BIOREMOVAL 0F SULPHATE LAYER FROM A 15TH CENTURY POLYCHROME MARBLE ARTIFACT

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1. Introduction

In recent decades, biotechnology has provided numerous technological innovations for the detection of microbial consortia able to induce the biodeterioration of artifacts [1-6], or of those that are present in the aerosol of confined spaces for cultural heritage [7, 8]. Although many bacterial species represent a threat to the conservation of cultural heritage and / or for operators, some can be used as agents for "biorestoring". The related literature reports that certain bacterial strains (*Pseudomonas stutzeri*, *P. aeruginosa*, *Desulfovibrio vulgaris*, *D. desulfuricans*) have been used for the removal of undesired layers from the surface of works of art. These microorganisms exploit their naturally occurring metabolic processes by transforming the surface layer components into non-toxic gases dispersed into the atmosphere [9].

Currently, bacteria exist that are able to transform organic substances (e.g. *Pseudomonas stutzeri*, nitrate reducers - NRB) or sulphates (e.g: bacteria sulfate reducers - SRB). Other bacterial species are able to trigger the process of biocalcification on stone substrates [10 - 12]. Bacteria such as *Desulfovibrio desulfuricans* and *D. vulgaris* have been successfully applied for the removal of black crust from a lunette in the Cathedral of Milan [13], for the removal of plaster from the basement of Michelangelo's Rondanini Pietà [14] and for cleaning the polychrome marbles of the Florence Cathedral [15].

In this study, *Desulfovibrio vulgaris* viable cells were utilized for the removal of sulfate crusts from the surface of a polychrome marble bas-relief depicting the Eternal Father in the act of blessing, exhibited at the Regional Gallery of Palazzo Abatellis (Palermo, Italy).

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The protocol developed for bioremoval in this case study is part of a complex intervention which, after a preliminary phase of art-historical research and assessment of the state of conservation, resulted in the restoration of the artifact, supported by specific technical-scientific investigations.

2. The case study

2.1 The artifact

Made by an anonymous Lombard sculptor of the fifteenth century, it has a frame with phytomorphic decorations that delimits a pentagonal space depicting the Eternal Father with a halo and his right hand raised in blessing while the other holds the world. The shape and decorative patterns are realized using a technique of low and high relief on a single marble slab of variable thickness. Small traces of colors, visible in limited areas of the surface, have led to the hypothesis that the work may originally have been a polychrome artifact. To confirm this hypothesis and to collect information on the technique of execution used, the artifact was investigated by *in situ* X-Ray Fluorescence, particularly on some areas characterized by the colors red, brown and green-blue. The materials found are those widely used during the Renaissance period, in accordance with the historical and artistic information regarding the use of specific colors for the decoration of marble surfaces [16,17]. The results of the analysis performed on the green-blue colored area revealed the presence of copper, to be correlated to the pigment azurite [$2CuCO_{3\cdot Cu}$ (OH)2]; in some points altered to malachite [$CuCO_{3\cdot Cu}$ (OH)2] by the action of moisture and / or other agents. In points presenting a red-brown color high levels of iron were recorded, related to the use of iron oxide-based pigments such as ochre or earths.

2.2 Conservation state

The state of conservation, mapped according to NorMal Recommendations 1/88 (established standards for the conservation of stone materials), showed that the degradation was mainly due to the presence of incoherent and coherent deposits. This very dark compact deposit forms an uneven layer on the surface, altering the correct reading of the sculptural details and colors.

In order to perform a selective and effective cleaning operation, the deposit was chemically characterized by diagnostic tests, which included X-Ray Fluorescence (XRF) and Fourier Transform Infrared Spectroscopy (FTIR).

2.3 Characterization of deposits

The XRF analysis was performed *in situ* on specific sample points (Figure 1). Results revealed high levels of sulfur, suggesting the deposits, which cover a large area of the bas-relief surface, consist mainly of sulfates (the main components of gypsum being CaSO4×2H2O). Gypsum has a marked tendency to accumulate on surfaces, forming crusts which thicken by including and cementing atmospheric particulates (coal, silicates and other salts), giving rise to “black crusts” [18 - 21].

The presence of gypsum was further confirmed by the FTIR spectra, which showed all the characteristic bands of this compound.

