Biocleaning

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Abstract

Cleaning is one of the first and most important steps in conservative restoration intervention, as it removes the unwanted layers of dirt and deposit from the surface of an artefact. It must be done selectively, however, by adapting the cleaning operation to the different zones and removing successive layers of deposit without acting directly on the original materials of the surface. Generally, cleaning protocols are based on chemical or physical procedures with potential negative effects for restorers' health and/or for the materials constituting the artworks. As an alternative, solvent gels, rigid gels and resin soaps can be used for selective cleaning. In recent decades, biological cleaning has greatly improved as a result of research into biotechnologies and today plays an important role in the preservation and restoration of cultural assets. Nowadays, biocleaning by viable bacterial cells or hydrolytic enzymes

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represents a resource with great potential in the restoration of cultural heritage, minimising risks for artworks and for human health. New methodologies based on sulphate-reducing bacteria or bioactive molecules with hydrolytic activity have been applied as selective and safer cleaning methods in the removal of black crusts from stone surfaces or organic materials such as glue and/or adhesives, from paintings and other substrates.

4.1 The Action of Cleaning in Restoration Projects

In the modern concept of cultural heritage restoration, the approach of minimal intervention is one of the main cornerstones established in several codes of ethics for restorers and conservators, such as that in the professional guidelines by the European Confederation of Conservator Restorers' Organisations (E.C.C.O. 2003). In summary, this approach consists in limiting interventions at minimum level, avoiding unnecessary ones and focusing on the control and/or mitigation of the causes of the decay, following the principles of 'preventive conservation' (Tabasso 2004).

From this perspective, cleaning treatments should focus essentially on the *controlled* dislodging from the surface of only those materials and substances that may interact negatively with the integrity of the underlying layers (Cremonesi 2003), re-establishing a *reasonable* aesthetic condition of the artwork and achieving a state of *legibility* (Bonsanti 2003). This means, for example, avoiding frequent and invasive artwork *maquillages* for media art exhibitions. In order not to consider cultural heritage merely as 'consumer goods', Cremonesi declares that a 'Charter of Rights' for works of art should be drawn up, so that their constituent materials are respected and they are enjoyed to the full in a sustainable way: care for the integrity of the layers through preliminary diagnosis and highly selective methods for the removal of undesired substances should therefore become a must for any advanced restorer (Cremonesi 2003).

The selectiveness of specific cleaning methods means that direct consequences are low impact for the artwork, environment and restorers involved. This is evident when comparing conventional cleaning methods (e.g. mechanical methods) with innovative protocols. For example, aqueous methods, able to couple the mild chemical action of water with other components such as chelating agents, surfactants, enzymes or living bacterial cells, are particularly selective and performing. In fact, thanks to the synergic effect of proper buffers and highly selective components, it is possible to act only against target compounds avoiding any interaction with others that we wish to preserve. As can be seen later, research on innovative cleaning methods is now moving towards biotechnology by searching for new chemicals, exploiting the specific metabolic capabilities of viable microorganisms and enhancing the proprieties of enzymes, for the bioremediation of *polluted* artworks.

4.2 Removing Undesired Layers from Artefact Surfaces

According to the illustrated glossary on stone deterioration patterns by ICOMOS[1] (Vergès-Belmin 2008), a stone alteration is a modification of the material that does not necessarily imply a worsening of its characteristics from the point of view of conservation. Thus, we may distinguish harmful from harmless alterations: the former should be eliminated or reduced because they threaten the conservation of the artwork; the latter need to be preserved because eliminating them would be pointless or even dangerous for the sound surface.

Among the harmful alterations, an additional distinction can be made between those that are endogenous or exogenous. Endogenous alterations derive from a synergic interaction between the artwork constituents and the surrounding environment (made up of pollutants and weathering), while exogenous ones derive from the accumulation of deposits or applied films on the surface, which may or may not interact with artwork constituents. Examples of harmful endogenous alterations are black and salt crusts, typical of stone or mural paintings, originated by an alteration of the constitutive minerals in sulphate (i.e. gypsum) or nitrate. They are considered undesired layers because of their disruptive action on the sound surface, which leads to loss of legibility and the gradual dissolution of the artwork. Several cleaning methods have been adopted to mitigate their presence, but many of them fail, causing loss of the constitutive materials due to the fact that altered layers are usually strongly bonded to the substrate.

On the other hand, altered layer called *patina nobile*, resulting from a natural ageing of artefact surface, contributes to the aesthetic significance of the artwork by inducing empathy in the spectator (Weil 1985) and is usually preserved. Furthermore, patina may represent a protection for the underlying layers from weathering and aggressive pollutants.

Biological deposits, residues from inaccurate past restoration and graffiti are usually harmful or undesired exogenous alterations, which have to be removed by cleaning, with some exceptions, for example, some past restoration or finishing treatments modifying positively by ageing, forming a protective and uniform layer on the surface, such as for oxalate films (Alessandrini 2005). Epilitic lichens showing low invasiveness on the substrate sometimes have aesthetical and historical value. Their removal, furthermore, could subsequently lead to serious damage exposing the bare surface to more aggressive biodeterioration agents such as black fungi or cyanobacteria (Pinna 2004).

This schematic discussion about alterations may conceal the complexity that each restorer faces in the cleaning process. Determining the nature and potential hazard of any single layer requires an accurate technical diagnosis and a careful historical and artistic interpretation of the artwork. A respectful and 'minimal' cleaning operation foresees a proper conservation project design (Mecchi and Sansonetti 2004) leading to modular and target-specific intervention on the artwork surface.

[1] International Council on Monuments and Sites.

4.2.1 Viable Bacterial Cells

It is only in the last few decades that microbiology applied to cultural heritage has been considered in the perspective of microbial contamination and the consequent degradation of artwork surfaces. The binomial correlation microorganism deterioration, deeply rooted in medical microbiology, dominated the scientific literature of the sector during the last century, promoting the development of specific tools for the precise identification of biodegradation agents and the study of their effects. However, microorganisms are not only decaying promoters. More recently, another field of study has focused its attention on 'good microbes' that are able to lead virtuous processes, useful in restoring practices. This field of study has been developed starting from the concept that specific microorganisms, by means of their metabolic properties, are essential to all biogeochemical cycling processes and their activity plays an important part in all facets of environmental and human life (Mapelli et al. 2012).

Moreover, as microbiology has always been a practical discipline, in the era of biotechnologies this assumes new importance due to the possibility of using new tools to manage microbes and their potentialities. The exploitation of the metabolic capabilities associated with microbes has been recently defined as microbial resource management (MRM) (Verstraete et al. 2007). According to this concept, microbial communities can be managed directly (e.g. by introducing into the environment-specific microorganisms with desired capabilities) or indirectly (for instance, by acting on environmental parameters for the growth of the desired microbial species) in order to induce a positive effect in their surroundings, such as, for example, in polluted soil and water, or on the surface of artistic objects. In fact, as microorganisms are able to degrade environmental contaminants from different sites, they can, in the same way, remove undesired substances from objects that we want to preserve.

