BIOTECHNOLOGY IN THE CONSERVATION FIELD: REMOVAL OF SULPHATES USING BACTERIA AND BIOCONSOLIDATION OF PAINTINGS AND STUCCOS

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1. Biorestoration

Biotechnologies, based on the use of non-pathogenic microorganisms, have been introduced into the field of cultural heritage conservation due to their positive properties and the absence of toxic effects for restorers [1]. During the past few years research has focused on two different areas: “biocleaning” and “bioconsolidation”, two biological processes involving the use of various bacterial strains as well as different application methods. The aim of this study was to explore the use of such procedures (usually applied on stone materials) in the restoration of wall paintings and stuccos, which often present constitutive materials and conservative conditions of great complexity. In particular, a bio-consolidation process was tested to re-establish cohesion in the materials of several superficial layers and a bio-cleaning method was used for the removal of sulphates which are often found on frescos as decay products. In both cases patented and marketed products were used. Experiments were conducted as part of the practical stage of a thesis on conservation by Eleonora Panella, in the *Istituto Superiore per la Conservazione ed il Restauro*. The actual research was carried out in the Chapel of St. Peter in the church of Santa Pudenziana in Rome, Italy, on wall paintings and stuccos from the sixteenth century.

2. Bioconsolidation

This treatment, aimed at restoring mechanical strength and internal cohesion in natural and artificial stone materials, is based on “biocarbonatogenesis”, a natural process that occurs in many environments and is related to the formation of carbonate sediments and rocks. The phenomenon of bioprecipitation is very com-

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plex and can be influenced by the physical-chemical parameters, metabolism and structure of the various bacterial strains.

Products derived from the metabolic activity of calcinogenic bacteria are new minerals, very similar to those that constitute the substrates of the artworks. Bioconsolidation treatments produce mainly two minerals, calcite and vaterite. Once released from the bacteria, these minerals accumulate in the porous spaces of the material and, by doing so, restore its cohesion.

The formation of calcite crystals begins on the surface of the bacterial cell, the cell wall, which contains a considerable number of functional groups such as phosphates, hydroxyls and carboxyls, highly reactive to metallic ions such as Ca^{++} found in the environment surrounding the cell. Since Ca^{++} ions are not used in large quantities in intracellular metabolic processes, they accumulate on the surface of the bacterial cell. When the metal ion Ca^{++} is complexed, it becomes the nucleation center on which the subsequent mineral crystals are formed. After the nucleation, crystalline structures develop, incorporating the bacteria themselves [2].

Studies on bio-induced calcite precipitation began in the 1980s in France and there are currently several European projects that are conducting experiments on the bioconsolidation of stone materials. Although most of the studies carried out so far relate to the consolidation of natural stone artifacts, for some years several research groups have been seeking to expand the types of artifacts on which such treatment can be carried out.

Among the various bioconsolidation treatments that are available, the present study focuses on the method developed by Dr. M.T. Gonzalez-Muñoz of the Department of Microbiology of the University of Granada, which provided the products and consulting for the application [3-8].

2.1. M-3P product

Initially, this research team proposed the use of Myxococcus xanthus as a bacterial strain capable of inducing the biomineralization of calcium carbonate.

Following further studies, a method was developed without the use of bacterial cells and therefore completely safe for the operator [9]. The procedure is based on the use of a culture medium called M-3P, which stimulates the calcinogenic activity of bacteria already present in the microbiota inhabiting the surfaces [10].

M-3P is a sterile nutritional solution made of 1% Bacto Casitone (a pancreatic digest of casein), 1% Ca $(CH_3\ COO)_2 \cdot 4H_2O$, 0.2% $K_2CO_3 \cdot 1/2H_2O$ in a 10 mM phosphate buffer (pH 8) [11].

The use of Bacto Casitone as a source of carbon and nitrogen causes alkalinization due to ammonia acidification involving the formation of ammonia, which results in an increase in pH. In the alkaline environment, with the lower concentration of H^+ ions, the balance of the reaction between carbonate and bicarbonate moves towards the production of carbonate ions:

$$HCO_3^- \leftrightarrow CO_3^{--} + H^+$$

In the presence of Ca^{++} calcite is produced

$$Ca^{++} + CO_3^{--} \leftrightarrow CaCO_3. \text{ [9]}$$

2.2. Experimental procedure and Application

The University of Granada provided the product M-3P, which was tested along with other consolidation treatments. This bioconsolidation process was compared with the conventional consolidants most frequently used to restore the cohesion of painted and stucco plasters: colloidal nano-particles of $Ca(OH)_2$[1] and acrylic resin[2].

The experimental procedure was structured in the following phases:

1) definition of materials and methods for investigations on samples and in situ;

2) production of samples, using the same techniques as for the original artefacts. For the stucco samples marble powder and lime were used, while painted plaster samples were made of pozzolana, lime and inorganic pigments;

3) identification of sample areas with severe lack of cohesion on frescos and stucco decorations in situ;

4) application of the consolidating products on painted plaster and Roman stucco samples, on detached erratic fragments of frescoes and stuccoes and on sample areas of the fresco and stucco in the Chapel of St. Peter.

5) evaluation of effectiveness by means of contact sponge method, peeling tape test, contact angle measurement on samples of painted plaster and Roman stucco, SEM analysis on detached erratic fragments from fresco and stucco.

Nanolimes were applied on the samples by impregnation, using a brush; acrylic resin and M-3P were sprayed onto the surfaces (Figure 1). After application of the products the samples were left in the Chapel of St. Peter for one month, to allow deposition of the same biocontaminants already present on the frescos and stuccos in situ, to take place.

