Cleaning and protection of historic objects – biotechnology and nanotechnology approach

Snežana Vučetić1, Jonjaua Ranogajec1, Helena Hirschenberger2, John Milan van der Bergh1, Ana Vidaković and Siniša Markov1

1 University of Novi Sad, Faculty of Technology, Laboratory for Materials in Cultural Heritage, Bul. cara Lazara 1, 21000 Novi Sad, Serbia, janjar@uns.ac.rs;
2 University of Novi Sad, Faculty of Technical Sciences, Trg Dositeja Obradovića 1, 21000 Novi Sad, Serbia

Abstract. Biocleaning procedure has been tested successfully on many materials and has proved to be more selective than mechanical and chemical cleaning techniques. Despite its success in treatment, biocleaning is not well-established method for the reduction of salt crystals. Therefore, the innovative approach combines biocleaning with the traditional (poultices) cleaning procedure. It is a new technology for salts reduction, starting from the design of clay poultices and joining their effects with targeted microorganisms. The chosen bacterial cultures increase the desalination capacity of the designed clay poultices, as a consequence of their metabolic activity, providing the mobilization of salts and salt reduction by the advection mechanism. Cleaned with this technology, architectural historic materials are properly prepared for long-term protection with photocatalytic coating based on TiO2 and LDH suitable for fragile heritage materials. This protective material, designed under the FP7 HEROMAT project, has been already proved in laboratory and in-situ to be effective in maintaining aesthetic and functional properties of the mineral substrates and ensuring their long-term resistance to environmental conditions. This promising two-stage treatment presents a method which is both environmental friendly and cost/time effective for the reduction of nitrate salts and continuous self-cleaning protection of the surfaces of historic buildings.

1. Introduction

Building materials are exposed to wind, sunlight, rain, snow, atmospheric pollution and temperature variations during their exploitation and consequently, they degrade over time. Even the most durable materials are modified by deposition of ambient dust which enables the colonization of biological organisms such as cyanobacteria, fungi and algae [1]. One of the principal outcomes of the interaction between the environment and historical buildings is the deterioration of materials and the formation of soluble salts [2]. Nitrates, chlorides, oxalates and sulphates are known to be among the most harmful soluble salts for porous materials. Under real environmental conditions these salts are subjected to cycles of crystallisation-dissolution. The growth of salts crystals within porous building materials can cause serious surface damage or even structural collapse [3].

The two-step method of cleaning and protection presents a promising way to restore and improve the characteristics of deteriorated constructive materials and objects. Currently, the most used method for desalination is based on the application of poultice [4]. The use of water based poultices for the extraction of salts is a well-established technique in conservation. The desalination poultices available
on the market are predominantly either cellulose powder based or mixtures consisting of cellulose fibres, clay materials (bentonite, kaolin, sepiolite) and sand or light aggregate. In practice, the composition of commercial products and self-made recipes do not undergo any modification in order to target individual properties of the substrates. However, the research findings have revealed that some variations of the textural properties of the poultices are needed to enhance the desalination efficiency [5]. In recent years, biocleaning (microbial cleaning) has been tested successfully on many materials and has proved to be more selective than mechanical and chemical cleaning techniques [6]. Biocleaning procedure with the use of non-pathogenic and non-spore forming microorganisms is a safe and environmental friendly way for cleaning the surfaces of architectural cultural heritage objects [7]. However, only about 30 articles related to bio cleaning of building materials (www.sciencedirect.com) can be found, which leaves room for further investigation [8]. In addition, publications exploring the usage of conventional water-based poultices as support for bacteria cultures are difficult to find.