Notably, artworks and monuments, especially outdoors, are subjected to the action of several physical and chemical factors that give rise to the accumulation, on their surface, of several undesired and harmful layers such as black crusts, caused by sulphation and/or nitration phenomena, deposition of organic substances and accumulation of acidic varnish or aged adhesive, caused by inappropriate restoration practices or negligence.

From the end of the 1980s, selected bacteria have been successfully employed as cleaning agents, leading to the development of a new and *green* method of restoration known as *biocleaning* (or *biorestoration* in a wider sense) (Ranalli et al. 2000). In principle, the method exploits the capability of specific bacteria to use undesired substances, such as oxidised compounds of sulphur or nitrogen as electron acceptors or, in the case of organic matter, such as a carbon source, inducing their gradual degradation. Similarly to enzymatic cleaning (see Sect. 4.2.2), this procedure may be included among the aqueous methods, sharing with them a high level of selectivity and low impact for artworks (Ranalli and Sorlini 2003).

A careful selection of the appropriate microorganisms, with good performance in the removal of the undesired substances (e.g. nitrates, sulphates or organic matters),

is one of the first steps in planning biorestoration strategies. Thus, the best approach in finding an effective biocleaning agent is to perform a proper chemical-physical characterisation of the decay and to isolate microorganisms from the most similar chemical-physical environment (Ranalli et al. 2000; Troiano et al. 2014). Of course, selected strains have to be non-pathogenic for humans or harmless for the environment and possibly non-spore forming to facilitate their dislodging after treatment. Moreover, in order to optimise their activity on the surface that we want to clean, bacteria need to be applied by using a matrix able to (*1*) provide them with the right environmental conditions, (*2*) keep them in contact with the alteration without interacting with the surface, and (*3*) be quick and easy to prepare, to apply and remove (Bosch-Roig and Ranalli 2014).

Over the years, several research groups have dealt with the different aspects of biocleaning, improving the method and in some cases leading to large-scale applications and industrial development of commercial products. In the present chapter, we will discuss several case studies set up for the removal of sulphates, nitrates and organic matter alterations from monuments and mural paintings.

4.2.1.1 Removal of Sulphate

Marble and stone sulphation occurs in the presence of moisture when sulphur dioxide, a major urban atmosphere pollutant, is converted to sulphuric acid, which reacts with marble and other soluble calcareous substrates to form gypsum (Böke et al. 1999). During gypsum crystallisation, airborne organic pollutants and carbonaceous particles accumulate on surfaces protected from rainfall and wash-out and are subsequently trapped in the newly formed mineral matrix to form a so-called *black crust* (Moropoulou et al. 1998; El-Metwally and Ramadan 2005). The cleaning of crusts is essential, not only for the conservation of deteriorated areas but also for preventing further erosion phenomena (Kapsalas et al. 2007).

In the traditional conservation approach, the main methods for the removal of black crust are mechanical and chemical and, more recently, laser cleaning treatments. Mechanical methods are largely used even if they can cause erosion of the sound stone; chemical treatments generally produce good results in a reasonable time but, because of their wide range of action, are not selective and can be dangerous for human health and the environment (Lazzarini and Laurenzi Tabasso 1986). The employment of laser is a more recent method that is spreading because of its high selectivity and faster application time, though there is still uncertainty concerning its real interaction with the different substances in the crust (Salimbeni et al. 2001).

An alternative cleaning technology employs sulphate-reducing bacteria. Sulphate-reducing bacteria (SRB) are able, in anaerobic environments, to dissociate gypsum into calcium and sulphates, the latter being reduced by the bacteria and the former, reacting with carbon dioxide, converted to new calcite (Gauri and Bandyopadhyay 1999). By reducing sulphates to hydrogen sulphide, these bacteria are able to obtain energy. Thanks to this anaerobic respiration, SRB are able to break the molecular structure of gypsum, which can be easily removed from the stone surface by using water and a soft brush, cotton wool or a sponge.

The first successful application of the anaerobic sulphate reducer *Desulfovibrio desulfuricans* was reported by Atlas and colleagues (1988). They obtained a partial removal of the crust (assessed only visually) by completely immersing different samples of marble with black weathering crusts rich in gypsum, in a broth containing the SRB. Calcite was also found on all the treated surfaces, suggesting that this microbe has both the potential to clean crusted marble monuments and to regenerate calcite. In 1992, using the same system, Guari and colleagues were able to remove the black crust after 84 h, from an entire old gypsum-encrusted marble statue previously consolidated (Gauri et al. 1992).

Even if the immersion system had obvious drawbacks (that we will discuss later), employing SRB as a cleaning agent was promising, and further research was carried out in order to optimise the method.

The first to employ SRB outside an underwater system were Ranalli and colleagues (1997). They tested different strains of *Desulfovibrio* in pure and mixed culture on marble specimens using sepiolite (a clay mineral) as a delivery system, in an anaerobic condition.

Later, in 2006, Cappitelli and colleagues improved the methodology by using *Desulfovibrio vulgaris* subsp. *vulgaris* ATCC 29579 and Carbogel (CTS, Vicenza, Italy) as cell carrier (Cappitelli et al. 2006). This strain is able to reduce sulphate even under low oxygen tension, making surface application easier. On the other hand, Carbogel guaranteed a faster and higher bacterial colonisation of the delivery system, higher water retention and better contact between the cells and surface reducing some other drawbacks encountered using sepiolite, as we will see later when discussing delivery systems. The obtained bacterial matrix was applied on a marble specimen fragment from Milan Cathedral, altered with a 2–3 mm thick black crust. The matrix was not applied directly on the crust. A Japanese paper layer soaked with phosphate buffer was placed between the treated surface and the matrix, in order to facilitate the removal of the delivery system at the end of the treatment. Finally, a plastic film was applied on top of the matrix to reduce oxygen diffusion and increase water retention. The treatment ceased only when removal of the black crust was visually satisfactory: three applications, each of 15 h, were needed. Two parameters were monitored to evaluate biocleaning effectiveness: ion-exchange chromatography and colour measurements. Analysis showed a 98 % removal of sulphates in the crust.

The improved biological method was further tested in two subsequent studies that demonstrated the superiority of biocleaning against chemical and physical methods. In the first one (Cappitelli et al. 2007), the SRB-Carbogel system was compared to an ammonium carbonate-EDTA mixture to remove a black crust from a *lunetta* of Candoglia marble from the Milan Cathedral. Results obtained by optical microscopy, SEM-EDS and FTIR analysis showed that the biological procedure produced a more homogenous removal of the surface deposits and a good preservation of the noble patina under the crust. In the second one (Gioventù et al. 2011), the prototype *D. vulgaris* ATCC 29579 in Carbogel was tested against chemical (ammonium carbonate-EDTA + Tween20) and laser (1064 nm, Nd:YAG laser) methods on three different lithotypes on the external walls of the Florence Cathedral: green

serpentine, red marlstone and Carrara white marble. Using the above-mentioned analysis and colour measurements, it was found that the chemical method led to nonhomogenous crust removal and occasional detachment of fragments and the laser technique left a thin yellow layer visible to the naked eye, and on Carrara marble in particular, it left a residual layer of gypsum. On the contrary, biological cleaning was satisfactory and showed none of the above-mentioned drawbacks.

