CURĂȚAREA PICTURILOR MURALE ȘI A MORTARELOR: REVIEW
CLEANING OF MURAL PAINTINGS AND MORTARS: REVIEW

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Both this paper and the identitification of the proper methods for cleaning of mural paintins and mortars (either originals or for restoration) have the great significance that aims scientific conservation and restoration of historical monuments. The review analyses both the main methods curently used for clening, pointing their efficiency in restoration activity, bioclening and case studies. Mechanical, physical, chemical and biological methods are used in the cleaning activity. The mechanical methods are based on the use of soft brushes and the physical ones on the use of the laser. For chemical cleaning, organic solvents, microemulions and gels are applied on the pictorial layer and mortars.

The findings show that biocleaning based on bacterial metabolic products perform better in this field. Green and nondestructive methodology for biocleaning of aged materials and deposits on murals based on products that contain gels with immobilized enzymes, active on salted surfaces is proposed. The new green technology based on metabolites (hydrolases and polysaccharides) obtained from non-pathogenic microorganisms grown under controlled conditions will be developed in the frame of the project "Murals biocleaning by new innovative green products based on microbial metabolites".

The case studies are regarding to: in situ biocleaning of mural paintings, of the murals restored with resins, of the extracted murals, bioremoving of sulphates, nitrates and graffiti.

Atât lucrarea, cât și identificarea metodelor adecvate pentru curățarea picturilor murale și a mortarelor (originale sau pentru restaurare) au o mare semnificație ce vizează conservarea științifică și restaurarea monumentelor istorice. Această lucrare de sinteză analizează atât metodele principale utilizate în mod curent pentru curățare, indicând eficiența acestora în activitatea de restaurare, cât și metode noi precum biocurățarea, exemplificate în studii de caz. În activitatea de curățare sunt utilizate metode mecanice, fizice, chimice și biologice. Metodele mecanice se bazează pe utilizarea periilor moi, iar cele fizice pe utilizarea laserului. Pentru curățarea chimică, se aplică solvenți organici, microemulsii și geluri pe stratul pictural și pe mortare.

Rezultatele arată că biocurățarea pe bază de produse metabolice bacteriene este mai eficientă în acest domeniu. Se propune o metodologie verde și nedistructivă pentru biocurățarea materialelor îmbătrânite și a depunerilor de pe picturile murale, pe bază de produse care conțin geluri cu enzime imobilizate, active pe suprafețe cu eflorescențe. Noua tehnologie verde bazată pe metaboliți (hidrolaze și polizaharide) obținuți de la microorganisme nepatogene cultivate în condiții controlate va fi dezvoltată în cadrul proiectului "Biocurațarea picturii murale cu produse ecologice noi bazate pe metaboliți microbieni".

Keywords: *cleaning, biocleaning, mural paintings, green methodologies, case studies*

1. Cleaning methods

The most common damages that require cleaning in the restoration activity are: black crusts, saline efflorescences, nitrates, sulfates, organic and mineral deposits or rest of materials used in previous restoration. Their removal is done by cleaning and this is a critical, time-consuming activity, sometimes irreversible due to the depth reached. The method chosen for cleaning must be non-invasive, efficient and environmentally friendly. Mechanical, physical, chemical and biological methods are used in the cleaning activity.

1.1. Mechanical methods

When the pictorial layer is in good condition but is covered with weakly adherent powdery deposits, it is cleaned with soft brushes. The restorer pays a special attention to the origin of the deposits by analyzing the chemical and biological expertises. The presence of sporulated fungi as biodeteriogens can be confused with powdery chemical deposits and therefore mechanical cleaning methods contribute to their spreading on neighboring uncontaminated surfaces.

1.2. Physical methods

The laser is used in the cleaning of different substrates: wood, mural, stone, polychrome painting and metal. The first results have been published at the end of 1960. The method is characterized as follows: high selectivity and precision, protects the component layers, the results can be appreciated immediately, does not involve mechanical intervention or abrasion of the substrate. Using spectrophotometric analysis (LIBS) the substrate can be characterized before and after cleaning so that all activity is under permanent control. The laser

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cleaning method is an alternative to the chemical one based on toxic organic solvents. Although both methods have comparable results, the one with laser is preferred because it does not penetrate the substrate in the depth. The cost price of this method is an impediment in some cases.