Erratic fragments were treated only with M-3P, in order to carry out investigations to evaluate the effectiveness of the product.

In situ, M-3P was applied to the previously selected sample areas, choosing different colour layers, to take into consideration the possible interference of the product with the pigments.

M-3P application was performed in the summer to ensure a suitable temperature for bacterial growth. The product was applied up to saturation three times a day for six consecutive days, on samples, on erratic fragments and in situ.

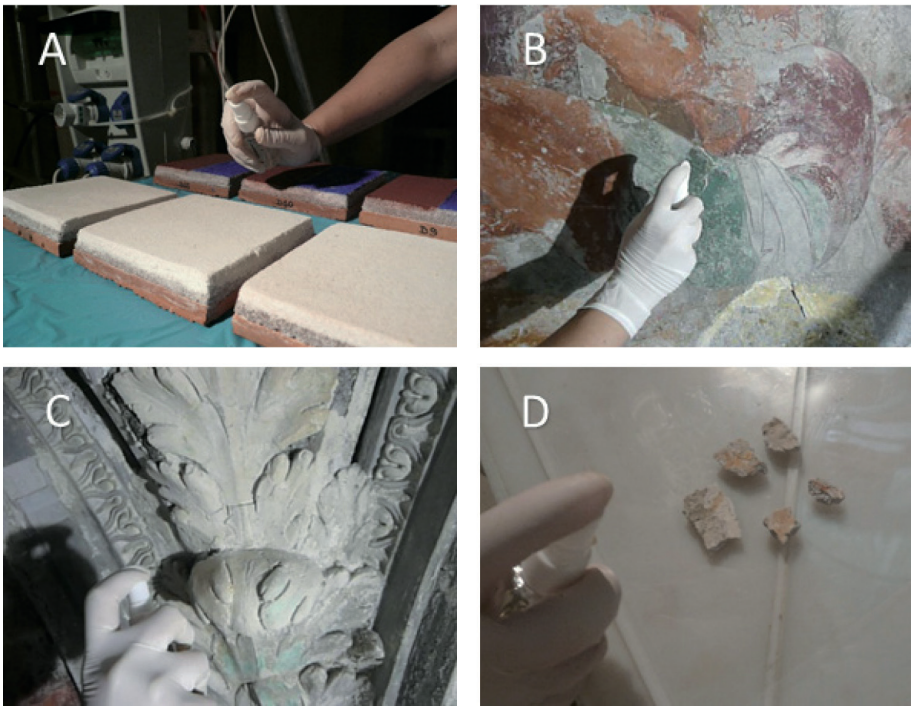

Figure 1. Application of the M-3P product by spraying it on the different surfaces: (A) on samples; (B) on wall paintings; (C) on stuccos; (D) on erratic fragments (Photo: Eleonora Panella).

2.3 Evaluation of efficacy

The tests were carried out before and after the treatments. Thanks to the use of samples, it was possible to make repeated measurements and so collect many data. These data were processed into charts that helped to highlight the results. Finally, an overall reading made it possible to evaluate the effectiveness of the different treatments.

Contact sponge method[3]: a reduction in water absorption was recorded for all the consolidation treatments analyzed. On the stucco samples, the best results were obtained with the M-3P product; on the plaster samples, the major reduction occurred with the acrylic resin (Figure 2).

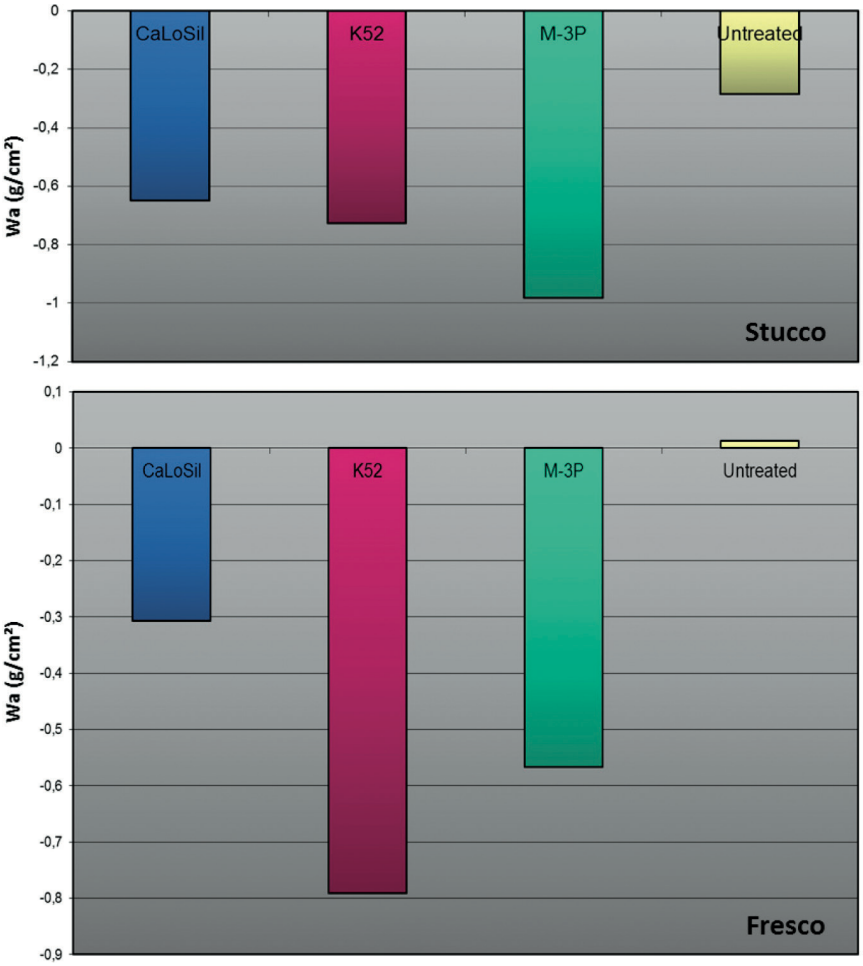

Figure 2. Reduction of water absorption after treatment on samples.