In 2010, *D. vulgaris* was applied for the first time on two limestone sculptures situated in the courtyard of Buonconsiglio Castle in Trento (Polo et al. 2010), obtaining successful removal of the black crust after three applications. Using the same system, biocleaning has been carried out in situ for the removal of black crust from the Pietà Rondanini by Michelangelo (Cappitelli et al. 2005) (located in the Sforzesco Castle in Milan), the sculpture 'Allegoria della Morte' by Lazzerini in Florence (Gioventù and Lorenzi 2013) and some areas of the façade of S. Maria delle Grazie, in Milan.

Despite the good results, there were still some drawbacks. Thickness and chemical heterogeneity of the crust occasionally determined long treatment times and uncompleted layer removal by the action of a single selective biocleaning agent. Indeed, black crust alterations are often not only composed of sulphate deposits but also by a complex mixture of nitrate and other various compounds such as carbonate salts, apatite and proteins (Mazzoni et al. 2014). It is worth noting that in case of powdery, an incoherent stone, prolonged contact of surfaces with water, even in a gelled state, can further exacerbate the degradation (Normandin and Slaton 2005). Several strategies, depending on the nature of the crust, have been proposed to overcome these limitations. Gioventù and Lorenzi (2010) obtained faster removal by a preliminary mechanical lowering of the crust before SRB-Carbogel applications. Troiano and colleagues (2013) suggested for the first time an integrated approach of chemical and biological methods, coupling on a stone column affected by black crusts the effects of SRB-Carbogel with a nonionic detergent pretreatment. The coupling of the two treatments removed the black crust without affecting the original sound marble, with 38 % reduction in cleaning time. The combined method was later applied to a century-old marble statue weathered by sulphate-based crusts and grey deposits. The detergent used alone effectively removed the grey deposit but not the black crust. However, the co-treatment synergy resulted in the complete removal of the black crust layers, with the added advantages, compared to biocleaning alone, of fewer biological applications (from seven to two) and a 70 % reduction in total cleaning time.

To remove black crust made of a mixture of nitrates and sulphates, Alfano and colleagues (2011) proposed a multilayer biosystem consisting of a Carbogel matrix enriched with *Pseudomonas pseudoalcaligenes* KF707, a nitrate-reducing strain, and *D. vulgaris* ATCC 29579. The effectiveness of this advanced system was confirmed by long-term data monitoring, after 6 years from the start of treatment.

The same multilayer strategy was attempted by Mazzoni and colleagues (2014) for the first time on wall paintings (in Casina Farnese, Rome) affected by a hard-to-remove inhomogeneous deposit layer. To solubilise the crust made of calcium sulphate, calcium oxalate, apatite and aged casein, researchers employed an innovative

modified laponite matrix (a colloidal clay consisting of a mixture of silicates of sodium, magnesium and lithium) containing three non-spore-forming bacterial strains: *Cellulosimicrobium cellulans* (able to solubilise calcium sulphate and carbonate), *Stenotrophomonas maltophilia* (a protein degrader) and *Pseudomonas koreensis* (able to solubilise inorganic compounds and to degrade protein material). According to the authors, laponite micro-packs containing the biocleaning agents showed high effectiveness in reducing and softening the complex deposit layer, without operating limitations. It was effective in aerobic conditions and in a wide range of temperatures (from 6 to 37 °C), safe for the restorer and with no damage to the pictorial layer or the underlying noble patina. Furthermore, micro-packs showed easy application and removal on vertical surfaces and ceilings.

In view of the interesting potential impact on the market, the employment of microorganisms in different carriers has been recently patented [MI2006A000776; RM2013A000519]. In particular, the use of *D. vulgaris* in a modified polyacrylic acid as cell carrier, thanks to a technology transfer process, is now a commercial product, available to restorers (Micro4Art sulfate produced by Micro4yoU S.r.l. and distributed by Bresciani S.r.l.).

4.2.1.2 Removal of Nitrate

As for sulphates, nitrate formation affects the surface of outdoor stones. Nitrogen dioxide is a by-product coming from industrial combustion installations and vehicles. In the atmosphere, it is first oxidised to N_2O_5 and then to nitric acid. When nitric acid interacts with the calcium carbonate of the stone, it produces calcium nitrate which is more soluble than the original mineral phases and causes the pulverisation of sound stones or the generation of microcracks in wall paintings (Warscheid and Braams 2000).

Nitrate salts may also arise from industrial agriculture or from the soil, climbing up the external wall of buildings, especially when bodies are buried in close proximity, as, for example, in the case of old cemeteries constructed around churches or cathedrals (Alfano et al. 2011).

Nitrate efflorescence is also frequent on indoor wall paintings as a result of biological processes, residual restoration products left on the surface because of inaccurate restoration or natural ageing processes of the painting constituents (Doehne and Price 2010).

However, as for sulphates, nitrates can be reduced by specific bacteria (nitrate-reducing bacteria, NRB). Ranalli and colleagues (1996) were the first to employ nitrate-reducing bacteria (NRB) in lab trials. Real samples of Vicenza stone altered by nitrates and artificially aged samples were treated with a strain of *Pseudomonas stutzeri* delivered in sepiolite. The application lasted 30 h, with removal of 88 % of nitrates.

During the BIOBRUSH (Bioremediation for Building Restoration of the Urban Stone Heritage in European States) European research programme (May et al. 2008), Matera Cathedral was a model for in situ NRB applications. Indeed, abundant soluble nitrates, causing darkening and pulverisation of the stones, affect the lower 2 m of the external walls of the church. The salts originate from the oxidation

of various N-organic compounds from bodies, which were buried in the ground when the area was used as a cemetery (Alfano et al. 2011). During the first trials, Carbogel or a mortar-alginate matrix was used as a delivery system for a NRB strain of *Pseudomonas pseudoalcaligenes*. The applications showed good nitrate removal; nevertheless, a significant contribution of the carrier especially Carbogel (up to 20% of nitrate removal) was also observed. On the other hand, mortar-alginate sustained a longer NRB activity in the interface between the surface area and the matrix.

In 2013, the system based on the use of *P. stutzeri* was applied for the first time on a wall painting altered by nitrate salt efflorescence (Bosch-Roig et al. 2013a). The research was carried out for the cleaning of wall paintings in Santos Juanes church in Valencia (Spain). In this case, among tested supports, agar was chosen as the most efficient in removing salts on vertical surfaces and as the most proper and safe because it reduces the amount of water released by the application system on the painted surface. Using this technique, a reduction of 92% in nitrate efflorescence was proved by ion-exchange chromatography.