1.3. Chemical methods

Most common chemical methods are based on *organic solvents*, *microemulsions, ionic solutions* and *gels*. Giordano et al. [1] revealed that the deposition of residues from cleaning materials brings negative impact because of future changes resulting from their aging during the time or microclimate. The mural cleaning is very difficult because of the physical-chemical properties of the substrate (porosity, chemical composition).

Organic solvents had been and are still used. The organic solvents action cannot be controlled due to their surface tension and common high wettability of the treated surface which goes to solubilization of soil material and its own spreading into the pores [2]. They may swell and partially solubilize the original material. All attempts to use mixtures of organic solvents reveal their aggressivity on murals. Organic solvents have also a high rate of evaporation and toxicity. Residual organic substances and salts are removed by ammonium carbonate solution [3]. As a case study, wax spots deposited over murals had been removed with dodecane but after solvent evaporation, the wax was re-deposited into the pores [4]. An oil-in water microemulsion containing Sodium Dodecyl Sulfate (SDS)/dodecane/n-butanol/H_2O provided a more efficient removal. Paraloid used for the restoration of 16th century wall paintings in Salvador Church in Venice that was found after 30 years compact, yellowed and shiny because of ageing, was removed with SDS/nitro diluent/1-pentanol/ H_2O [5]. To remove Mowilith DM5 used in restoration of the archaeological area of Mayapan in Mexico, ethyl acetate in the propylene carbonate [6] or nanomaterials [7] had been used.

As alternatives to classical cleaning procedures, *microemulsions and ionic solutions* (innovative low impact cleaning methods for restoration currently outdate) had been proposed [8]. Removal of wax spots of art works dates back to the end of the 1980s. Microemulsions (ECOSURF EH-3, ECOSURF EH-6, ECOSURF EH-9) were applied in several projects having the advantages of water-based cleaning systems and thus limiting the chemical solvents associated risks [9].

Microemulsions are cleaning products used for mural painting and stone. They are considered environmentally friendly because of their small amounts of solvents and capacity to reduce the degree of toxicity and impact on the environment.

To remove degraded polymers from the surface of the murals (nitrocellulose, acrylic polymers) and soluble salts, Baglioni et al. [10] recommend dimethyldodecan-l-amine oxide and diethyl carbonate (DDAO-DC) dispersed in water. This product is a biodegradable and eco-friendly surfactant.

Ionic solutions have special characteristics, among which can be mentioned: low vapor pressure, they are not flammable, they are thermally stable. Depending on the combination of cations and anions, some properties can be modified (viscosity, pH, melting temperature). In the laboratory phase, ionic solutions were used to remove natural and artificial resins from the surface of the paintings. Finally, the appearance of the painting was as no varnished. In the same phase, the ionic solution with the addition of proteases (obtained from *Aspergillus sojae*) gave good results for the removal of protein deposits [11]. The advantages of ionic cleaning are versatility and selectivity. However, there is a disadvantage that arises from maintaining the residue on the substrate due to the low vapor pressure that determines the chemical interaction with it. Additional studies on solubilization parameters, degradation kinetics and compatibility with traditional materials are needed for the widespread use of ionic solutions.

Gels were also proposed as a tool for cleaning because they decrease the risk of the pictorial layer swelling, reduce the diffusion of the solubilized molecules allowing a control between the viscous gel and support; they are efficient for embedding of organic solvents, micellar solutions and microemulsions [2]. Unfortunately, they do not selectively remove deposits or damaged materials, reduce but not eliminate swelling of pictorial layer. All gels have to contain water soluble macromolecules (gelator), natural (cellulose ethers) or synthetic polymers (Carbopols-[12]); should be chemically inert and physical-chemical stable. Nanomagnetic sponge was proposed as an alternative [2] because gelators develop transparent rigid hydrogels that retain water in contact with porous support, avoiding their penetration. Nanomagnetic sponges made from nanoparticles loaded with solvents or other cleaning agents are cutt in different shapes and applied on the murals. Then, conservators removed the sponge with a magnet.

Gels of microbial origin (Vanzan NF-C, Gelano Kelcogel and Agarart) are also used. For the removal of Dammar varnish, the gel containing ammonium / polyethylene carbamate is used but it loads the substrate with toxic solvent [5]. Significant results were obtained in the case of the mural painting executed in the secco technique in the sacristy of the church of Santa Maria della Scala (Siena, Italy); the analytical techniques did not reveal any remains from the gel structure. Research is needed about the diffusion of gel components in the pictorial layer.