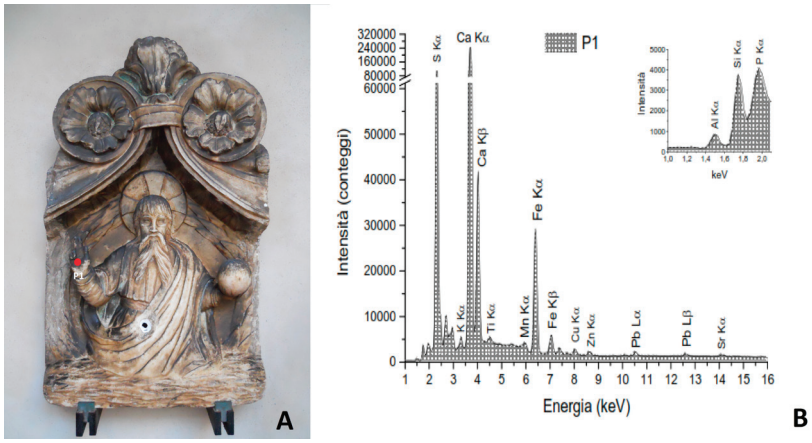

Figure 1. XRF analysis: A) Set point (in red) on artifact surface; B) corresponding XRF profile (relevant Sulphur amount).

3. Bioremoval

The restoration process involved different phases, one of the most important being the removal of the black crust, which was performed respecting the physical-chemical nature of the constitutive materials.

Considering that extensive portions of the surface were characterized by deposits with a significant presence of sulfates, a number of sulfate removal strategies were examined and their different peculiarities assessed.

3.1 Viable bacterial cells

Desulfovibrio vulgaris viable bacterial cells were utilized for the removal of the black crusts. The viable bacteria, available as lyophilized cells (*Micro4Art solfati*© produced by the company *Micro4you* S.r.l.), were hydrated and applied to the surface of the artifact as a gelled solution (modified polyacrylic acid gel - Carbogel) [22]. Bacterial cells carry out a desulfating action according to the following reaction: $6CaSO4 + 4H2O + 6CO2 \rightarrow 6CaCO3 + 4H2S + 2S + 11O2$ [9].

Tests were performed on the consistent uniform deposit present on the surface of the marble artifact by comparing the results after application of the Bacteria + Carbogel solution with the control solution, consisting only of Carbogel in aqueous solution (Figure 2).

Both applications were performed by inserting a sheet of Japanese paper between the artifact surface and the aqueous gelled solutions and subsequently covered with a polyethylene film (Figure 3) so as to maintain conditions of both humidity and partial anaerobiosis, necessary for bacterial metabolism [23].

Compared to the Control solution, the effectiveness of the bioremoval was evident from the first application. The desired level of cleaning, however, was only achieved after three applications.

Successful removal of the sulfate crusts was confirmed by XRF analysis, which

showed a very low sulfur content in the cleaned area when compared to the untreated area.

In view of these results an *ad hoc* protocol was defined for each area of the artifact characterized by a chalky coherent deposit, applying the solution once or twice in relation to the thickness (Fig. 4).

Figure 2. Preliminary test performed by Desulfovibrio vulgaris viable cells solution, gelled by Carbogel (A); Control, Carbogel alone (B). The gelled solutions were covered with a transparent polyethylene-sheet, in order to keep adequate humidity and partial anaerobic conditions, needed for bacterial metabolism.

Figure 3. Bioremoval: (from left) before processing; Japanese paper and gelled solution application; covering by transparent polyethylene sheet; black crust removal.

Figure 4. Bioremoval, detail: (from left) before; during; after cleaning.

At times, the bacterial solution gelled by Carbogel proved difficult to apply, mainly due to the fact that the artifact was positioned vertically in a ventilated space and had small sections with complex geometries. In addition, tests were carried out by supporting the viable bacterial cells with Agar, which unlike Carbogel, is not a “direct” gelling agent requiring cycles of heating and cooling [24]. Using the Agar gelling agent, the result of the cleaning operation was much poorer than that performed with Carbogel.

3.2 Alternative methods

In this study, the action of Ammonium carbonate added to direct gelling agents, such as polyacrylic acid (Carbogel) and Xanthan Gum (Vanzan), were evaluated as biocleaners. Ammonium carbonate performs a desulfating action according to the following reaction: $CaSO4 \cdot 2H2O + (NH_4) 2CO3 \rightarrow CaCO3 + (NH_4) 2SO4$ [25].