Peeling tape test: the application of acrylic resin showed the best result when compared with the untreated sample. Where the acrylic resin was used, in fact, the peeling tape caused less pigment detachment from the surface than where M-3P was used (Figure 3).

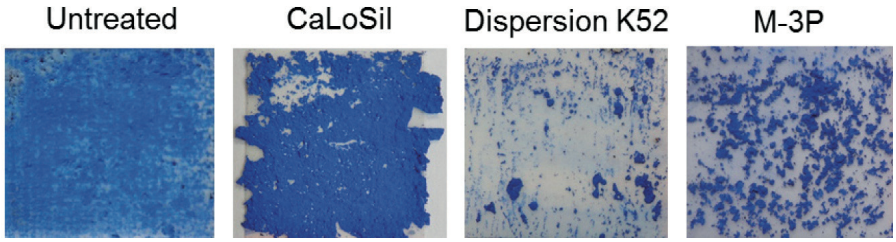

Figure 3. Amount of pigment detached after consolidation compared to the untreated surface (Images from the ISCR biology lab).

Contact angle measurement[4]: the contact angle could not be measured before the treatments as the drop was absorbed too quickly by the surface. After the treatments, it was possible to measure the contact angle on both stucco and painted plaster samples treated with acrylic resin and stucco samples treated with M-3P (Figure 4).

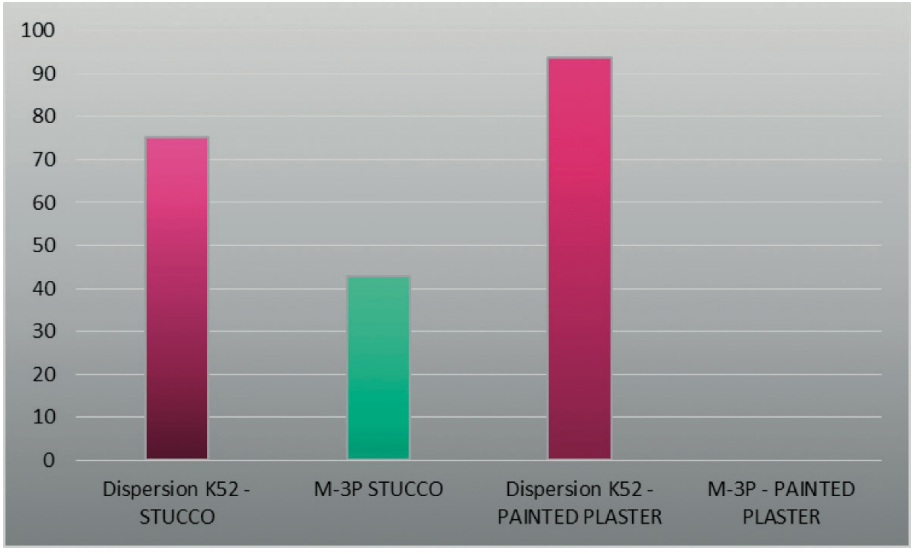

Figure 4. Contact angle measurements after treatments

SEM and XRD: these tests were run only on erratic stucco and plaster fragments treated with M-3P in order to evaluate the efficacy of the bio-consolidation process.

Calcium enrichment was observed on the fragments (Figure 5). However, it was difficult to quantify the formation of bacterial calcite because it could have been masked by the large amount of calcite present in the untreated sample (before treatment).

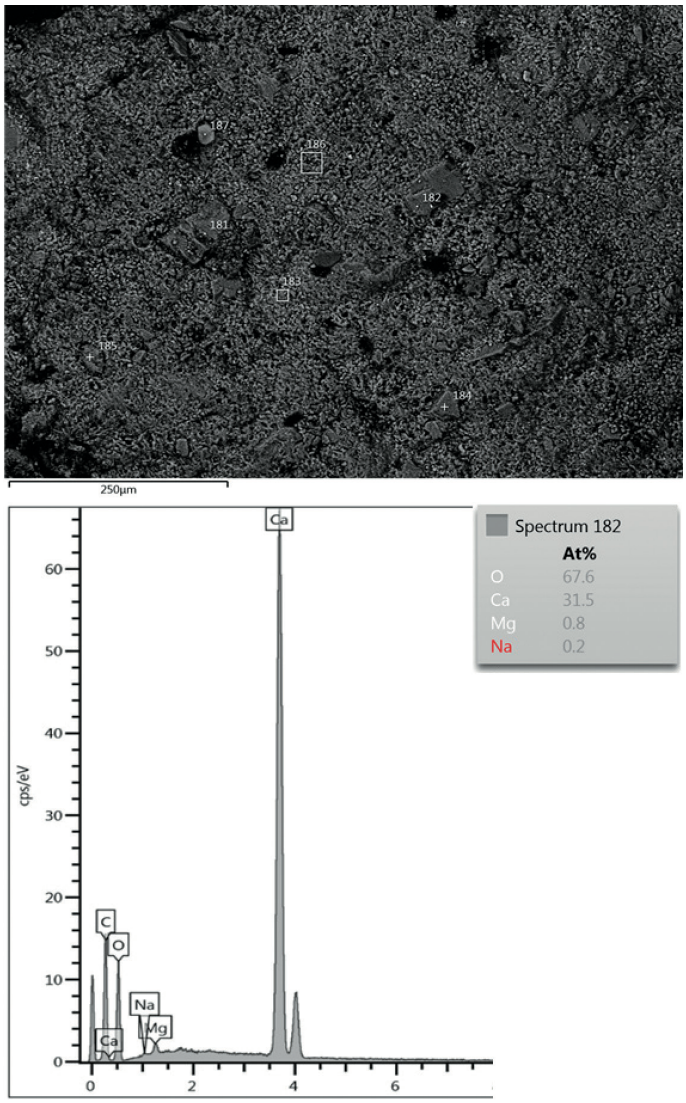

Figure 5. SEM-EDS of the stucco surface after treatment. Calcite crystals are visible (Images from the ISCR biology lab).