Bonini et al. [13], used a magnetic nanosystem for cleaning. $CoFe_2O_4$ magnetic

nanoparticles were successfully and homogeneously embedded in the gel structure (polyethylene glycol and acrylamide). The nanomagnetic gel was "charged" with a microemulsion (mixture of nitrodiluents and p-xylene) and then applied to the marble surface coated 8 years ago with Paraloid B72. Analytical methods showed that the resin was completely removed and, on the treated surface no residue, remained. After cleaning, the nanomagnetic gel can be dried and reused, thus reducing cost and pollution.

Carretti et al. [14] worked with a hydrogel (polyvinyl alcohol-borate) "loaded" with 1-propanol (1-propanol PVA borate) for the solubilization of oxidized varnishes but also the hydrogel "loaded" with other solvents (cyclohexanone, 1-pentanol, 1-butanol) because their toxicity is lower. The gradual removal of varnish from the wood panel was controllable and selective, showing that the method is effective for removing deposits and has a little impact on the health of the restorer.

Dominigues et al. [15] applied a high biocompatible hydrogel containing poly (2-hydroxyethyl methacrylate) or p (HEMA) and poly (vinyl pyrrolidone = PVA) on a textile painting executed in the tempera technique covered with hydrophilic deposits. The favorable results showed the efficiency of the product in cleaning. Hydrogel solves the problem of swelling and residues. In this gel, organic solvents are eliminated and the lipophilic layers are effectively removed. Also, other characteristics of the hydrogel can be modified (water ratio, PVP, HEMA). The hydrogel is transparent, easy to apply, has a good adhesion and does not require subsequent mechanical cleaning.

1.4. Biological methods

Biological cleaning started with trypsin in the beginning of 1970 when it was performed on paper-based artifacts and on polychrome canvases [16]. In 1977 double enzymatic treatments was used to remove starch, in 1982 a mixture of amylase/protease was used to remove glue paste and in 1999 aged acrylic resins were removed with lipase. Five pure commercial enzymes were applied on a swab, on a paper disc or with a brush [4] to remove unwanted organic matter from the frescoes. In 1990, Bosch-Roig [17] having as target the removal of nitrates, sulfates and organic matter used microbial metabolites or living cells. Cremonesi [18] was a pioneer in the application of enzymatic treatment to hydrolyze protein/oily binder (biocleaning, as an alternative to chemical solvents). Biocleaning can be also performed either with whole bacterial cells (*Pseudomonas stutzeri*) or with commercial enzymes [3]. The first one is an approach difficult to perform due to the compulsory monitoring of the microbial activity and cell removal.

Cold-active molecules extracted from marine invertebrate organisms (sponges, jellyfish, sea-anemones and shellfish) were used to remove protein layers from painting surface. Bioactive molecule swith protease activity act in a temperature range of 4-30^{0}C. Also had been isolated biomolecules with esterase activity for removal of waxes. Unfortunately, the productivity of the marine ecosystem is very changeable and marine invertebrate organisms are not always available. The method based on microbial metabolites obtained in laboratory in controlled conditions avoids the biological features of different invertebrates and other additional activities. Barresi et al. [19] used bioactive molecules with proteolytic and antimicrobial activity for the biocleaning of casein layers. The comparative treatment with commercial enzymes revealed that biological enzymes did not need heating to 37^{0}C which is an advantage for on-site cleaning. Extracted bioactive molecules with hydrolase activity-esterases and proteases [20] were applied on a laboratory specimen mimicking "the strappo" of mosaic tesserae, carried out by gluing tesserae on a canvas sheet and led to positive results. Palla et al. [20] evaluated different gelling agents such as Klucel-G, Pluronic F 108 and Vanzan NFC 2 as proteases support, and then the team performed the removal of the protein layer from canvas painting, polychrome wood and paintings. Giordano et al. [1] used Velvesil Plus (crosspolymer that consists of cyclosiloxanes with polyethylene glycol) combined with a novel hydrolase isolated from invertebrate marine organisms for cleaning of contemporary paintings. The team used the same product to remove adhesive tape covering the protective paper of a contemporary painting. There are not excluded changes in time.