After the cleaning, the surface should be properly protected, since many deterioration processes start from the surface. For this, the application of a suitable surface coating is required. Surface treatments such as water repellent coatings [9] and antigrafiti coatings [10, 7] (based on silicones, fluoropolymers, polyurethanes and alkoxy silanes) strongly reduce water and vapour transport and consequently are not considered compatible with most historical building materials. A very promising approach has been found in the use of nano-photocatalyst based material. By applying inorganic-inorganic coatings, the compatibility of these materials with the mineral substrates (built-in materials) increases, compared to the organic coatings available on the market. Different types of coatings with additional functions have been developed so far. A specific problem of these applications is the lack of compatibility of photocatalysts with the surface of building materials. This difficulty could be solved by the appropriate immobilization of TiO2-nano-particles onto the photocatalyst support, layered double hydroxides (LDHs) [10]. Since the degradation processes start on the material surface, the deposition of suitable protective coating might be a very useful tool for surface protection.

Considering all above mentioned, the aim of this work was to study the possibility of coupling good properties of the designed cleaning procedure (combination of the traditional poultices and bio- cleaning) with a long-term protection using the photo-catalytic coating based on TiO2 and LDH. The chosen cleaning procedure was performed using the already proven methodology based on effective bioactive denitrification poultices [8]. Combining the effects of targeted designed water-based poultice and already verified bacterial culture for the removal of nitrate (Pseudomonas stutzeri ATCC 17588), an effective bioactive system was prepared. In this cleaning system, the poultice ensures the transport of soluble salts from the interior of the mineral substrate to the surface. Based on our previous studies, poultice was developed in order to suit the properties of the targeted mineral substrate (carbonate stone material). It possesses a wide pore size distribution incorporating large pores that can act as reservoirs for wetting, and small pores to ensure advection from the substrate to the poultice. At the same time, the poultice needed to take the role of a good microbiological carrier (water activity and appropriate pH values). When both requirements were achieved, the salt cleaning procedure (denitrification) was tested. Following the denitrification, the treated stone substrates were protected with a photocatalytic coating system designed under the FP7 project HEROMAT [11]. This protective coating (doped clay material) was used directly on the stone substrates surface, with or without prior cleaning. It enabled the monitoring and examination of the joint effects of the used cleaning and protection procedures on the treated stone substrate.

2. Materials and methods
The experimental procedure includes three main groups of experiments: 1 - contamination and examination of stone mineral substrates; 2 - preparation and application of bioactive poultice and characterization of treated samples; 3 - protection and examination;

2.1. Contamination and examination of stone mineral substrates
The uniform samples of carbonate stone (marble) were prepared in the form of cubes of 5 cm x 5 cm x 2 cm (length x width x height). The obtained samples were contaminated with potassium nitrate at a level of about 500 mg ml⁻¹. The desired contamination level was achieved using the following protocol: (1) drying samples in the oven at 105°C for 2 h and (2) complete immersion of the samples in a saturated solution of potassium nitrate for 22 h. The described steps were repeated in 5 cycles.

Textural characteristics, pore size distribution and total porosity values, were measured using Mercury Intrusion Porosimetry (MIP). MIP is a well-known and often used method for the determination of pore size distribution in stone substrates. It is commonly used in the investigation and assessment of degradation caused by salt crystallization. The prepared samples were investigated using Hg-porosimeter Autopore IV 9500 (Micromeritics, USA) with mercury intrusion pressures ranging up to 228 MPa, which enabled the determination of pores with diameters ranging from about 390 µm to 0.005 µm. The assumed contact angle between mercury and stone was standard 141°.