The diffractometric investigation, in this case, proved ineffective because calcium carbonate is the main constituent material of the plaster in the fresco and of the Roman stucco.

It is important to specify that the results of the analyses are related to the specific areas where the samples were taken from and therefore may not be representative of the general situation.

2.4 Evaluating interference

A first evaluation of interference of the treatment was performed on the basis of the visual comparison between the samples. In particular, two aspects were considered: the yellowing potential of the nutrient medium, which is of a slightly yellowish colour and possible whitening from the newly formed calcite. Colorimetric measurements were performed in situ on all fresco samples.

Colorimetric measurements: on stucco samples treated with M-3P, colorimetric measurements showed a mild yellowing, while no remarkable variations were observed on the fresco samples (Figure 6). In addition, the surfaces treated in situ with M-3P did not show any relevant variation in colour.

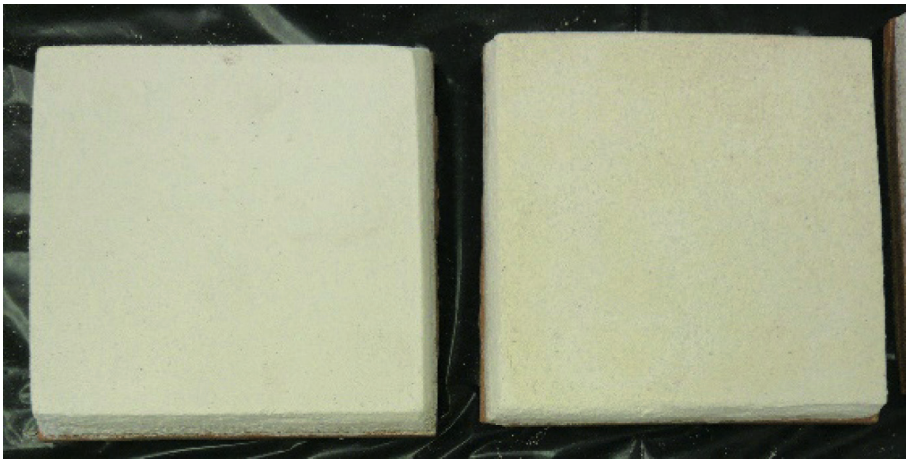

Figure 6. On the left the untreated stucco sample; on the right the sample treated with M-3P, slightly yellowed (Photo: Eleonora Panella).

Biological growth control: it was also necessary to check the growth of potential biodeteriogens, such as fungi, due to M-3P application. To this end, half the surface of a painted plaster sample was treated with M-3P following the same procedure used in situ. Subsequently, the sample was left in the Chapel of St. Peter for about 2 months, after which it was placed in a dryer, maintaining relative humidity conditions close to 100% for about 30 days. At the end of this period visual and stereo-microscope observation showed the growth of fungi colonies on the surface of the sample where M-3P had been applied (Figures 7-8), while no growth was detected on untreated areas. At least three species of fungi had developed in small colonies, distributed homogeneously on the treated areas.

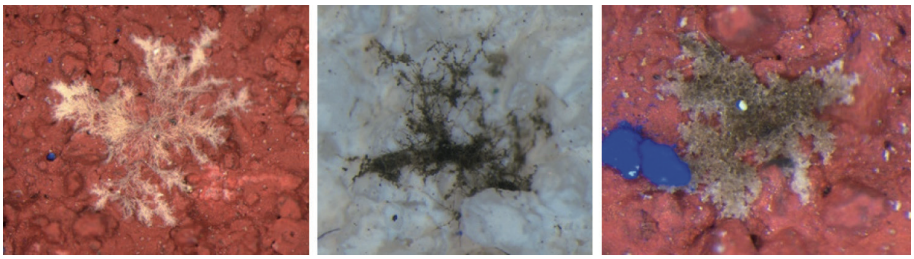

Figure 7. Stereomicroscope images of fungi grown on the part of the sample treated with M-3P (Images from the ISCR biology lab).

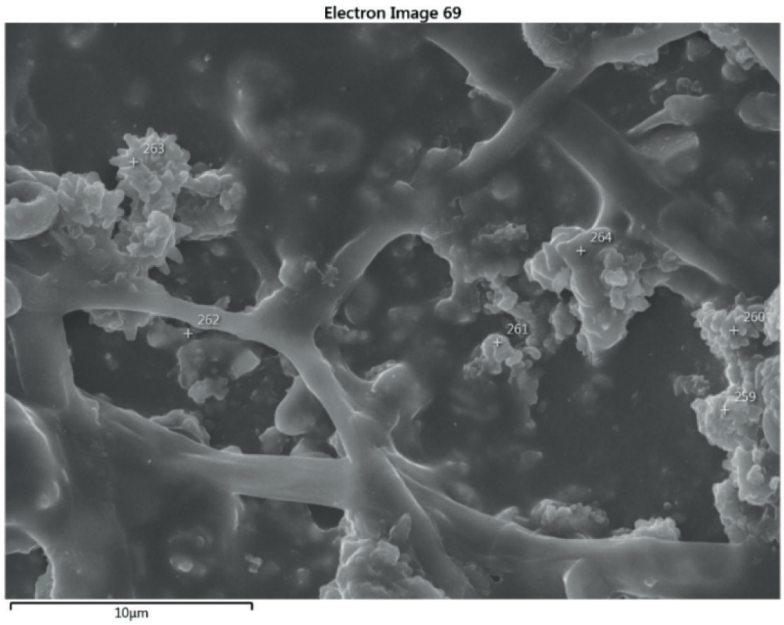

Figure 8. SEM image of plaster fragments treated with M-3P. Presence of fungal hyphae and extracellular polymeric substances (Images from the ISCR biology lab).