The biological removal of nitrates has not received as much attention from scientists as sulphates, possibly because the former are more soluble than sulphates and do not produce a marked and anti-aesthetic crust as the latter (Webster and May 2006). Nevertheless, as we have seen in the above-mentioned works by Alfano et al. (2011) and Mazzoni et al. (2014), their use in combination with other biocleaning agents appears promising especially for the removal of inhomogeneous and hard-to-remove black crust made of a mixture of sulphates, nitrate and other substances.

4.2.1.3 Removal of Organic Matter

In addition to air pollutants and biofilms, the surfaces of man-made artistic stonework can also be altered by organic matter that has been applied, but then not completely removed, during restoration. Residuals often act as a good growth substrate for microorganisms and mycetes that destroy the surface and allow hyphae penetration (Ranalli et al. 2005).

Fourteenth-century Pisa frescoes in the Monumental Cemetery (Camposanto), painted by famous artists such as Buonamico Buffalmacco and Spinello Aretino, represent the most important example of biocleaning for the removal of aged organic matter from painted surfaces. During an air raid by the allies in 1944, some bombs fell on the cemetery damaging the frescoes that were removed from the walls using the 'tear-off' technique, in order to be restored at a later data. The 'tear-off' technique consists in the application of a gauze on the fresco surface, by using animal glue as a consolidating agent. After glue adhesion, the cloth and the fresco form one single layer, which is then detached from the wall. One of these frescoes, 'Conversion of S. Efisio and Battle', that was restored and replaced in the original site was again removed in the 1980s, using the 'tear-off' technique, since discoloured and damaged (air humidity and pollution). Twenty years later, curators attempting a second restoration found great difficulty in removing the gauze from the previous restoration, which still adhered to the paint layer on the front surface, even when treated with a mixture of the most aggressive proteolytic enzymes available on the market

(Ranalli et al. 2005). The failure was ascribed to the presence of formalin responsible for the formation of insoluble compounds during the long storage (Antonioli et al. 2005). Based on previous lab trials (Ranalli et al. 2003), a suspension of *Pseudomonas stutzeri* (DSMZ 5190) was applied to the fresco embedded in hydrophilic cotton strips, which were laid over it. After 8–12 h, the bacteria were able to digest the glue, allowing the removal of the gauze (Ranalli et al. 2005). By proteomic analysis, Antonioli and colleagues (2005) in a lab trial showed that in the presence of glue or aged glue, *P. stutzeri* DSMZ 5190 produces caseinolytic and collagenase activity, two fundamental enzymes needed for the digestion of the main animal glue constituents. This experiment shows once again the wide versatility of bacterial metabolism. In fact, bacteria are known to produce not only constitutive but also inducible enzymes whose synthesis takes place only in the presence of a specific substrate. These enzymes can attack and degrade different types of molecules only when the bacterial cells are exposed to them, creating a regulatory effect.

However, in this case, after gauze removal from the fresco surface, to avoid direct and longer interaction between the bacterial suspension and the painted layer, a mixture of proteolytic enzymes was employed to remove the glue residues completely (Ranalli et al. 2005).

In 2013, Bosh-Roig and colleagues improved the above-mentioned method. Using agar instead of cotton as a carrier for *P. stutzeri* (DSMZ 5190), authors were able to reduce to 2 h the duration of the biocleaning on an eighteenth-century fresco, sited at the central vault of the Santos Juanes Church, in Valencia, Spain. Compared with the cotton carrier, agar reduced the amount of the required bacteria by ten times and supported their activity better. Apart the obvious economic advantage, the shorter interaction between bacteria and the painted surface has prevented the integrated use of expensive proteolytic enzymes (Bosch-Roig et al. 2013b).

In 2012, glue removal with *P. stutzeri* was attempted on another fourteenth-century fresco (*Stories of the Holy Fathers* by Buonamico Buffalmacco) belonging to the complex of the Monumental Cemetery in Pisa (Lustrato et al. 2012). This time the aim was to remove proteinaceous material residues from past incorrect restorations. Over the years, the proteinaceous materials had caused serious alterations and, as in the previous case, had become very hard and resistant to commonly used solvents. For the first time, a fully computerised laboratory batch fermenter (20 l useful volume) was used to obtain a suitable amount of viable bacterial cell biomass for a full-scale biocleaning (about 95 m^2 of surface area). Before application, the detached fresco was laid flat, in a horizontal position and completely covered with silk tissue paper. The bacterial culture was manually applied to the paper by using a large roller, using gentle brush strokes all over the surface. Sterile cotton swabs soaked with an abundant, activated, bacterial biomass were finally applied over the fresco, adhering to the tissue paper. After preliminary testing, ex situ biorestoration treatment was performed by a single-step application lasting only 2 h. In this case, due to the short duration of the treatment, cotton was chosen as the best carrier for the bacterial suspension. After the biocleaning, the fresco was subjected to short- and medium-term monitoring to assess microbial colonisation, activity and the presence of any undesired viable *P. stutzeri* cells. The absence of any viable cells

in the fresco after bio-treatment, and thus of any potential negative effects due to their metabolism, was confirmed.

Another application in the biocleaning field may be seen in the removal of different hard-to-remove organic substances, such as organic synthetic polymers used in restoration – such as adhesives or protective coatings – or original constituents of contemporary artworks, which cause deterioration by ageing processes.

A valid strategy for the identification of microorganisms able to remove these materials could be to isolate them directly from varnish, paint or polymer manufacturing, industrial wastewater and sludge (Chen et al. 2007; Arutchelvan et al. 2005; Saleem et al. 2008). In 2014, Troiano and colleagues tested for the first time the ability of five bacteria to attack a 4-year-old Paraloid B-72. Bacteria were isolated from biodeteriorated acrylic paintings or wastewater treatment plants. Despite the presence of cell clusters over the surface of the Paraloid samples inoculated with one of the strains, observations from the solubility tests, scanning calorimetry and FTIR analysis and the stereomicroscope and SEM showed that the selected bacteria were not able to attack the polymer (Troiano et al. 2013). Nevertheless, this methodology could be considered a reference for future research on the bio-removal of synthetic resin.

4.2.1.4 Bacterial Cell Applications

In order to implement marketable applications of biocleaning products, two main goals have to be pursued: the first is to search for mixed populations of strains with different metabolic profiles for heterogeneous degraded layers, and the second is to make a ready-to-use product with a cheap delivery system.