Biocleaning is a sustainable alternative to the traditional chemical cleaning method. Microorganisms are biodeteriogens of the artwork but can also be used for their ability to synthesize metabolic products with a role in cleaning of salts, graffiti, natural and artificial polymers. Metabolic products obtained from *Desulfovibrio vulgaris* has been successfully used to remove the black crust from a marble sculpture placed in a cemetery in Florence [21]. Removal of phosphates, sulfates and protein deposits was obtained by inoculation with *Pseudomonas koreensis*, *Cellulosmicrobium cellulans* and *Stenotrophomonas maltophilia* [22]. It is estimated that biocleaning is less expensive than chemical cleaning and has a high degree of control. There is perspective to obtain bioleaning kits but their use must be correlated with the training of operators.

In the field of cultural heritage there can be applied both biotechnologies based on the treatment of degraded restoration materials (shellac, casein dispersion, animal and vegetable glues) and deposits accumulated over time (proteins, lipids) with hydrolytic enzymes and biotechnologies based on direct inoculation of

microorganisms which have the metabolic ability to use materials as a source of nutrition.

2. Case studies

2.1. In situ biocleaning of mural paintings

Ranalli et al. [23] carried out biocleaning on Cristo che salva Pietro dale acque = La Navicella in the Vatican Museum and on the Incarnato in the dome of the Cathedral of Pisa.

Before biocleaning, analyzes of Py / GC-MS surfaces (Pyroliser TY3030 coupled with Gas Chromatograph 6890N and Mass Spectrometer detector 5973) and FTIR (Fourier transform infrared spectroscopy) were performed. Thus, on La Navicella were identified: traces of protein material that suggested the presence of egg yolk and fatty acids that indicated the lipid component of a material with animal origin. Calcite, gypsum and basic copper carbonate (indicates the azurite) were also highlighted.

Agar - biogel was prepared for the vertical surfaces and agar - gauze - biogel for the vault. Both biogels contained *Pseudomonas stutzeri* A 29 cells in a concentration of 2 - 5 x 10^6 viable cells / cm^2. The treatment was performed on surfaces of 60 cm^2 – 4000 cm^2 for 10 - 150 minutes on the painting from La Navicella in the Vatican Museum and for 12 hours on the one in the dome of the Cathedral of Pisa. In parallel, gels containing sterile water were applied as control. After removal of the biogels, the treated surfaces were cleaned with a sponge moistened in sterile distilled water and samples were taken for the viability test to demonstrate if *P. stutzeri* cells were still viable or not. Thus, it was found that during 2 months of monitoring, *P. stutzeri* colonies were not identified. In some places total cleaning was observed but at the edges of the biogels this was only partial. The advantages of using biogels are the following: they adhere better to the surface on which they are applied, they reduce the risk of dehydration and detachment, they are easy to apply and remove.

The success of biocleaning is attributed to the versatility of the metabolism of *P. stutzeri* cells that synthesize the enzymes needed for the hydrolysis of organic compounds from the murals to ensure nutrients. Factors such as: temperature, humidity (in the substrate and relative), the presence of toxic ions (metals) and the carbon source influence both the metabolic activity of bacteria and the activity of enzymes synthesized. The efficiency of biocleaning depends on the duration of the treatment but decision of the application has to be correlated with the state of conservation of the painting layer. Prolonging the duration of treatment does not increase its effectiveness because the number of bacterial cells is increased and can affect the pictorial layer (by number and metabolic products). Their number is controlled by measuring of ATP (adenosine triphosphate that reflects biological activity). It is the responsibility of the restorer to choose how to apply the treatment to avoid inactivating the biogel by dehydration.

Biocleaning is an ecological method because protein compounds are decomposed to CO_2 and H_2O. In addition, the health of restorers is not affected because no toxic compounds are used and *P. stutzeri* is not pathogenic. During the treatment there is no overcontamination of the treated surface although in the structure of the biogel or in gauze there are organic compounds. It is also the responsibility of the restorer to avoid contamination of the environment by ensuring the incineration of products used for biocleaning.

2.2. Biocleaning of the restored mural with resins

Synthetic (Paraloid B-72) and natural resins (Shellac) are used to restore the murals. Over time, they are chemically transformed so that the surface on which they were applied undergoes aesthetical changes. They are removed using chemical solvents but they have different degrees of toxicity for both restorers and the environment.

Shellac is a natural resin produced by a parasitic insect (*Kerria laca*) in the phloem of trees, where it forms galleries. It produces a yellow varnish and a resin with a complex structure (mono and polyesters of aliphatic hydroxy acids and sesquiterpenoids). The resin was used to restore the paintings in the Ajanta cave as fixatives due to its specific characteristics (good penetrability, adhesion and resistence to microbial decomposition). Over time, Shellac has changed aesthetically (yellowing and blackening), has hardened and become shiny.