2.5. Considerations

M-3P showed different capacities depending on the materials on which it was applied. In fact, a good level of cohesion to the stucco was obtained but no evident difference was obtained on the painted surfaces.

The acrylic resin, on the other hand, showed good consolidating properties both on the stucco and painted plasters as proven by the reduced water absorption, the mild water-repellent effect on the plaster and the results of the peeling tape test.

The present study has therefore shown that this bioconsolidation process has a consolidating effect despite having lower efficacy than the acrylic resin. If the constitutive material is not excessively degraded, the M-3P product offers some advantages over chemical processes; however, if there is a particularly severe lack of cohesion, this method may be inefficient.

The advantages of M-3P over chemical processes are that it does not require the use of chemicals and solvents, it is easy to apply, and it is totally compatible with carbonate substrates.

However, it is less effective than acrylic resin, especially on plaster; the application procedure so far proposed is rather rigid and cannot be adapted to the different situations as in the case of conventional treatments; the treatment also requires relatively high temperatures and, if applied in environments with high humidity, could cause potentially harmful biodeteriogenic growth.

3. Biocleaning

Biocleaning is an eco-friendly technology that uses living organisms and/or their enzymes as cleaning agents through their metabolic processes [12]. The bacteria used to remove specific substances from a substrate, are non-pathogenic and non-sporogenic, therefore this method is safe both for the worker and the artwork.

Cleaning with bacteria can have advantages over other biological methods already in use in the cultural heritage field (e.g. enzyme processes). That is because enzymes are very selective but when it comes to more complex decay products, they are often incapable of removing them completely. Bacteria, on the other hand, have a pool of enzymes which guarantee selectivity as well as the ability to act on a wider range of substances. Furthermore, microorganisms can adapt very well to various environmental conditions which means application is easier for the operator to perform.

Early studies on the use of bacteria to remove decay products or other substances date back to the end of the 1980s and have been directed especially at the removal of protein substances and at salt reduction on stone materials and wall paintings [13-21].

This study has focused on the removal of sulphates using a bacterial formulation consisting of sulphate-reducing bacteria. For the bio-cleaning tests a partnership was established with the company Micro4you (Dr. A. Balloi), a spin-off company of the University of Milan, which supplied the product Micro4Art.

3.1. Micro4Art

Micro4Art is a lyophilized formulation characterized by a high concentration of *Desulfovibrio Vulgaris*. This bacterial species is part of the anaerobic soil bacterial flora, so it does not require the presence of oxygen to survive. It uses sulphate as an electron acceptor, transforming it into hydrogen sulphide, which, at ambient temperature, evaporates without leaving any residues.

$$6CaSO_4 + 4H_2O + 6CO_2 \rightarrow 6CaCO_3 + 4H_2S + 2S + 11O_2$$

Calcium ions (Ca^{2+}), derived from the gypsum during the process, react with carbon dioxide, resulting in the formation of calcite ($CaCO_3$) which penetrates into the substrate, producing a mild consolidating effect [22-23].

The application method includes the following:

– application of the bacterial suspension by means of a suitable supporting gelling agent which guarantees surface humidification throughout the treatment;

– use of a plastic film covering the surface to prevent water evaporation and to reduce the oxygen supply in order to improve the ability of the bacteria to metabolize sulphates;

– bacteria removal followed by a soft cleaning with sponges and deionized water to eliminate residuals.

Since the treatment is highly influenced by the application method, the tests were mainly directed at defining an application method that would facilitate its use in situ and, in particular, to find a supporting agent suitable for vertical surfaces.

The trial procedure was structured in the following phases:

1) verification of the presence of sulphates as decay products on the fresco in the Chapel of St. Peter by conductivity measurements and chemical kits for the detection of sulphates and nitrates;

2) selection of the most suitable supporting agent for the application of bacteria and development of the treatment procedure through laboratory tests on samples;

3) identification of sample areas on the fresco, characterized by uniform sulphate efflorescence;

4) removal of sulphates using the bioformulation applied with different gelling agents, compared to traditional methods;

5) evaluation of its effectiveness by means of conductivity measurements; sulphate analyses with chemical kits on extractive compresses of cellulose pulp; SEM investigations with EDS analysis on small samples of the fresco.

The bio-formulation was tested, in situ, along with three different supporting agents:

– neutralized polyacrylic acid[5] + Micro4Art

– gellan gum[6] + Micro4Art + synthetic mesh

– natural seaweed agar agar[7] + Micro4Art

The gelling agents were also applied on the surface without bioformulation in order to evaluate their influence on the biocleaning procedure.

Finally, the bioformulate was compared to a traditional chemical method:

– a solution of 5% ammonium bicarbonate in deionized water gelled with hydroxypropyl cellulose[8] and cellulose pulp (Figure 9).