Although both gelling agents gave good results, for the removal of black deposits from the pigmented areas with copper residues, the use of ion exchange resins (anionic) was preferred. Desulfation was performed according to the following reaction: $R2(OH)2 + CaSO4 \cdot 2H2O \rightarrow R2SO4 + Ca(OH)2 + 2H2O$, followed by: $Ca(OH)2 + CO2 \rightarrow CaCO3 + H2O$ [26].

4. Conclusion

Among the advantages of using the viable bacterial cells developed by Micro4you, as well as the speed and ease of use, considerable importance is represented by the guarantee of a gradual, selective and repeatable activity that fully respects the surface of the work of art (Figure 5).

Figure 5. The artifact before and after restoration.

Another advantage is that cleaning can be done without the use of polluting substances, guaranteeing high safety standards, both for the operator and the environment [27].

These results confirm that the use of microorganisms satisfies the selective characteristics of the cleaning, at the same time respecting the constitutive materials of the artifact, as required by modern conservative restoration. This method, therefore, is a viable alternative to traditional methods, oriented towards sustainable restoration.

The results of this study, moreover, provide useful information for the controlled biocleaning of polychrome stone surfaces.

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Biographical notes

Manuela Martino, is a qualified restorer (L.D, 42/2004 - specialization in Stone Materials and Products, decorated surfaces of architecture; she has a degree (MRL / 02) in Conservation and Restoration of Cultural Heritage from the University of Palermo and is now studying a Master's Degree, "Expert researcher in nano materials and nano technologies for Cultural Heritage" at the University of Palermo**.**

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Annalisa Balloi, graduated in Biological Sciences at the University of Cagliari with a thesis carried out at the research center in Alnarp, Sweden and a PhD at the Faculty of Agriculture of the University of Milan. In 2010, with some colleagues, she participated in the university spin-off "Micro4yoU Srl", of which she is the CEO. Since its establishment, Micro4yoU has received numerous awards for its commitment and achievements in Technology Transfer in the field of environmental biotechnology. In addition to continuing her work in Micro4yoU, she is currently a professor at the Academy of Fine Arts of Brera where she is responsible for the course, "Elements of Biology Applied to Restoration."

Lorella Pellegrino, qualified as a Restorer of Cultural Heritage, with a Specialization in Restoration of Stone Artifacts at the OPD in Florence, and in Restoration of Wall Paintings at the ICR in Rome. She has an executive role as Restorer of Cultural Heritage and head of the Laboratory of Restoration of Artifacts of Inorganic Origin at the *Centro Regionale per la Progettazione e Restauro* of Sicily. She coordinates ordinary and extraordinary restoration of movable, architectural, archaeological, historical and artistic heritage. Chief Operating Officer and Director of works in complex interventions on sculptures and on sites such as the Greek archaeological areas of Halaesa Arcidiaconea, the Greek theater in Taormina, the Villa Romana del Casale in Piazza Armerina and Patti. She carries out educational activities, which include theoretical, technical and practical aspects for internships of the degree course "Cultural Heritage Restorer" at the University of Palermo. She coordinates senior and junior Italian and Cambodian restorers, who carry out restoration work at the archaeological site of ANGKOR, Cambodia, on sculptures and architectural complexes of Khmer temples.

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Franco Palla is Associate Professor of Applied and Environmental Botany at the University of Palermo, Italy. He is Professor and Vice-Coordinator of the Five-Year Degree in Conservation and Restoration of Cultural Property (MRL 02, certified professional restorer). He is a member of the Permanent Commission for the Protection of Cultural Heritage of the National Order of Biologists. Scientific head of UNIPA Research Unit, for the Research Project PON01_00625, It@cha (Italian technology for advanced applications in Cultural Heritage). He was one of the members of the working groups in the cooperation project Italy-Cambogia for Training Experts of Cultural Heritage, University of Palermo-Royal University of Fine Arts and the Ministry of Culture and Fine Arts, Angkor, Cambodia. He is Coordinator of the Laboratory of Biology and Biotechnology for Cultural Heritage at the Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF) of the University of Palermo.