By cleaning with organic solvent it was not possible to restore the original shades of the paintings; the fragility of the color layer, the appearance of gaps, white crusts and epieflorescences were remarked [24]. The resin was not completely solubilized and the successive restoration works were not carried out according to the previously established plan.

Researchers from ENEA, Italy [31] inoculated 3 strains of bacteria CONC 11 (genus *Pseudomonas*), CONC 18 (genus *Achromobacter*), and LAM 21 (genus *Acinetobacter*) on nutrients containing Shellac as a carbon source. Depending on the growth rate and consumption of Shellac (80%) they selected the strain of *Acinetobacter*. In order to use that strain for the removal of Shellac from Ajanta cave, aged experimental models were inoculated with bacterial cells. The treatment efficiency was demonstrated microscopically and by mass spectrometry.

2.3. Biocleaning of extracted murals

Lithic materials and murals are deteriorated over time due to aging, action of pollutants and

biodeteriogens. If they have been previously restored, organic compounds (which have not been completely removed) are identified in most cases. There is a risk to start the biodeterioration process because microbiodeteriogens use them as nutrients. There had been put in evidence rests of organic materials from previous restoration, both on the surface and into the pores of mural paintings and mortars. Among the microbiodeteriogens, the fungi can penetrate the pores by their branched hyphae and they produce biochemical, physical and finally aesthetical damages.

In the case of extracted frescoes, before removing them from the wall, organic compounds (glue and casein) are used. By applying the classical methodology of using ammonium carbonate solution and organic solvents, the glue and casein are not completely removed.

For the development of an efficient biotechnology for cleaning the frescoes extracted from the Monumental Cemetery in Composanto (Pisa, Italy), Ranalli et al. [3] analyzed and identified the original organic materials used in the previous restoration and the components remaining after their transformation over time. They proposed biotechnology for extracted frescoes in two distinctive stages: hydrolysis of glue and casein by using bacterial cells and treatment with enzymes of microbial origin.

Three types of gels had been obtained from halophilic microorganisms in order to be used for the immobilization of esterases, proteases as well as cellulases and then to be applied for biocleanng murals restored with Paraloid B 72 and transparent casein dispersion or having different deposits. The main steps for obtaining gels with entrapped enzymes for biocleaning of murals are presented in Fig1. developed based on the experimental results made by the authors of this paper.

To apply this biotehnology, under laboratory conditions, biomass of *Pseudomonas stutzeri* A29 was obtained in biofermenters. The surface of the mural was inoculated with bacterial suspensions by three methods: spraying, brushing and on cotton swab and incubated at temperatures of 20^0C and 10^0C in order to establish optimal conditions for application. The best results were obtained when the bacterial suspension was applied on the cotton swab at 20^0C. Later on, it was found that the compresses immersed in bacterial suspension ensured good cell adhesion, efficient and permanent direct contact of bacterial cells and the rapid dehydration was prevented. After 8-12 hours from the application of the compresses with bacterial cells, approximately 80% of the organic material was removed by the metabolic activity of the bacteria (they synthesized and released extracellular protases and lipases). During treatment, the pH slightly decreased (from 7.2 to 6.7). Bacterial cells were removed with a sponge soaked in distilled water. The viability test is the one that determines the number of applications of the wet sponge. The degree of glue removal depends on the thickness of the applied layer. It is not recommended to prolong the treatment time because it can lead to swelling and detachment of the paint layer. An 80% removal of of casein glue was obtained using bacterial cells.

Final treatment with enzymes of microbial origin was performed. Microbial enzymes (collagenase obtained from *Clostridium histolyticum*, proteases from *Streptomyces griseus* and *Aspergillus sojae* as well as lipase from *Candida cylindracea*) were applied separately and in mixture by brushing.