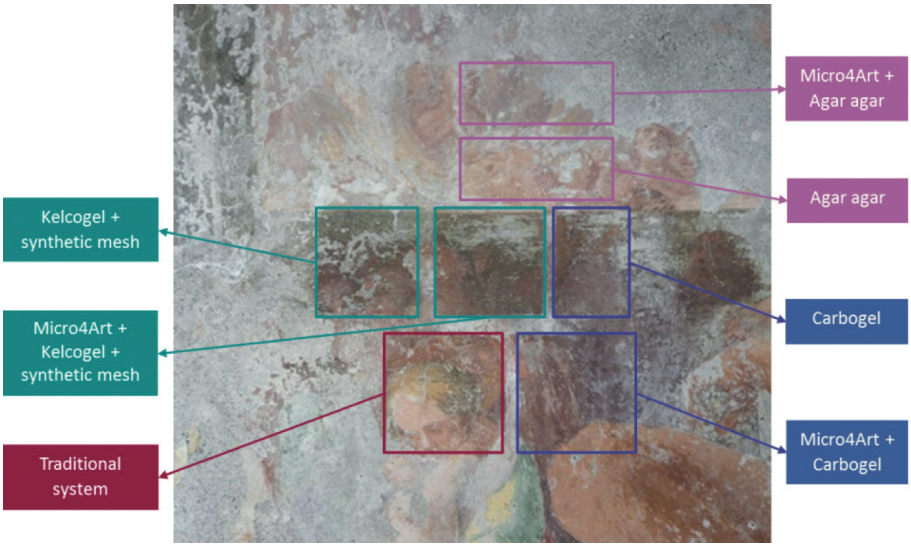

Figure 9. Test showing the different cleaning systems on the fresco surface (Photo: Eleonora Panella).

The best system to apply the Micro4Art bioformulation proved to be the one with gellan gum + Micro4Art + synthetic mesh (Figure 10) because:

– it allows the bacteria to be inserted in the gel;

– it gradually releases the water on the surface while maintaining the right degree of moisture for the bacteria;

– it leaves very few residues.

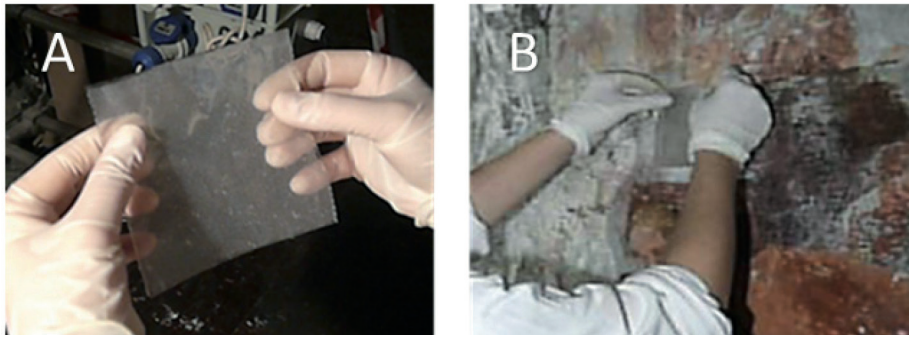

Figure 10. (A) Micro4Art + gellan gum Kelcogel ready to be put on the surface; (B) application on the fresco surface (Photo: Eleonora Panella).

3.2 Evaluation of efficacy

The efficacy of the various methods was evaluated by measuring the amount of residual sulphates after treatment compared with the untreated surface.

Conductivity measurements: showed a significant reduction of ions on the areas treated with the traditional system and with agar agar + Micro4Art (Figure 11).

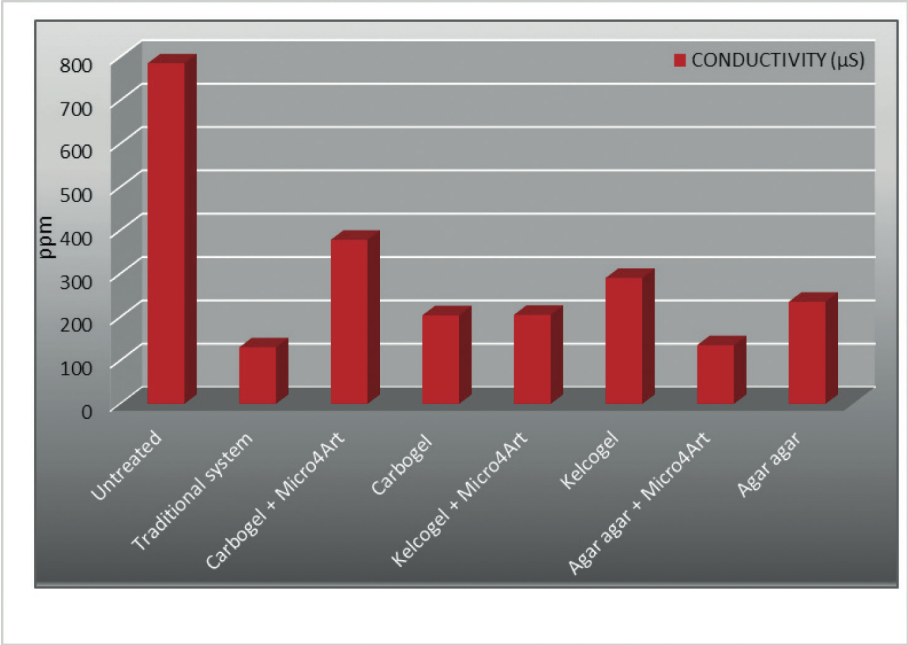

Figure 11. Histogram with conductivity measurements

Sulphates control test: the assay showed that the traditional system produces a considerable decrease in sulphates, while the agar agar + Micro4Art and gellan gum Kelcogel + Micro4Art procedure only slightly reduce sulphate concentrations. In the area treated with Carbogel + Micro4Art the sulphate concentration was equivalent to the untreated area (Figure 12).

