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# The use of high-frequency electromagnetic radiation to remove biofilms from canvas

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***Abstract*** **– A methodology based on pulsed electromagnetic radiation in an unlicensed radiofrequency band is proposed to eliminate biodeteriogens from biofilms encrusting canvas of artistic interest. Molecular techniques allowed to identify species of bacteria and microfungi in the biofilms. Experimental results showed that the area covered by biofilm disappeared after exposure over five days.**

## I. INTRODUCTION

Microorganisms forming biofilms play a significant role in the deterioration of canvas of artistic interest. Many factors are involved in the growth of the microorganisms on these artefacts, such as the chemical nature of the substrate and the environmental conditions [1,2].

Many heterotrophic organisms use for their growth the inorganic and organic compounds present in the paintings. The organic materials that constitute the paintings, such as the fibres of cellulose contained in the support material of the canvas, the animal glue, the gypsum used to prepare ground layers and the linseed oils of the paint layers, are all easily degraded [3]. There is a variety of phenomena involved in the biodeterioration of paintings, such as depigmentation, degradation of compounds and hydration of the materials. The successive colonization of the paintings, the excretion of aggressive metabolic products (organic or inorganic acids) and the production of extracellular enzymes increase the loss of material [3].

Only the identification of the microbial communities associated with the different materials and the understanding of the role of such communities on the biodeterioration processes enable the prevention and/or remediation of the problems related to biodecay [1,2].

The analysis of microbial communities colonizing cultural assets was traditionally based on classical cultivation approaches, which are useful for understanding the physiological capabilities of pure isolated strains and for the development of metabolic studies, but the molecular identification is necessary to determinate exactly the species present in the biofilms.

After the identification it is important to choose the appropriate methodologies to remove the biofilm without causing damage to the artefacts.

Several techniques are adopted to eliminate the deteriogenic microorganisms, among which the most widespread is based on use of biocides, applied on several types of substrates, such as stones, wood, tissue and other materials [1,2,3]. The use of biocides is controversial, because the chemical compounds involved, although very effective, can alter the substrate and the surrounding environment and can be dangerous to operators as well.

For this reason, we adopt a methodology based on the use of electromagnetic radiation (EMR). The methodology was developed by the authors and proved to be non-invasive for the substrates and not dangerous for the operators and the environment [4]. In addition, this innovative methodology was implemented in such a way that no relevant temperature increase occurred, as usually happens when using radiofrequencies [4]. The methodology was already applied to substrates formed by tuff and terracotta [4-5].

The success achieved in the previous applications has encouraged to verify the applicability of the methodology to biofilms growing on substrates made of canvas of artistic interest.

## II. EXPERIMENTAL SETUP

Samples of canvas for the analyses were collected from paintings at the Laboratory for Restauration Techniques of the University Suor Orsola Benincasa of Naples, Italy, where the paintings were temporary transferred to undergo restoration interventions. The paintings were subject to a significant biological attack by bacteria and fungi. High values of air temperature and relative

*Fig. 1. Sampling on the versus of a painting.*

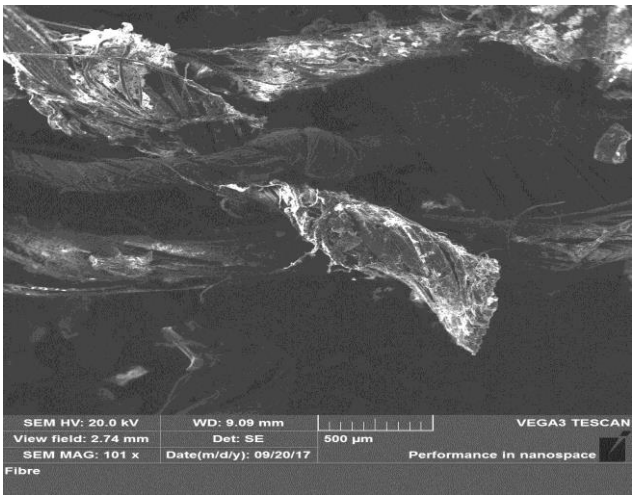


*Fig. 2. SEM of a canvas covered by biofilm.*

humidity measured in the rooms hosting the paintings were retained as favouring the microbial growth.