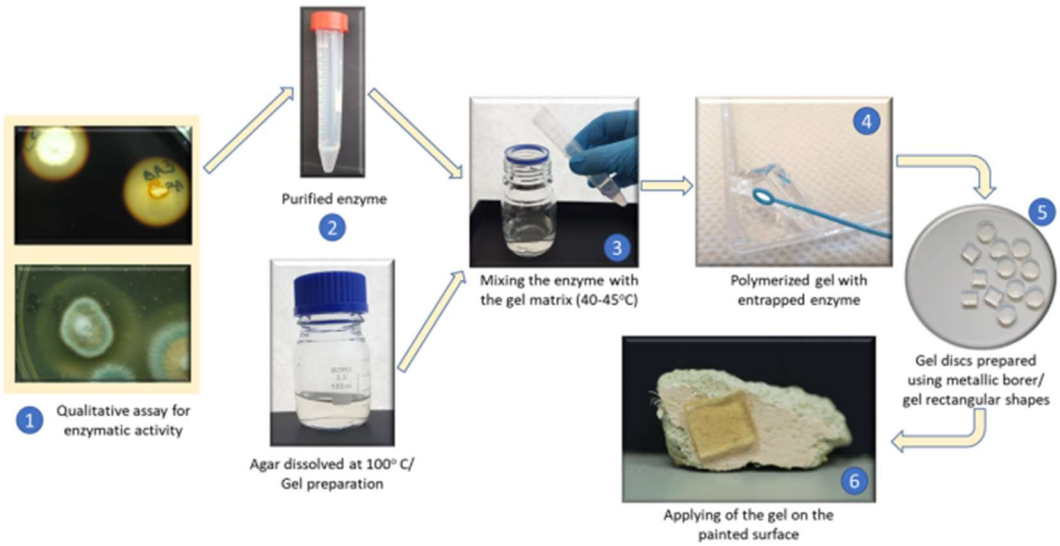

Fig.1 - Obtaining of gels with microbial entrapped enzymes for biocleaning of murals / *Obținerea gelurilor cu enzyme imobilizate pentru biocurățarea picturii murale*

2.4.Bioremoving of sulphates and nitrates

Black crusts are identified on stone works and walls exposed in the open air. It is formed as a result of the transformation of calcite into gypsum as well as of the incorporation of pollutants and mineral deposits.

Sulfur-reducing bacterium *Desulfovibrio desulfuricans* was used in biocleaning attempts. It transformed the gypsum into biocalcite, the biocleaning and conservation of the surface taking place at the same time [25].

In the case study represented by the Cathedral of Milan, Cappitelli et al. [25] covered the black crust with wet filter paper and applied the paste containing *D.vulgaris* cells distributed in the Carbogel powder. Then, the surface was covered for 48 hours with a film of polyvinyl chloride. The film and paper were taken off and the residue was mechanically removed (with a brush). The efficiency of the biocleaning was evaluated spectroscopically and colorimetrically. Even with the naked eye, it could be seen that the surfaces have not been completely cleaned. A whitish layer under which there is a yellowish one was noticed.

Nitrates are deposited both on lithic works and on the walls of historical monuments or on their pavement.

Alfano et al. [26] proposed removing nitrates from the walls of Matera Cathedral (Italy) with nitrate-reducing bacteria. The efficiency of the application of this biotechnology was demonstrated during the 6 years of monitoring by physical and chemical methods. During this period, no newly colonized areas were identified, so the treatment was also preventive.

Bosch-Roig et al. [27], removed insoluble nitrates from the mural in the church of Santos Juanes in Valencia (Spain) using *Pseudomonas stutzeri* included in bacteriological agar. This support allows a good adhesion of bacteria to the surface the pictorial layer and also a uniform biocleaning of the vertical surfaces.

Bosch-Roig et al. [28] tested the efficiency of nitrates removal on the floor of the monastery of Santa María de Conxo (Santiago de Compostela, Spain) by 3 classical methods (with cellulose, sepiolite and mixture of cellulose and sepiolite) and 2 methods of biocleaning (*Pseudomonas stutzeri* cells included in 2% agar and cotton swab). Of these, the best results were obtained by applying biocleaning methods.

2.5 Bioremoving of graffiti

Presently, graffiti is identified on any type of material (glass, metal, stone and walls). In addition to the fact that the respective surfaces are aesthetically affected, the execution technique is polluting because it includes organic solvents. Their removal is very expensive and incomplete. The difficulty of removing graffiti is due to the type of substrate (chemical composition, texture, porosity, strength, etc.), the state of conservation and the time passed from the application of the paint to start the removal work. Porous substrates can not be cleaned.The use of high-pressure water-based cleaning methods and organic solvents causes irreparable damage. Techniques based on the use of laser visibly alter the surface (it turns yellow), removes minerals, causes the formation of microcracks.