SEM / EDS: this investigation proved to be more reliable in evaluating the efficacy of treatments, since the sulphur mapping allows clearer observation of the residues.

They showed that the traditional method is the most effective in the removal of sulphates, followed by gellan gum Kelcogel + Micro4Art. In fact, only a few sulphur residues are visible on the samples collected from the areas treated with these two procedures. On the other hand, on the areas treated only with the gelling agents, a consistent and uniform layer of sulphates is visible (Figure 13).

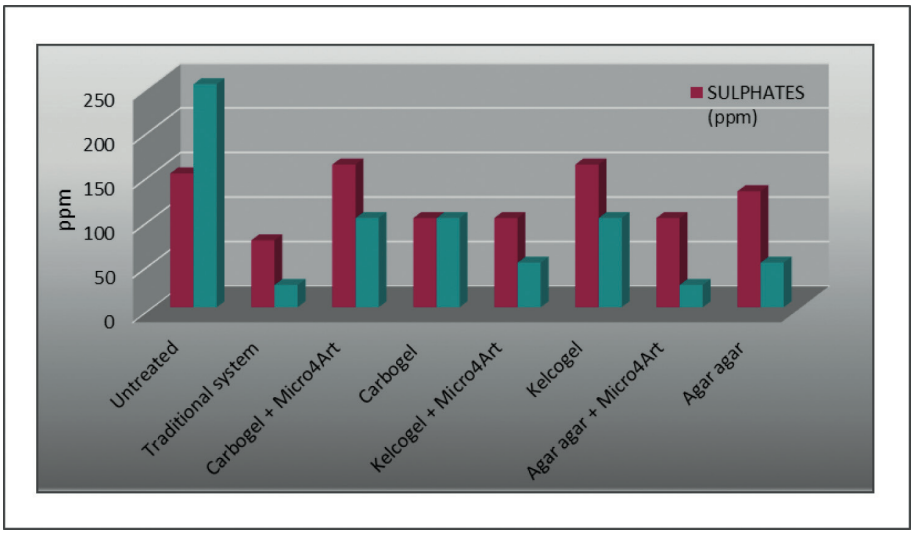

Figure 12. Histogram with sulphate and nitrate concentrations after cleaning.

4. Conclusion

This study highlighted that the traditional procedure is slightly more effective than the gellan gum Kelcogel + Micro4Art system and that the gelling agent, supporting the bioformulation, highly influences the efficacy of the treatment. The advantage, is the high selective action that does not involve the use of solvents or chemical substances, making it completely non-toxic. Among the disadvantages, especially if compared to the traditional procedure with ammonium bicarbonate, is the high cost of the bioformulation-support system, along with the preparation time of the products and the rather lengthy application times.

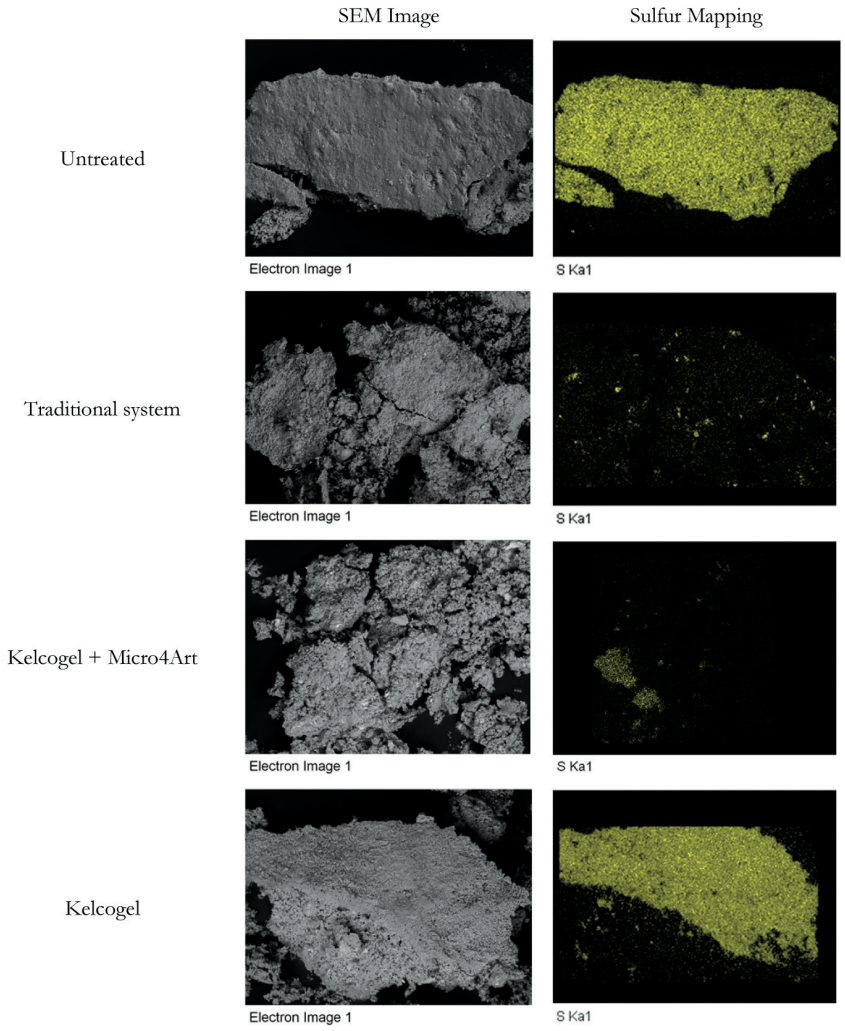

Figure 13. SEM-EDS sulphur mapping of fresco fragments (Images from the ISCR biology lab).

