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BACTERIAL CLEANING TECHNOLOGY FOR MARBLE SURFACES AFFECTED BY BLACK CRUST: COMPARISON WITH CHEMICAL AND LASER TREATMENTS

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Abstract

An alternative cleaning technique for the removal of black crust from stone surfaces is that using sulphate-reducing bacteria. Studies in this field were first performed by Gauri and Chowdhury (1989) and the technology has been recently improved by Cappitelli et al. (2006, 2007).

The aim of this research was to compare the efficacy and damaging effects caused by a traditional chemical cleaning procedure (ammonium carbonate poultice) and laser treatment (1064 nm, Nd:YAG laser) with the efficacy and damaging effects of the biological method using sulphate-reducing bacteria. The research was carried out on stone materials from the Cathedral of Florence and on the sculpture *Allegoria della Morte* (1870) in the English Cemetery in Florence.

The effects of the different procedures on the original surfaces were evaluated by Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM/EDS) Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, and colour measurements.

Results show that microbial cleaning is the most easily controlled process, and the most efficient for sulphate removal. Its main drawback appears to be the extended time required to remove thick black crusts since numerous applications were necessary. This problem could be solved by a mechanical crust-thinning pre-treatment.

Keywords: microbial cleaning, black crust, stone artworks

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. Trials in this sector have been going on for about ten years, in the form of ancillary activities to the main lines of research in the environmental sector. It was found that, while certain microorganisms proliferate on the artworks, adopting constitutive materials or materials used for conservation (biodeterioration) as a growth substrate, other microorganisms can be used, on the other hand, for the selective removal of unwanted chemical compounds or materials used in previous conservation treatments (bioremediation) (Cifferi et al. 2000).

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. A major conservation problem of outdoor stone artworks is surface sulphation and their consequent blackening in sheltered areas (Moropoulou et al. 1998; Bugini et al. 2000). Interaction between a calcareous substrate and the sulphuric acid of polluted air leads to the chemical transformation of insoluble calcium carbonate to the more soluble calcium sulphate dehydrate, or gypsum. In areas exposed to the rain, soluble salts are washed away, whereas in sheltered areas, salts such as gypsum embed airborne pollutants, such as carbonaceous particles, leading to the development of black crusts during crystallization/solubilization cycles.

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.

2. Materials and methods

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 Bacterial cleaning technology

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 (SO_4^{2-}) into sulphide (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. Furthermore, by varying the concentration of the gel, a mixture can be obtained with the desired compactness and consistency, appropriate for the morphology of the surfaces, so that the bacteria can be brought into contact with the surface to be treated, and then kept in position.

Application is usually performed by positioning tissue paper, followed by a coating of the gel with cling paper. This makes it possible to retain the bacteria in conditions of partial anaerobiosis. It also prevents the gel from drying out, maintaining the cells in a humid environment.

The poultice is left on for a time considered appropriate, and then the softened black crust is removed using a damp cotton swab.

The contact times may vary depending on a series of parameters such as: the thickness, degree of stratification, the composition of the crust, the absorption level of the treated surface, and the external environmental conditions.

Since the microorganisms work in direct contact with the substance to be removed, the cleaning takes place in layers, so repeated applications are nearly always necessary in order to achieve complete crust removal.

2.2 Chemical and laser cleaning

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^2 to 8.8 J/cm^2. The ablation test was performed under water-assisted conditions (Bromblet et al. 2003).

Both chemical and microbial treatments were applied in Carbogel poultice (the best for microrganisms) applied with the interposition of tissue paper, for 10 hours, covered with cling film and removed with a wet cotton swab.

3. Research objectives and organization

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

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.

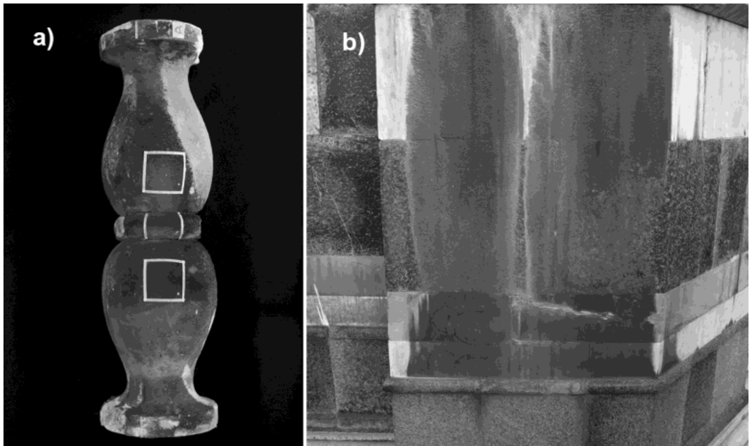

Figure 1. Selected areas for the different cleaning methodologies. a) Column and b) External pilaster of the Florence's Cathedral showing three different lithotypes (from top: green serpentine, red limestone , Carrara marble) uniformly covered by black alteration.