Baglioni et al. [29] proposed the cleaning of graffiti on the decorated stone from the archeological site Ba 'Cuana, Asunciòn Ixtalpetec, Oaxaca, Mexico, with micellar solutions or microemulsions. They contain water, surfactants and at least one organic solvent. They have low toxicity if compared to methods that use only organic solvents. It is even less invasive because it causes the paint to swell and soften, making it easily removable by mechanical means.

Strains of microorganisms that tolerate the chemical components of graffiti and that have at the same time the ability to synthesize the enzymes to degrade them must be isolated. The results of the cultivation of microorganisms on graffiti powder are encouraging because they have shown that one of the tested strains (*Pseudomonas sturzeri* DSMZ 5190) can use it as an energy source.

Sanmartín and Bosch-Roig [30] conducted research to develop a strategy for the graffiti removal, using different bacteria (*Pseudomonas sturzeri* DSMZ 5190, *Aerobacter aerogenes* ATCC 13048 and *Comamonas* sp. ATCC 700440), applied by brushing or in agar gel.

The evaluation of the efficiency of biocleaning was tested by means of spectroscopical (FTIR), colorimetric, microscopic and microbiological techniques. The best support for bacteria was considered to be the agar gel, due to its good adhesion to the surface and the capacity to maintain moisture.

3. Future perspectives

According to our research activity, we propose the development of two cleaning methodologies: biocleaning using metabolic products like hydrolytic enzymes produced by halophilic microorganisms and biocleaning by direct inoculation of halophilic microorganisms able to use deposits as nutrients. Both methodologies have two common steps: the selection of microorganisms and the establishing of optimal growth conditions (temperature, pH, cultivation).

The assesment of the cleaning methodology with hydrolytic enzymes is the result of passing the following steps:

• Establishing the optimal conditions for the cultivation of microorganisms in order to synthese the hydrolytic enzymes;

• Isolation of hydrolytic enzymes;

Establishing the optimal conditions for the enzymatic activity (pH, temperature, substrate concentration, salt concentration);

• Enzymatic treatment of the experimental models containing protein, lipid and cellulose deposits;

• Evaluation of the enzymatic treatment efficiency (under the optical microscope and by colorimetry) in order to establish the parameters for the development of the enzymatic treatment (pH, temperature, substrate concentration, salt concentration, duration); the photographic documentation will be elaborated;

• Examination of the treated experimental models under the scanning electron microscope (SEM) to confirm the efficiency of the treatment in terms of enzymatic hydrolysis and the integrity of the surface; the photographic documentation will be elaborated.

In order to elaborate the cleaning methodology by inoculating the experimental models with microbial cells, the following steps will be followed:

• Inoculation of the experimental model in areas with deposits with a dense suspension of microbial cells prepared in the laboratory;

• Placing the inoculated sample in the optimal development conditions of the selected microorganisms;

• Microscopic monitoring of the decomposition process of the deposits and multiplication of the inoculated microorganism; elaboration of photographic documentation;

• Removal of microorganisms by repeated application of compresses containing biocide;

• Evaluation of the treatment efficiency by optical and electron microscopy examinations, colorimetry, spectroscopy (special attention is paid to the identification of by-products and the viability of inoculated cells).

After the elaboration of the cleaning methodology, the activity will continue with:

• Elaboration of the cleaning guide;

• Dissemination of the cleaning method by organizing meetings with restorers in which new cleaning methodologies are presented and practical applications are made;

• Standardization of methods and products for biocleaning;

• Providing technical assistance by the institution that developed the methodologies;

• Monitoring the efficiency of biocleaning of cultural heritage (case studies) and its contribution to preventive conservation.

The scope of the project *"Murals biocleaning by new innovative green products based on microbial metabolites"* is to develop and validate a new green and nondestructive methodology for biocleaning of aged materials and deposits from experimental models and frescoes, based on new innovative products such as different gels in a new shape with immobilized microbial hydrolases, active on salted surfaces to be implemented on historical monuments, mainly murals. Four microbial gels and non-ionic cellulose ethers with immobilized hydrolases (esterases and proteases) will be obtained as innovative smart biocleaning systems exploiting their main features in order to be used for restoration and conservation of murals. The targets of biocleaning are: materials used in a previous restoration but damaged over time, such as Paraloid B-72 (acrylic resin based on methacrylate-ethyl-methacrylate) and transparent dispersion of calcium caseinate (artisanal Romanian fixative).

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