As we have seen previously, researchers over time have employed different materials and methods to deliver biocleaning agents on artwork surfaces. In general, according to a recent review by Bosch-Roig et al. (2014), a carrier should have the following characteristics:

1. Be able to retain the microorganisms and provide them with the right conditions (aerobic or anaerobic) and the water they need in order to remove the cause of decay but without any damage to the art work itself and any undesirable changes in the colour of the surface
2. Be applicable to all types of surfaces (horizontal, vertical, oblique, rough, smooth, etc.)
3. Be quick and easy to prepare, but also easy to apply and to eliminate at the end of the treatment, and using as far as possible only a few inexpensive materials

Apart from the pioneering experimental applications by immersion, the principal carriers commonly employed have been sepiolite, Carbogel, cotton wool, agar and laponite. It's hard to indicate the best material to employ because it mostly depends on the single circumstances encountered by the restorer, namely, nature and position of the layer to remove, state of conservation of the artwork and, of course, the physiological requirements of the biocleaner. Thus, strongly degraded and sensitive surfaces (such as wall paintings) need short applications and low water release by the

carrier. In this case, delivering bacteria together with agar on Japanese paper may be the best solution. On the other hand, when a stronger and homogenous application of the treatment or a friendly environment for anaerobic bacteria is needed, it may be better to use Carbogel. Low-cost applications on pre-consolidated surfaces may be obtained using cotton wool, with low risk to the artworks and easy-to-use applications. Finally, on vertical surfaces, good results have been achieved employing micro-packs of laponite.

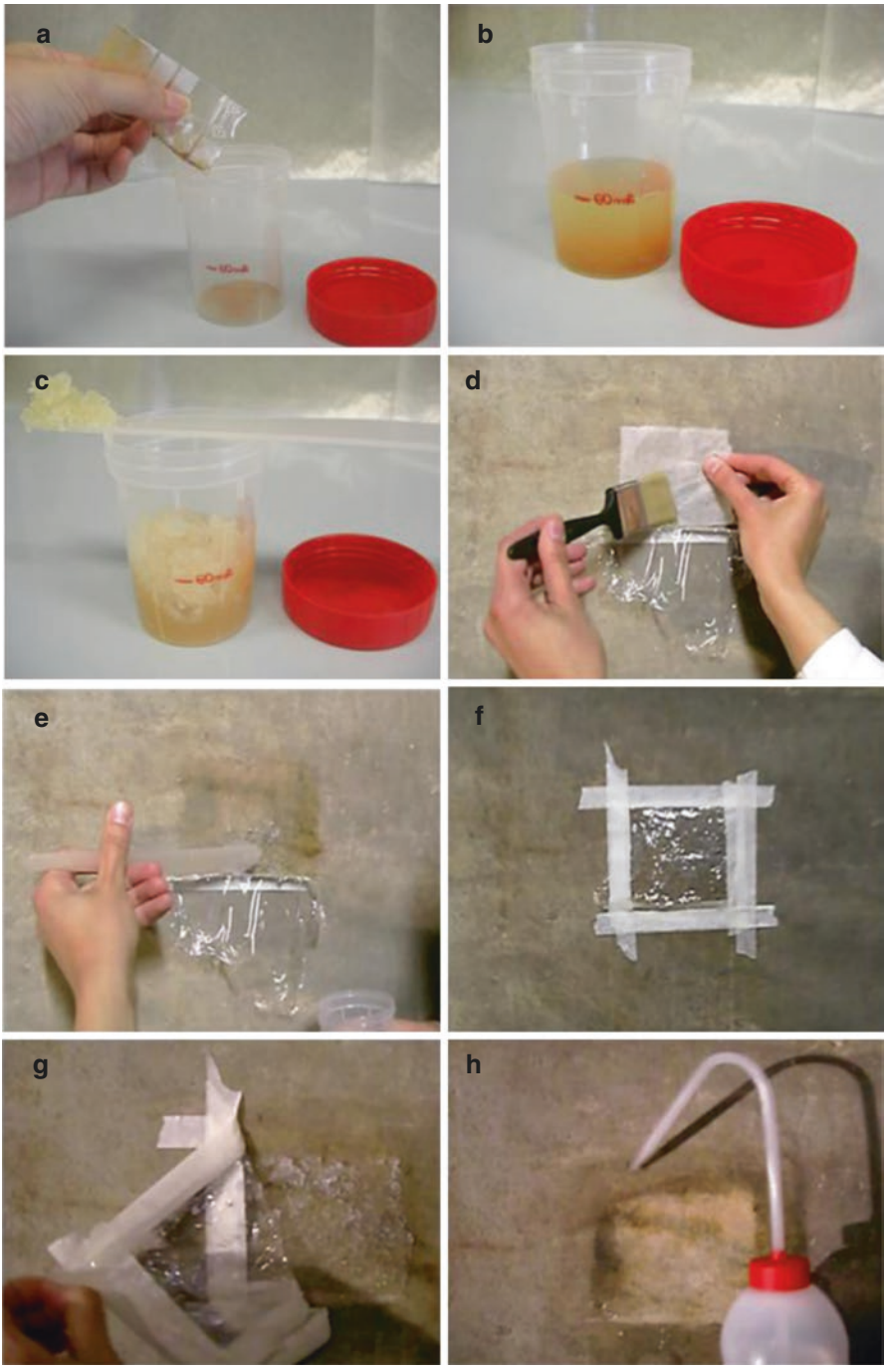
A biotechnological product for non-specialised end users should be intuitive and ready to use. Restorers should be able to prepare it quickly even in difficult environments, such as those usually found in restoration sites. Figure 4.1 reported the simple steps to prepare *Micro4Art sulfate*, a biocleaning market product based on sulphate-reducing bacteria in Carboneutralgel.

4.2.2 Enzymatic Cleaning

Hydrolytic enzymes represent a very helpful tool in the biocleaning of a variety of artworks, particularly to remove dirt, adhesives and other organic residues from paintings, mural paintings and paper, wooden and stone artworks (Ranalli et al. 2005; Schwarz et al. 1999; Hamed 2012; Valentini et al. 2012; Barbabietola et al. 2016). Commercial hydrolases, such as proteases, amylases, lipases and esterases, are isolated from animal (pancreas, stomach), vegetal (seeds of oats and wheat) and microbial (bacteria or fungi) sources and utilised in biocleaning treatments in order to remove specific substrates (Palla et al. 2013; Ranalli et al. 2005). Several enzymatic treatments in cleaning have been performed on different kinds of objects with good results, representing a sustainable methodology and a safer approach to the artworks as well as a valid alternative to conventional acids and alkaline products (Bosch-Roig and Ranalli 2014; Cremonesi 2013).

Since the 1970s, enzymatic cleaning has found application in conservation treatments to remove starch paste, animal glues or protein adhesives by amylase or protease (Wendelbo and Fosse 1970; Segal and Cooper 1977), aged acrylic coatings on painting by lipase (Bellucci et al. 1999), animal glue and protein/oily binder from paintings by mixed enzymatic solutions (Makes 1982). Enzymes, such as trypsin, amylase and protease, were mainly used for the treatment of glue

Fig. 4.1 Steps to prepare Micro4Art solfate, a biocleaning market product based on sulphate-reducing bacteria in Carboneutralgel: (**a**) transfer the freeze-dried sulphate content of one sachet into the supplied jar; (**b**) add deionised water (preferably pre-reduced water) to the freeze-dried bacteria until the indicated volume; (**c**) add the appropriate cell carrier to obtain the desired density; (**d**) apply a layer of Japanese paper on the surface to be treated using a moist brush (preferably moistened with deionised water); (**e**) apply the bacterial suspension on the Japanese paper; (**f**) cover the area with a plastic film; (**g**) remove the wrap (plastic film, bacterial formulate and Japanese paper); (**h**) rinse the stone delicately with a damp sponge or a brush, and remove eventual residuals with a cotton bud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stains on paper (Wendelbo 1976; Segal and Cooper 1977). During the 1990s, many restorers promoted other cleaning practices by evaluating the toxicity of the chemical products used since then (Signorini 2013). The technological biocleaning approach is also based on the combined use of viable microbial cells and hydrolytic enzymes in order to obtain the total removal of undesirable layers on artwork surfaces (Ranalli et al. 2005; Bosch-Roig et al. 2013b). Preliminary to enzymatic cleaning, characterisation of the undesired layer must be determined, for example, using chromatography techniques (Barresi et al. 2015; Cremonesi 2013; Bosch-Roig et al. 2013a, b).

