraphic analysis and FTIR spectroscopy, showed that the other two cleaning methods had not been fully effective.

Chemical cleaning, implemented using the same measurements and times of application as for microbial cleaning (four applications of ten hours each), did not attain the total removal of the crust, and the result was therefore non-uniform. In addition, images of the sequence of application show that microbial cleaning is more gradual and controllable than chemical cleaning. The non-homogeneity of the latter technique is particularly marked (Fig. 3). The laser technique, while not causing adverse effects on the surface of the stone and attaining a uniform level of cleaning, did not achieve complete removal of the crust. In fact, stratigraphic analysis and FTIR spectroscopy showed the continuing presence of gypsum, oxalates and silicates. Colour analysis also showed a marked variation ( $\Delta b$ ) on the b axis (blue-yellow).

The investigation conducted on the surfaces of the external pilaster revealed, overall, that the microbial cleaning operation was successful. In fact, the results of the analysis confirmed that there were no crust residues left on the treated surfaces.

However, on the external pilaster, the greater efficacy of microbial cleaning with respect to other methods of intervention (when compared to the results obtained on the column) is less marked, as one additional application was required with respect to the chemical poultice (three applications of ten hours each for microbial treatment, and two for chemical treatment).

As regards laser cleaning, surface residues of gypsum and silicate and a greater change ( $\Delta b$ ) on the b axis (blue-yellow) in colorimetric examinations were always detected.

Analyzing the different substrates, serpentine, marlstone and white marble, it was seen that best results were obtained for white marble and serpentine, while as regards the marlstone, problems of delamination of the surface material itself meant that the use of compresses was not the most appropriate.

The application of microbial cleaning techniques to the sculpture *Allegoria della Morte* attained satisfactory results, because the process, very gradual and controlled, led to an excellent result in terms of visual appearance (Fig. 2), even though a number of successive applications were necessary for the complete removal of the crust on the cloak that envelops the skull. In this case the extended time required for this technique is caused essentially by two factors: the marked thickness and greater compactness of the crust (Fig. 4), and the objective difficulties in applying Carbogel to the more complex forms of the sculpted surface.

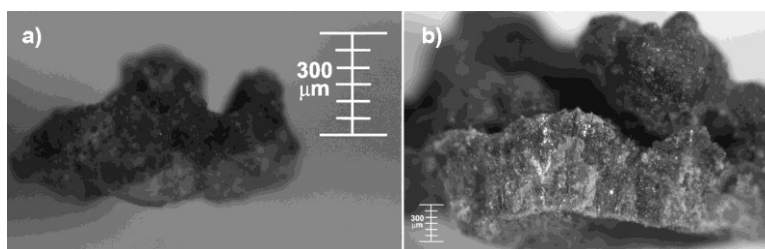


Fig. 4. Comparison of the crust thickness coming from a) the preliminary test area and from b) the area of the cloak enveloping the skull on the sculpture.

The microscopic observation of samples taken respectively from the area of the mantle around the skull and the preliminary test area in the lower part of the mantle reveal the marked differences between the two crusts: the first is at least five times thicker than the second, and has a much more compact and layered morphology (Fig. 4). These are factors that limit the action of microorganisms, which therefore penetrated the different layers slowly. A further factor that can inhibit bacterial metabolism is the presence of heavy metals because of their potential toxicity.

During this work, it was seen that the amount of crust removed after each application varied greatly depending on the area, probably due to the crust's differing stratigraphic cross-linking and composition. In fact, while the non-homogeneous characteristics of the crust may not affect other cleaning methods, it has a great influence on microbial activity.

#### 4. Discussion

First of all we have to underline the fact that the analyses of the sampled surfaces before treatment showed that the black crust consisted of components and layers that differed in various areas of the same monument. Therefore it is important to remember that the differences in the results could be due not only to the cleaning methodologies employed but also to the non-homogeneous distribution of the alteration products.

Using the chemical cleaning technique, the surface was cleaned unevenly, some areas being cleaned perfectly at the first application, while others were left with thick residual black crust. Moreover, the chemicals removed the crust in a rough way, sometimes detaching relatively large fragments. In contrast, bacterial cleaning could be controlled better, probably because the method is more selective in removing specific compounds (e.g. sulphates) from the altered layer (Cappitelli et al. 2007). It is for this reason that the number of applications necessary with this method was so closely related to crust thickness. During the biological application, the black crust progressively and uniformly decreased in thickness.

With regard to the laser cleaning of the white marble of the pilaster and column, we were able to observe that the use of a lower range of fluences removed the crust but left a thin yellow layer easily visible to the naked-eye (data not shown). Raising the range of fluences led to a thinner yellow layer but damage appeared on the stone surface, so it seems that in order not to damage the surface, there will always be a residual yellow layer. It is known that Nd:YAG laser on pale-coloured materials leaves the substratum slightly yellowed (Delivré, 2003; Vergé-Belmin, 2003). Opinions regarding the yellowing are still being discussed, not without controversy; some restorers consider the yellowing to be part of the object's genuine appearance, while others consider it to be additional damage (Prasad and Siano, 2010).

The aesthetic impact of these treatments was quantified by colour measurement, SEM and FTIR analyses, showing that both microbial and chemical cleaning managed to remove all the gypsum residues from the surfaces, except for the area of the column cleaned with chemicals. In contrast, laser cleaning, particularly on the white marble, left a residual layer consisting mainly of gypsum, with some calcium oxalate and impurities (silicon and phosphorus).

In terms of colour, the multivariate analysis showed that the best results were attained using the chemical and biological types of treatments, whereas laser cleaning was the least satisfactory method.

Despite the variety of techniques used for cleaning stone surfaces, one reason for exploring new treatments is to find a method capable of removing even very thin layers (down to a few micrometers) of materials, with great precision and efficiency, thus allowing the operative to halt the cleaning process at any selected level (Sabatini et al. 2000). In our case-study, only the microbiological treatment seemed to fulfil this requirement. This is a notable advantage of this technique over other methods. However the uniform removal of the black crust layer by layer can certainly be a disadvantage in terms of time, especially when the black crust is very thick.

The results of these tests confirm the efficacy of bacterial cleaning and show that it is potentially competitive with respect to traditional cleaning methodologies. Although microbiological cleaning appears to be a valid tool for black crust removal, the feasibility of using this treatment has to be assessed on a case-by-case basis.

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