2.2. Preparation and application of bioactive poultice and characterization of treated samples
Based on our previous study [8], the poultice material was prepared using the easily available industrial components - kaolin:quartz sand:talc = 1:1:1. The composition of the prepared poultice material was designed to be of adequate textural properties required for the advection mechanism of desalination and at the same time, to retain the water necessary for a normal bacterial activity. The usage of talc was beneficial due to its surface adsorption capacity [12], while kaolin was used for its moderately shrinking properties. The particle sizes of the used components were as follows: kaolin – particles less than 43 µm, quartz sand –particles less than 710 µm, with 45mass. % of the particles with diameter of 180 µm and talc particles less than 6,5 µm. All used components were firstly mixed in dry state. In order to achieve consistency, bacterial suspension (for bioactive poultice) was added after mixing in the following manner - dry components: bacterial suspension =1:0.5. For the preparation of bioactive poultice the strain Pseudomonas stutzeri ATCC 17588 was used. The selected strain was stored in Nutrient Broth (HiMedia, Mumbai, India), with the addition of glycerol (Lach-ner, Neratovice, Czech Republic) as a cryoprotectant and kept in the freezer for ultra-low temperatures (Snijders Lab, Tilburg, The Netherlands) at -80 °C. The strain was grown overnight at 37 °C in Nitrate Broth (DifcoTM, Becton, Dickinson and Company, Le Pont-de-Clair, France). After centrifugation (3 x 4200 rpm for 10 min), the obtained pellet was washed twice and suspended in sterile distilled water. The final cell concentration was adjusted to approx. 3x10⁸ CFU ml⁻¹ corresponding to McFarland No.1 standard.

After the application of the prepared bioactive poultice the denitrification process was monitored through only one face of the carbonate stone substrate. The other five were completely covered with silicon, to ensure unidirectional diffusion. The freshly prepared poultices were applied on the surface of contaminated models (1 cm thick layer). Denitrification process was monitored after 24 h, 48 h and 6 days. In the case of the samples that were used for monitoring of the denitrification process for 6 days a new fresh layer of poultice was applied after 48 h (two-stage application). The poultice was removed from the surface prior to nitrate content determination. In order to determine the nitrate content in the bulk, the stone samples were powdered. The nitrates from the powdered substrate were extracted with distilled water (1:1) and the nitrate content was determined by Quanofix® test stripes (Macherey-Nagel, Düren, Germany).

2.3. Protection and examination
The used protective coating was developed under the FP7 HEROMAT project and its self-cleaning properties had already been proven on different mineral substrates (brick, mortar, render, sandstone…) in laboratory, as well as in real environmental conditions [11]. The synthesized suspension is based on the layered double hydroxides (LDH) and photocatalytically active TiO₂. The LDH structure was selected in order to prevent the aggregation of the TiO2 nanoparticles and to provide adequate porosity of the deposited coatings. Another reason for its selection was the compatibility with the chosen mineral substrates: mortars, renders, bricks.
Four types of stone substrate samples were investigated: 1) B - blank sample (non-contaminated and non-protected); 2) NC+P - non-contaminated and protected sample, 3) C+P – contaminated and protected; 4) C+CL+P - contaminated, cleaned and protected. All sample groups were prepared for the colourimetric tests using the following procedure: non-polished side of the stone substrate sample (dimensions 5 cm x 5 cm) was covered with 1.5 ml of the protective suspension, evenly distributed over the whole surface using a brush. Protected samples were then dried in a laboratory dryer for 30 min at 105°C. After that, the protective suspension was again applied on the same side of the samples and dried using the same methodology as described before. Dried samples were then left to cool down to room temperature.

2.3.1. Aesthetic appearance test. The influence of the photocatalytic coating on the visual appearance of stone substrates was examined by measuring the colour change. All samples were recorded before and after the application of the coating with Konica-Minolta CM700-d spectrophotometer (2 cm diameter spot size) in CIE LAB 76 colour space. Total colour differences (ΔE) before and after the application of the coating was calculated as follows:

\[ \Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \]

where \( \Delta L^* \), \( \Delta a^* \) and \( \Delta b^* \) are the differences in the respective values of colourimetric coordinates before and after the application of the coating.

2.3.2. Contact angle measurements. Contact angle measurements between the experimental fluid (glycerol) and the coated surfaces of the investigated stone samples were performed by the Surface Energy Evaluation System (Advex Instruments). The liquid drops, of about 5 μl in volume, were gently deposited on the substrate surface using a micro syringe. The measurements of the initial contact angle (\( \theta_0 \), after 1s) were performed at five different points of the examined samples. The examined samples were the same ones used for photocatalytic activity measurements.