Moreover, enzymatic cleaning is preferable in many cases in relationship to safety and selectively to remove organic compounds under specific pH and temperature conditions. Because commercial enzymes require a temperature ≥37 °C, their application can represent a limitation. Another feature to be taken into account is that the inhibitors present on the artwork, such as salts, metal ions, pigments or other molecules, may induce changes by binding to the proteinaceous structure of the enzyme (Bellucci and Cremonesi 1994).

Recently, novel purified enzymes, in particular proteases and esterases from marine invertebrates, have been isolated and assayed in bio-removal tests performed on artworks and laboratory specimens. The peculiarity of these cold-active enzymes is that they can be utilised at temperatures <30 °C, without heating enzymatic solutions or artwork surfaces, in order to successfully hydrolyse aged proteinaceous (animal glue, casein binder) or Paraloid B-72 layers (Salamone et al. 2012; Palla 2013; Palla et al. 2013, 2016; Barresi et al. 2015).

After biocleaning, the effective removal of undesirable layers and the presence of residues must be checked in order to evaluate potential chromatic changes in the surface (Pruteanu et al. 2014; Micheli et al. 2016; Hrdlickova Kuckova et al. 2014; Bosch-Roig et al. 2013a, b; Palla et al. 2016).

References

Alessandrini G (2005) Patine su materiali lapidei. In: Le patine: genesi, significato, conservazione. In: Workshop promosso da Mauro Matteini. Acts by Piero Tiano e Carla Pardini. Kermes Quaderni, Nardini Editore, Firenze, pp 15–28

Alfano G, Lustrato G, Belli C, Zanardini E, Cappitelli F, Mello E, Sorlini C, Ranalli G (2011) The bioremoval of nitrate and sulfate alterations on artistic stonework: the case-study of Matera Cathedral after six years from the treatment. Int Biodeter Biodegr 65(7):1004–1011. doi:10.1016/j.ibiod.2011.07.010

Antonioli P, Zapparoli G, Abbruscato P, Sorlini C, Ranalli G, Righetti PG (2005) Art-loving bugs: the resurrection of Spinello Aretino from Pisa's cemetery. Proteomics 5(9):2453–2459. doi:10.1002/pmic.200401182

Arutchelvan V, Kanagasabai V, Nagarajan S, Muralikrishnan V (2005) Isolation and identification of novel high strength phenol degrading bacterial strains from Phenol-Formaldehyde resin manufacturing industrial wastewater. J Hazard Mater 128(1–3):236–242. doi:10.1016/j.jhazmat.2005.04.043

Atlas RM, Chowdhury AN, Gauri KL (1988) Microbial calcification of gypsum-rock and sulfated marble. Stud Conserv 33(3):149–153. doi:10.2307/1506207

Barbabietola N, Tasso F, Alisi C, Marconi P, Perito B, Pasquariello G, Sprocati AR (2016) A safe microbe-based procedure for a gentle removal of aged animal glues from ancient paper. Int Biodeter Biodegr 109:53–60. doi:10.1016/j.ibiod.2015.12.019

Barresi G, Di Carlo E, Trapani MR, Parisi M, Chillè C, Mulè M, Cammarata M, Palla F (2015) Marine organisms as source of bioactive molecules applied in restoration projects. Herit Sci 3(3):17–20. doi:10.1186/s40494-015-0046-1

Bellucci R, Cremonesi P (1994) L'uso degli enzimi nella conservazione e nel restauro dei dipinti. The use of enzymes in the conservation and restoration of paintings. Kermes arte e tecnica del restauro 21:45–64

Bellucci R, Cremonesi P, Pignagnoli G (1999) A note on the use of enzymes in conservation: the removal of aged acrylic resin coatings with Lipase. Stud Conserv 44(4):278–281. doi:10.1179/sic.1999.44.4.278

Böke H, Gokturk EH, Caner-Saltik EN, Demirci S (1999) Effect of airborne particle on SO_2-calcite reaction. Appl Surf Sci 140(1–2):70–82. doi:10.1016/S0169-4332(98)00468-1

Bonsanti G (2003) Storia ed etica della pulitura. In: Atti del Convegno Metodi Tradizionali ed Innovativi nella Pulitura dei Dipinti e delle Opere Policrome Mobili di Piazzola sul Brenta, 25–26 Ottobre 2002. Il Prato, Padova, pp 14–15

Bosch-Roig P, Ranalli G (2014) The safety of biocleaning technologies for cultural heritage. Front Microbiol 5:155. doi:10.3389/fmicb.2014.00155

Bosch-Roig P, Regidor-Ros JL, Montes-Estellés R (2013a) Biocleaning of nitrate alterations on wall paintings by *Pseudomonas stutzeri*. Int Biodeter Biodegr 84:266–274. doi:10.1016/j.ibiod.2012.09.009

Bosch-Roig P, Regidor-Ros JL, Soriano-Sancho P, Montes-Estellés RM (2013b) Biocleaning of animal glue on wall paintings by *Pseudomonas stutzeri*. Chem Today 31(1):50–53

Bosch-Roig P, Lustrato G, Zanardini E, Ranalli G (2014) Biocleaning of cultural heritage stone surfaces and frescoes: which delivery system can be the most appropriate? Ann Microbiol 65(3):1227–1241. doi:10.1007/s13213-014-0938-4

Cappitelli F, Zanardini E, Toniolo L, Abbruscato P, Ranalli G, Sorlini C (2005) Bioconservation of the marble base of the Pietà Rondanini by Michelangelo Buonarroti. GRA 7:06675

Cappitelli F, Zanardini E, Ranalli G, Mello E, Daffonchio D, Sorlini C (2006) Improved methodology for bioremoval of black crusts on historical stone artworks by use of sulfate-reducing bacteria. Appl Environ Microbiol 72(5):3733–3737. doi:10.1128/AEM.72.5.3733-3737.2006

Cappitelli F, Toniolo L, Sansonetti A, Gulotta D, Ranalli G, Zanardini E, Sorlini C (2007) Advantages of using microbial technology over traditional chemical technology in the removal of black crusts from stone surfaces of historical monuments. Appl Environ Microbiol 73(17):5671–5675. doi:10.1128/AEM.00394-07