The samples of canvas exposed to the EMR were pieces of tissue 2 cm x 2 cm x 0.3 cm thick collected on the versus of a painting (Fig. 1).

Following the procedure by Muller et al. [6], artificial biofilms were created on the samples. The bacteria *Bacillus mycoides* and *Bacillus subtilis*, and the microfungi *Aspergillus versicolor, Cladosporium cladosporioides, Cladosporium oxysporum* and *Fusarium oxysporum* were used for the inocula. These species were collected and isolated from biofilms occurring on the versus of canvas of several paintings.

Small fragments of biofilms, after critical-point drying, were gold-coated in an Emitech k550 Sputter Coater and observed by SEM (Philips EM 208S).

Three samples with newly formed biofilms were used for the EMR treatments and three samples with newly formed biofilms were used as the control group.

The EMR exposure protocol consisted of three applications of two hours each, with a day relaxation in between. The EMR strength was set at 360 V/m. The protocol is described in detail in Cennamo et al. [4].

To measure the biofilm areas during the growth of the artificial biofilms and during and after EMR exposures, photographs of the biofilms were analyzed using the open source software Gimp® for manipulating images (http://www.gimp.org).

During each of the three phases, the microbial composition of the biofilms was also examined by molecular techniques, such as Denaturing Gradient Gel Electrophoresis (DGGE) and Agarose Gel Electrophoresis [4-7].

## III. RESULTS AND DISCUSSION

DGGE analyses carried out before the EMR treatments showed occurrence of all species used for the inocula (Table 1).

SEM observations showed that the microbial community is embedded in an exopolysaccharidic matrix that facilitates the establishment of tight bonds between biofilms and substrata (Fig. 2). In all samples, tissues showed to offer an ideal environment for the growth of bacteria and microfungi: while bacteria strictly adhered to the canvas, microfungi generally occurred more superficially.

EMR exposure proved to be effective in removing the biofilms after exposure over five days.

Growth patterns of both exposed and control samples measured by Gimp® are shown in Fig. 3. In this figure, dots indicate control samples while triangles indicate exposed samples. Red, green and blue colours indicate extension of biofilm between inoculum and exposure, during EMR exposure, and after the end of exposures, respectively (Fig.3). In Fig. 3, each point is the average of three measurements and standard deviations are indicated by vertical segments.

DGGE analyses conducted during the treatments showed that after the first exposure bacteria species disappeared, whereas the microfungal species were still alive; after the second exposure, all microfungal species disappeared, but *Fusarium oxysporum*; after the third exposure, *Fusarium oxysporum* disappeared, too (Table 1).

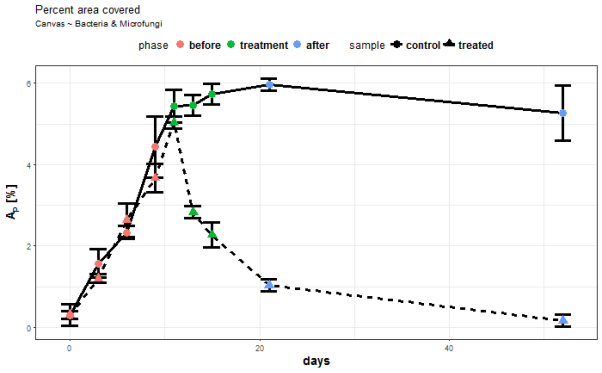


*Fig. 3. Mean growth of biofilms on canvas measured as percent area ($A_p$) covered by biofilm.*