Acknowledgments

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Notes

1 Calosil IP5, IBZ- Salzchemie GmbH & Co.KG.
2 Dispersion K52, Kremer Pigmente.
3 The tests were performed in accordance with the UNI 11432: 2011 standard.
4 The tests were performed in accordance with the NORMAL – 33/89 standard.
5 Carbogel.
6 Kelcogel, CP Kelco.
7 AgarArt.
8 Klucel G at 3% in the ammonium bicarbonate solution.

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Biographical notes

Eleonora Panella was born in Rome in 1982. From 2001 to 2003 she attended the professional institute for restoration Ars Labor. In 2015 she obtained her degree from the Istituto Superiore per la Conservazione ed il Restauro in Rome with a dissertation on the restoration of late sixteenth century wall paintings and stuccos and on biotechnologies applied to cultural heritage. Since 2004 she has worked in the conservation field and currently collaborates with ISCR.

Carla Giovannone is conservator of cultural heritage, graduate and majored in Restoration and Conservation of Cultural Heritage at the *Istituto Centrale per il Restauro* in Rome and also in Medieval Art History at "La Sapienza University" in Rome; she is a Restoration Officer at the Higher Institute for Conservation and Restoration (ISCR), Laboratory of Wall Paintings and Plasters. From 2018, she is a member of the Scientific Council of the aforementioned Institute. She is a lecturer at the ISCR Higher Education and Study School for the Master's Degree in Restoration and Conservation of Cultural Heritage (LMR / 02), Decorated Architectural Surfaces. She carries out restoration activities on wall paintings and stucco decorations, natural stone materials and decorated surfaces. From 2012-2016 she was a lecturer at "La Sapienza University" of Rome in the School of Specialization in Architectural and Landscape Heritage for the study and restoration of monuments, with specialist theoretical lectures on inorganic binders and mortars and on consolidation systems. From 2016 she is a contract professor for MIUR (*Ministero dell'Istruzione, dell'Università e della Ricerca*) at the Academy of Fine Arts in L'Aquila for the degree course in Restoration and Conservation of Cultural Heritage (LMR / 02), Decorated Architectural Surfaces. She has authored 29 scientific publications on topics related to studies, experiments and conservative interventions on wall paintings, stuccos, mosaics, architectural decorated surfaces.

Marco Bartolini graduated in Biological Science in 1994 at Tor Vergata University of Rome. He coordinates the mycology and bacteriology Units and is responsible for both the Instrumental and biochemical investigations section and Instrumental Techniques of the Biology Laboratory at the *Istituto Superiore per la Conservazione ed il Restauro* (ISCR). Since 2017, he is a member of the Scientific Council of the aforementioned Institute. Since 2017 he is vice-director at ISCR Higher Education and Study School for the Master's degree course in Restoration and Conservation of Cultural Heritage (LMR/02). He is lecturer of "Applied Biology to Cultural Heritage" at the ISCR Higher Education and Study School, and at the University of Fine Arts in Macerata. His research focuses on the role of fungi and bacteria in the biodeterioration of inorganic materials and on the evaluation of the effectiveness of biocides for the control of biodeteriogens on artefacts. Member of the Italian UNI -NORMAL Committee for the definition of standard methods on the study and the conservation of natural and artificial stones. Author of over 70 scientific publications.

Veronica Fondi was born in Marino (Rome) in 1987. After obtaining a degree in Cultural Heritage at the University of Tor Vergata she attended the *Istituto Superiore per la Conservazione ed il Restauro* in Rome, where she graduated in 2015 with a dissertation on the restoration of the roman frescoes in Massenzio's Villa on the Appia

Antica. Since then she has worked in the conservation field and is currently a Restorer-Conservator Official at the Ministry of Cultural Heritage and Activities.

Summary

The aim of this study was to explore the use of biotechnologies in the restoration of wall paintings and stuccos which often show a great complexity regarding constitutive materials and conservative conditions. 'Biological methods' were compared to traditional methods, focusing on restoring the cohesion of the superficial layers (bioconsolidation) and the removal of sulphates (biocleaning) often found on the frescoes as decay products. The experimentation took place as part of a graduate thesis and was divided in two stages: the first was spent in the laboratory working on samples of painted plaster and the second was spent in situ, on the frescoes and stuccos in the Church of Santa Pudenziana in Rome, Italy. Application of the products was followed by a campaign of investigations aimed at determining the efficacy, and the advantages and disadvantages of these methods compared to conventional ones.

Riassunto

Lo scopo di questo studio era di esplorare l'uso delle biotecnologie nel restauro di pitture murali e stucchi che spesso mostrano una grande complessità per quanto riguarda i materiali costitutivi e le condizioni conservative. I "metodi biologici" sono stati confrontati con i metodi tradizionali, concentrandosi sul ripristino della coesione degli strati superficiali (bioconsolidamento) e la rimozione dei solfati (biopulitura) che si trovano spesso sugli affreschi come prodotti di decadimento. La sperimentazione si è svolta nell'ambito di una tesi di laurea ed è stata suddivisa in due fasi: la prima, in laboratorio, su campioni di intonaco dipinto e la seconda, in situ, sugli affreschi e gli stucchi della chiesa di Santa Pudenziana in Roma, Italia. L'applicazione dei prodotti è stata seguita da una campagna di indagini volte a determinare l'efficacia, i vantaggi e gli svantaggi di questi metodi rispetto a quelli convenzionali.