Chen J, Zhang Y, Du GC, Hua ZZ, Zhu Y (2007) Biodegradation of polyvinyl alcohol by a mixed microbial culture. Enzyme Microbiol Tech 40(7):1686–1691. doi:10.1016/j.enzmictec.2006.09.010

Cremonesi P (2003) Un approccio alla pulitura. In: Atti del Convegno Metodi Tradizionali ed Innovativi nella Pulitura dei Dipinti e delle Opere Policrome Mobili, Piazzola sul Brenta, 25–26 Ottobre 2002. Il Prato, Padova, pp 59–63

Cremonesi P (2013) Rigid gels and enzyme cleaning, in new insights into the cleaning paintings. In: Mecklenburg MF, Charola AE, Koestler RJ (eds) Proceedings of cleaning 2010 international conference Universidad Politécnica de Valencia and Museum Conservation Institute, vol 3, Smithsonian contributions to museum conservation. Smithsonian Institution Scholarly Press, Washington, D.C, pp 179–183

Doehne E, Price A (2010) Stone conservation: an overview of current research. Technical report. Getty Conservation Institute, Los Angeles, p 158

E.C.C.O. professional guidelines: code of ethics (2003) http://www.ecco-eu.org/about-e.c.c.o./professional-guidelines.html

El-Metwally AA, Ramadan AB (2005) The role of air pollutants and sewage waste in acceleration of degradation of the Islamic cultural heritage of Cairo. In: Comparative risk assessment and environmental decision making, vol 38, Part 3. Springer, Dordrecht, pp 363–370

Gauri KL, Bandyopadhyay JK (1999) Carbonate stone, chemical behaviour, durability, and conservation. John Wiley & Sons, New York

Gauri KL, Parks L, Jaynes J, Atlas R (1992) Removal of sulfated-crusts from marble using sulphate-reducing bacteria. In: Webster RGM (ed) Proceedings of the International conference on stone cleaning and the nature, soiling and decay mechanisms of stone. Donheadd, Edinburgh, pp 160–165

Gioventù E, Lorenzi P (2013) Bio-removal of black crust from marble surface: comparison with traditional methodologies and application on a sculpture from the Florence's English Cemetery. Procedia Chem 8:123–129. doi:10.1016/j.proche.2013.03.017

Gioventù E, Lorenzi PF, Villa F, Sorlini C, Rizzi M, Cagnini A, Griffo A, Cappitelli F (2011) Comparing the bioremoval of black crusts on colored artistic lithotypes of the Cathedral of Florence with chemical and laser treatment. Int Biodeter Biodegr 65(6):832–839. doi:10.1016/j.ibiod.2011.06.002

Gioventù E, Lorenzi PF (2010) Valutazione dell'efficacia nell'utilizzo di microrganismi per la biorimozione delle croste nere dai materiali lapidei ed approfondimento delle metodologie applicative, OPD Restauro 22:127–138

Hamed SAM (2012) A preliminary study on using enzymes in cleaning archaeological wood. J Archaeol Sci 39(7):2515–2520. doi:10.1016/j.jas.2012.02.026

Hrdlickova Kuckova S, Crhova Krizkova M, Pereira CLC, Hynek R, Lavrova O, Busani T, Branco LC, Sandu ICA (2014) Assessment of green cleaning effectiveness on polychrome surfaces by MALDI-TOF mass spectrometry and microscopic imaging. Microsc Res Tech 77(8):574–585. doi:10.1002/jemt.22376

Kapsalas P, Zervakis M, Maravelaki-Kalaitzaki P (2007) Evaluation of image segmentation approaches for non-destructive detection and quantification of corrosion damage on stonework. Corros Sci 49(12):4415–4442. doi:10.1016/j.corsci.2007.03.049

Lazzarini L, Laurenzi Tabasso M (1986) Il restauro della pietra. CEDAM, Padova, p 320

Lustrato G, Alfano G, Andreotti A, Colombini MP, Ranalli G (2012) Fast biocleaning of mediaeval frescoes using viable bacterial cells. Int Biodeter Biodegr 69:51–61. doi:10.1016/j.ibiod.2011.12.010

Makes F (1982) Enzymatic consolidation of a painting: seventeenth century landscape from Skokloster Palace. Stud Conser 27, Issue sup1:135–138. 10.1179/sic.1982.27.Supplement-1.135

Mapelli F, Marasco R, Balloi A, Rolli E, Cappitelli F, Daffonchio D, Borin S (2012) Mineral–microbe interactions: biotechnological potential of bioweathering. J Biotechnol 157(4):473–481. doi:10.1016/j.jbiotec.2011.11.013

May E, Webster AM, Inkpen R, Zamarreño D, Kuever J, Rudolph C, Warscheid T, Sorlini C, Cappitelli F, Zanardini E, Ranalli G, Krage L, Vgenopoulos A, Katsinis D, Mello E, Malagodi M (2008) In: May E, Jones M, Mitchell J (eds) Heritage microbiology and science. Microbes, monuments and maritime materials. RSC Publishing, Cambridge

Mazzoni M, Alisi C, Tasso F, Cecchini A, Marconi P, Sprocati AR (2014) Laponite micro-packs for the selective cleaning of multiple coherent deposits on wall paintings: the case study of Casina Farnese on the Palatine Hill (Rome-Italy). Int Biodeter Biodegr 94:1–11. doi:10.1016/j.ibiod.2014.06.004

Mecchi AM, Sansonetti A (2004) Le operazioni di pulitura alla luce dei dettami del minimo intervento: prassi già consolidata o obiettivo da perseguire? In: Il minimo intervento nel restauro. Arkos, Nardini Editore, Firenze, pp 81–90

Micheli L, Mazzuca C, Palleschi A, Palleschi G (2016) Development of a diagnostic and cleaning tool for paper artworks: a case of study. Microchem J 126:32–41. doi:10.1016/j.microc.2015.11.052

Moropoulou A, Bisbikou K, Torfs K, Van Grieken R, Zezza F, Macri F (1998) Origin and growth of weathering crusts on ancient marbles in industrial atmosphere. Atmos Environ 32(6):967–982. doi:10.1016/S1352-2310(97)00129-5

Normandin K, Slaton D (2005) Cleaning techniques in conservation practice. Donhead Publishing Ltd, Dorset, p 160

Palla F (2013) Bioactive molecules: innovative contributions of biotechnology to the restoration of cultural heritage. Conserv Sci Cult Herit 13:369–378

Palla F, Cammarata M, Trapani M, Ghersi G, Salamone M, Sebastianelli M (2013) Novel proteases from marine organisms with potential interest in restoration procedure. In: Rogerio-Caldera MA, Lazzari M, Cano E (eds) Science and Technology for the Conservation of Cultural Heritage, Taylor & Francis Group, London, pp 279–282. doi:10.1201/b15577-65