Figure 2. The sculpture *Allegoria della Morte* before and after the restoration treatment.

3.1 Description of the surfaces treated

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 Lazzerini, 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. 3) 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. This high difference in thickness meant that varying numbers of applications had to be made in order to attain complete crust removal, depending on the treated area.

Figure 3. Selected area for the microbial cleaning application on the cloak enveloping the skull of the sculpture, before and after treatment.

4. Results

From the results of tests performed on the column (Fig. 4) it was possible to evaluate the effectiveness of the microbial poultice in removing the black crusts.

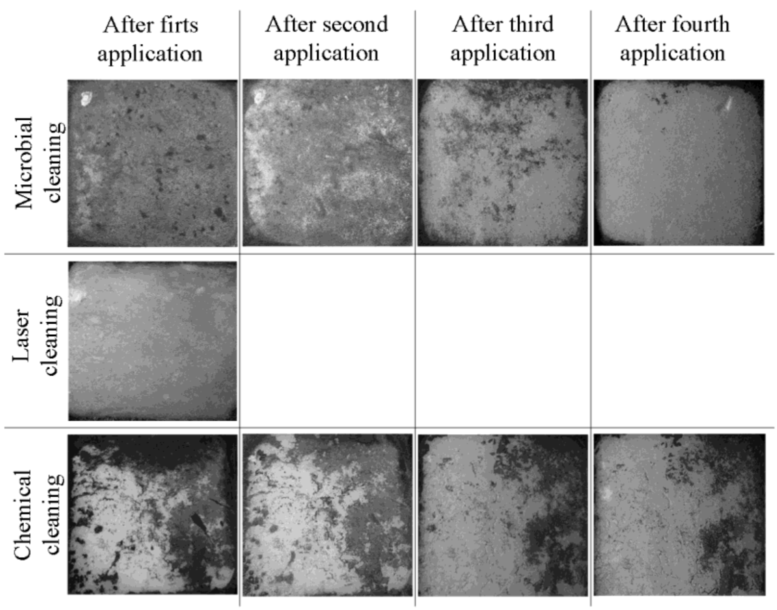

Figure 4. Sequence images of cleaning applications on the Carrara marble column.

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. 4).

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 (Fig. 5) showed the continuing presence of gypsum, oxalates and silicates. Colour analysis also showed a marked variation (Δb) on the b axis (blue-yellow).

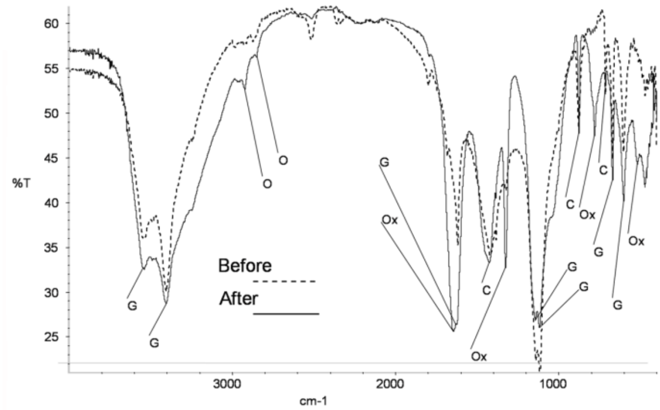

Figure 5. FTIR analyses before and after laser cleaning of the column surface. C, calcite; G, gypsum; O, traces of organic compounds; Ox, calcium oxalate.

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 (Δb) 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. 3), 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.

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. 6). 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.

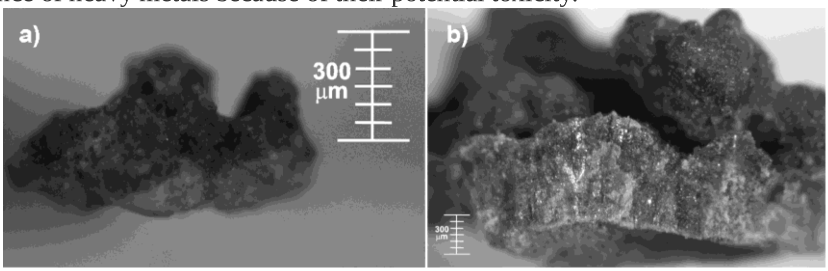

Figure 6. Comparison of the crust thickness coming from a) the preliminary test area and from b) the area of the cloak enveloping the skull on the sculpture.

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.

5. 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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