*Table 1. Reduction of species of bacteria and microfungi during EMR exposures as resulted by DGGE analyses (+ presence - absence).*

| Species | EMR exposures | | |
|---|---|---|---|
| | First | Second | Third |
| **Bacteria** | | | |
| *Bacillus mycoides* | - | - | - |
| *Bacillus subtilis* | - | - | - |
| **Microfungi** | | | |
| *Aspergillus versicolor* | + | - | - |
| *Cladosporium cladosporioides* | + | - | - |
| *Cladosporium oxysporum* | + | - | - |
| *Fusarium oxysporum* | + | + | - |

Agarose Gel Electrophoresis carried out on EMR treated biofilms after the third exposure showed that DNA damage occurred in the biofilms.

Coloured areas still detected by Gimp® on exposed samples after the third day of treatment (Fig. 3) were optically observed. They resulted void of live cells and formed by crusty materials deriving from the degraded biofilms, that gradually decreased and completely disappeared after about 35 days from the end of the treatments (Fig. 3). Decrease of control samples after the third exposure (Fig. 3) was attributed to the depletion of nutrients in the substrata.

Samples of superficial layers of canvas collected after the third EMR exposure and transferred to a culture medium at 24 °C for 72 h did not show any re-growing of organisms.

The average temperature measured on the substrata during treatments was 24-26 °C. No alteration in the colour and structural consistency of all treated samples resulted after visual observations.

Samples of canvas observed before and during EMR exposures are shown in Fig. 4.

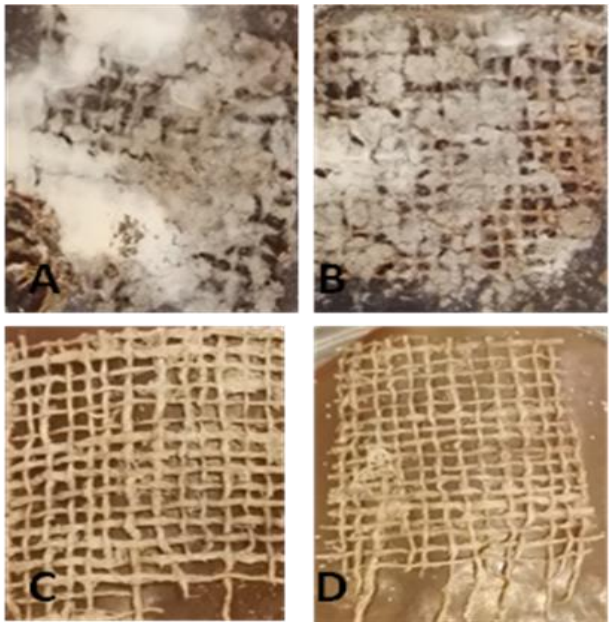


*Fig. 4. Samples of canvas before exposure to EMR (A) and after the first (B), the second (C) and the third (D) exposure to EMR.*

## IV. CONCLUSIONS

The knowledge of biodeteriogenic species and the type of colonized substrates was crucial for the choice of a suitable technique to apply in the removal of biofilms.

The methodology based on the use of electromagnetic radiation in the radio frequency range showed to be very effective to remove biofilms from substrates made of canvas. Exposure to radiation resulted in the elimination of all microorganisms, did not cause structural and chemical alterations in the substrates, were not hazardous for operators at the adopted exposure levels, and did not contribute to environmental pollution. In this last respect, the methodology appears to be a useful alternative to biocides and other chemical agents that present all the above described drawbacks.

Moreover, the electromagnetic field strength applied in our tests do not generate any significant increase in the temperature of exposed substrates. This appears very useful in the application to delicate artefacts. The results significantly differ from those obtained by using other control techniques based on hyperthermia, like those adopted for wood pest control.

A constant control of temperature and humidity in the rooms where the paintings are exhibited is here suggested to prevent microbial development on the canvas.

Since tissues of canvas represent common materials to make artefacts of historical and cultural interest, the biofilm removal methodology presented in this paper can be successfully used in the field of conservation of cultural heritage.

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