Palla F, Barresi G, Giordano A, Schiavone S, Trapani MR, Rotolo V, Parisi MG, Cammarata M (2016) Cold-active molecules for a sustainable preservation and restoration of historic-artistic manufacts. Int J Conserv Sci 7(Special Issue 1):239–246

Pinna D (2004) Il minimo intervento nel controllo del degrado biologico. In: Proceding of Il minimo intervento nel restauro. Nardini Editore, Siena, pp 69–74

Polo A, Cappitelli F, Brusetti L, Principi P, Villa F, Giacomucci L, Ranalli G, Sorlini C (2010) Feasibility of removing surface deposits on stone using biological and chemical remediation methods. Microb Ecol 60(1):1–14. doi:10.1007/s00248-009-9633-6

Pruteanu S, Vasilache V, Sandu ICA, Budu AM, Sandu I (2014) Assessment of cleaning effectiveness for new ecological systems on ancient tempera icon by complementary microscopy techniques. Microsc Res Tech 77(12):1060–1070. doi:10.1002/jemt.22437

Ranalli G, Sorlini C (2003) Application of microorganisms for the deteriorates surfaces recovery. Coalition 6:2–4

Ranalli G, Chiavarini M, Guidetti V, Marsala F, Matteini M, Zanardini E, Sorlini C (1996) The use of microorganisms for the removal of nitrates and organic substances on artistic stoneworks. In: Riederer J (ed) Proceedings of the 8th international congress of deterioration and conservation of stone. Möller, Berlin, pp 1415–1420

Ranalli G, Chiavarini M, Guidetti V, Marsala F, Matteini M, Zanardini E, Sorlini C (1997) The use of microorganisms for the removal of sulfates on artistic stoneworks. Int Biodeter Biodegr 40(2–4):255–261. doi:10.1016/S0964-8305(97)00054-1

Ranalli G, Matteini M, Tosini I, Zanardini E, Sorlini C (2000) Bioremediation of cultural heritage: removal of sulphates, nitrates and organic substances. In: Ciferri O, Tiano P, Mastromei G (eds) Of microbes and art. The role of microbial communities in the degradation and protection of cultural heritage. Kluwer Academic/Plenum Publishers, New York, pp 231–245

Ranalli G, Belli C, Baracchini C, Caponi G, Pacini P, Zanardini E, Sorlini C (2003) Deterioration and bioremediation of frescoes: a case-study. In: Saiz-Jimenez C (ed) Molecular biology and cultural heritage. A.A. Balkema Publishers, Lisse, pp 243–246

Ranalli G, Alfano G, Belli C, Lustrato G, Colombini MP, Bonaduce I, Zanardini E, Abbruscato P, Cappitelli F, Sorlini C (2005) Biotechnology applied to cultural heritage: biorestoration of frescoes using viable bacterial cells and enzymes. J Appl Microbiol 98(1):73–83. doi:10.1111/j.1365-2672.2004.02429.x

Salamone M, Cuttitta A, Seidita G, Mazzola S, Bertuzzi F, Ricordi C, Ghersi G (2012) Characterization of collagenolytic/proteolytic marine enzymes. Chem Eng 27:1–6. doi:10.3303/CET1227001

Saleem M, Brim H, Hussain S, Arshad M, Leigh MB (2008) Perspectives on microbial cell surface display in bioremediation. Biotechnol Adv 26(2):121–186. doi:10.1016/j.biotechadv.2007.10.002

Salimbeni R, Pini R, Siano S (2001) Achievement of optimum laser cleaning in the restoration of artworks: expected improvements by on-line optical diagnostics. Spectrochim Acta B At Spectrosc 56(6):877–885. doi:10.1016/S0584-8547(01)00197-5

Schwarz I, Blüher A, Banik G, Thobois E, Maurer KH (1999) The development of a ready-for-use poultice for local removal of starch paste by enzymatic action. Restaurator 20(3–4):225–244. doi:10.1515/rest.1999.20.3-4.225

Segal J, Cooper D (1977) The use of enzymes to release adhesives. Paper Conservator 2(1):47–50. doi:10.1080/03094227.1977.9638498

Signorini E (2013) Surface Cleaning of Paintings and Polychrome Objects in Italy: The Last 15 Years, in New Insights into the Cleaning Paintings. In: Mecklenburg MF, Charola AE, Koestler RJ (eds) Proceedings of cleaning 2010 international conference Universidad Politécnica de

Valencia and Museum Conservation Institute, vol 3, Smithsonian contributions to museum conservation. Smithsonian Institution Scholarly Press, Washington, D.C, pp 17–23

Tabasso ML (2004) Il criterio del minimo intervento: possibili interpretazioni e applicazioni alla conservazione dei materiali lapidei. Il minimo intervento nel restauro, atti del convegno, 18–19 giugno, Siena. Nardini, Firenze, pp 9–16

Troiano F, Gulotta D, Balloi A, Polo A, Toniolo L, Lombardi E, Daffonchio D, Sorlini C, Cappitelli F (2013) Successful combination of chemical and biological treatments for the cleaning of stone artworks. Int Biodeter Biodegr 85:294–304. doi:10.1016/j.ibiod.2013.08.011

Troiano F, Vicini S, Gioventù E, Lorenzi PF, Improta CM, Cappitelli F (2014) A methodology to select bacteria able to remove synthetic polymers. Polym Degrad Stab 107:321–327. doi:10.1016/j.polymdegradstab.2013.12.029

Valentini F, Diamantia A, Carbone M, Bauer EM, Palleschi G (2012) New cleaning strategies based on carbon nanomaterials applied to the deteriorated marble surfaces: A comparative study with enzyme based treatments. Appl Surf Sci 258(16):5965–5980. doi:10.1016/j.apsusc.2012.01.076

Vergès-Belmin V (2008) Illustrated glossary on stone deterioration patterns. Glossaire illustré sur les formes d'altération de la pierre. English-French ed, Monuments & Sites no. 15. ICOMOS and (ISCS) International Scientific Committee for Stone, Paris

Verstraete W, Wittebolle L, Heylen K, Vanparys B, de Vos P, van de Wiele T, Boon N (2007) Microbial resource management: the road to go for environmental biotechnology. Eng Life Sci 7(2):117–126. doi:10.1002/elsc.200620176

Warscheid T, Braams J (2000) Biodeterioration of stone: a review. Int Biodeter Biodegr 46(4):343–368. doi:10.1016/S0964-8305(00)00109-8

Webster A, May E (2006) Bioremediation of weathered-building stone surfaces. Trends Biotechnol 24(6):255–260. doi:10.1016/j.tibtech.2006.04.005

Weil P (1985) Patina: historical perspective on artistic intent and subsequent effects of time, nature and man. In: Naudé V (ed) Sculptural monuments in an outdoor environment. Pennsylvania Academy of the Fine Arts, Philadelphia, pp 21–27

Wendelbo O (1976) The use of proteolytic enzymes in the restoration of paper and papyrus. University Library of Bergen, Bergen

Wendelbo O, Fosse B (1970) Protein surgery: a restoring procedure applied on paper. Restaurator 1(4):245–248