Session 4. Bioremediation

APPLICATION OF MICROORGANISMS FOR THE DETERIORATED SURFACES RECOVERY

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Among the pathologies present in artistic works, those related to lithoid materials (stones, frescoes, paintings) are the most well known. In fact, in the last decades, particularly in urban areas, alterations such as (1) "nitratation", (2) "sulphatation" black crusts, and, in particular conditions, (3) dust, residual hydrocarbons and other organic pollutants have strongly increased.

All the studies confirm that "nitratation" and "sulphatation" are caused by inorganic atmospheric pollutants such as nitrogen oxides and sulphur dioxide produced by the combustion of petroleum and its derivatives. The compounds, which are the main cause of accelerated deterioration of exposed stoneworks, are oxidised in the air into nitric and sulphuric acid, respectively, that attack carbonates of stones, converting them into sulphates and highly soluble nitrates, which are easily washed away by rain.

Another kind of alteration is represented by black crusts, which are composed of crystals of gypsum (including re-crystallized calcite) mixed with atmospheric particles (spores, pollen, dusts, and soot containing aromatic and aliphatic hydrocarbons series) entrapped in the mineral matrix. It is commonly believed that black crusts originate from wet and dry deposition processes in which sulphuric acid (a sulphur dioxide oxidation product) attacks carbonatic rocks causing the gypsum formation.

Finally on the surface it is possible to find organic matter; this can be due to inadequate past restorations, to the lysis of microbial cells and to the compounds originating from oil combustion. This last phenomenon appears to be particularly evident in artistic stone works located in urban open air. Organic matter can favour microbial colonisation, with alteration consequences.

The pathologies of the materials composing the works of art can generally be treated by chemical and/or physical techniques, whereas biological methods are scarcely used. In particular only recently the use of enzymes became more frequent in the recovery of alteration, while whole living microorganisms as agents of bio-recovery have never been used, with very few exceptions.

However, investigators and experts reported that the conventional physico-chemical cleaning methods to remove gypsum, organic matter, nitrates and sulphates are in some cases disadvantageous, because they can cause colour changes in the rocks or excessively remove the original rock material. In particular water based methods to remove nitrates can damage the stones because nitrates can be transferred in the deep by water.

For this reason during the last years investigations and experimentations were carried out to verify the possibility to use some bacterial species to recover the above described alterations.

Whole bacterial cells

In order to remove nitrates produced during the "nitratation" process, nitrate reducing bacteria can be used. The capacity to use nitrates as final electron acceptor in the anaerobic metabolism is largely sprayed among bacterial taxa. In fact this functional large group lives in several natural aerobic ecosystems; it is facultative anaerobic and, as a consequence, it can grow both under aerobic and anaerobic conditions. Under anaerobic conditions this microbial group reduces nitrates to molecular nitrogen, which at room temperature evolves into the atmosphere (NO^{3-}, N_2). By this way nitrate-reducing bacteria remove nitrates in a soft, not disruptive for the art work manner and without environmental damages, because molecular nitrogen is a natural component of the atmosphere (around 79%). Among the nitrate reducing microorganisms, bacteria belonging to the *Pseudomonas* genus occur more frequently in the natural environments.

Similarly sulphate removal from stone and other material can be obtained by another functional microbial group named sulphate-reducing bacteria, that are strictly anaerobic. Sulphate-reducing activity is characteristic of bacteria belonging to genera of *Desulfovibrio, Desulfomonas, Desulfobacter, Desulfococcus, and Desulfosarcina*, that live in anaerobic mud, freshwaters sediments in marine environments, soil and in the gastrointestinal tract of man and other mammalians. Their metabolism is based on the capacity to oxidise organic low molecular weight compounds transferring the electrons to sulphate as final acceptor. By this way sulphates are reduced to hydrogen sulphide (SO_4^{2-} H_2S), that, being a gas at room temperature, evolves in the atmosphere.

The use of these microorganisms is suggested to remove sulphates not only when they are present alone, but also when they are associated with other contaminants as in the case of the black crusts. After the treatment with sulphate reducing bacteria, some authors found the presence of new calcite crystals (Atlas and Rude, 1988). They suggested that the formation of crystals occurred by a combination of dissolution-precipitation and diffusion processes; calcium ions (Ca^{2+}), released from gypsum when the bacteria reduce sulphates, react with carbon dioxide and result in the formation of calcite. However the authors concluded that the mechanism of the crystal formation was not enough clear. Deeper studies on the calcite precipitation have been performed by Tiano et al. (1999).

Finally the removal of organic patina composed by different compounds requires the use of heterotrophic bacteria able to attack and mineralise organic contaminants to water and carbon dioxide. Microbial strains to be used in this intervention must be selected on the basis of their capacity to remove the specific organic compounds present on the surface of artistic work. For example, to remove casein (present as a residue of a previous restoration intervention) it is necessary to select bacteria able to produce specific proteases able to hydrolyse casein. If the organic patina is constituted by unburned hydrocarbons, selected bacteria to be used must be able to degrade these compounds.

In order to elaborate a strategy of intervention, it is necessary to consider the following steps:

1. Selection of microbial cultures that showed *in vitro* the best performance in nitrate, sulphate and organic matter removal.
2. Identification of the best environmental conditions in order to favour their activities.
3. Selection of the inert matrix to use as a carrier of microbial cells. In fact microbial cells cannot be applied to the surface directly, but by means of an adapt carrier on which microbial populations formed a biofilm. The affinity between carrier and microbial cells depends on the charge present on the external cell envelopes and those of the inert matrix. For this reason it is possible that the support matrix good for a microbial population could not work with another one. Moreover the supports must offer adequate hydratation without interference with the sound substrate deriving from release of ions or metabolic activities. Furthermore, the matrix should be easy to remove and not toxic for the operators and the environment.
4. Select the best environmental conditions (pH, temperature, humidity, aerobiosis/anaerobiosis etc.) in order to optimise the removal process.
5. Test the support matrix activated by microbial biofilm on specimens under laboratory conditions.
6. If the results are satisfactory, apply the colonised carrier to the real altered surfaces.

7. Finally it is necessary to remove with care any microbial cells at the end of the treatment in order to avoid any secondary effect.

Experiments carried out by us demonstrated that, among microbial screened species, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* gave the best results in the investigations carried out on small specimens. *Pseudomonas aeruginosa* was eliminated because it is not safe.

As far as the sulphate removal is concerned, among the screened bacteria, *Desulfovibrio vulgaris* resulted the most effective. When applied to stones altered by the presence of black crusts, very satisfactory results were obtained.

Moreover in order to remove collagen present on a fresco surface *Pseudomonas stutzeri* strain A29 was used. Other tools applied in order to remove this collagen resulted ineffective. In fact, collagen showed a very low solubility in water solutions with or without the addition of specific enzymes, even at different room temperature. Moreover, a semi-mechanical approach was inadequate because it took too long and resulted in some damages. This strain of *Pseudomonas* was selected on the basis of its high degradation capability of the organic compounds generally used in the restoration intervention.

The best support matrix, among the different materials tested, resulted to be sepiolite, that was used as a carrier for all three microbial strains selected for the recovery.

The results obtained from the activated sepiolite to the altered surfaces of real samples were satisfactory.

In fact in the case of Vicenza stone altered by the presence of nitrates, biological treatment allowed to remove as much as 88% of them.

In the case of sulphates, 81% of sulphates were biologically removed.

Finally in the case of XV century frescoes of the Camposanto Monumentale (historical Cemetery) of Pisa, collagen started to disappear after three days from the application of activated sepiolite and disappeared completely after five days.

Enzymes

The use of enzymes is more known and applicated, but only to remove organic matter mainly from paints, frescoes and paper.

As in the case of whole cells, enzymes are used when the traditional non biological methods resulted ineffective or damaging.

The most used enzymes are: amilases to remove starch paste, gum-arabic, vegetal gums; proteases, to remove glues, animal gelatins, albumin, casein, egg. Generally proteases are used for paint surfaces to eliminate organic compounds, used in previous restoration interventions: esterases and lypases are used to remove oil paints, wax, synthetic resins, as acrylic and vinyl esters.

Their application requires attention to the environmental conditions (temperature, pH, etc.).

The application of biological tools can be a not disruptive, soft way to recover works of art without damage for the environment, on condition that safe microorganisms are used.

Suggested bibliography

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