2.3.3. Rhodamine B colorimetric test. In order to examine the photocatalytic activity of the coated stone samples, a modified Rhodamine B colourimetric test was performed [13]. Rhodamine B is a fluorone dye that exhibits a characteristic red-pink colour and is commonly used as a test dye for photocatalytic activity. The chemical structure of this dye is related to polycyclic aromatic hydrocarbons, which are often found among soiling agents in real urban conditions.

The 100 μmol/L Rhodamine B water solution was applied to previously prepared dried samples–1.5 ml of solution (per sample) was uniformly distributed with a brush on the sample surface covered with the protective suspension. The samples were then left to dry in the dark at room temperature for 24 h.

The dyed samples were put in a chamber with a UV-light source (2 Sanyo Denki 20 W UV-lamps, 368 nm) located 20 cm above the samples. The UV-A light intensity absorbed by the samples was 0.80 mW/cm². The samples were irradiated during 24 h, and their colour was measured using Konica-Minolta CM700-d spectrophotometer (0.6 cm diameter spot size) in CIE LAB 76 colour space. Even though CIE LAB 76 colour space uses 3 colourimetric coordinates (\( L^* \), \( a^* \)and \( b^* \)) to define colour, only coordinate \( a^* \) was considered, since Rhodamine B solution dyed the sample surface reddish-pink. The measurements of \( a^* \) coordinated were averaged over the entire sample surface and were performed after irradiation periods of 30 min, 1.5 h, 2.5 h, 3.5 h, 4.5 h and 24 h. The photocatalytic activity (A) was calculated using the following equation (1):

\[ A = \frac{a_0^* - a_x^*}{a_0^*} \times 100\% \]  \hspace{1cm} (1)

where \( a_0^* \) is coordinate \( a^* \) that corresponds to the substrate before UV irradiation (0 hours), and \( a_x^* \) is coordinate \( a^* \) that corresponds to the substrate after \( x \) hours of UV-irradiation.
3. Results and discussion

3.1. Cleaning: reduction of nitrate content in stone substrates (biotechnology approach)

3.1.1. Textural characteristics of the stone substrate and of the applied poultice. The total open porosity and the pore size distribution of poultice and contaminated stone sample measured by MIP are presented in figure 1 and table 1.

![Figure 1: Pore size distribution of the contaminated stone sample and the used poultice](image)

| Sample               | Total porosity (%) | Total cumulative pore volume (ml/g) |
|----------------------|--------------------|------------------------------------|
| Contaminated stone sample | 1.73               | 0.019                              |
| Poultice             | 47.79              | 0.347                              |

Table 1. Textural properties of poultice and contaminated stone sample

In the case of the advection mechanism of desalination, the dissolved salts are transported from the mineral substrate into the poultice by capillary forces during the drying process [14]. The saline solution travels from large pores of the mineral substrate into the fine pores of the poultice. Accordingly, the poultice must have a larger quantity of fine pores than the stone substrate, but for the wetting phase (the first phase of desalination) the poultice has to contain large pores.

Based on the results presented in figure 1 and table 1, the advection mechanism of desalination could be expected. Namely, the measured values of pore size distribution in the developed poultice are in very good correspondence with the pore size distribution of the contaminated stone samples, allowing denitrification by the advection mechanism, figure 1. The total porosity of the developed poultice is 40 times higher than the same value measured for the contaminated stone, thus a very good desalination capacity of the developed poultice could be expected, figure 2. Moreover, the developed poultice has a higher amount of both small and large pores compared with the contaminated stone samples. It also has a very well developed network of fine pores, which are necessary for the advection mechanism of denitrification.

3.1.2 Application of the designed bioactive poultice. Based on the obtained results, figure 2, it could be concluded that the application of the developed cleaning system led to the significant nitrate reduction in the contaminated stone samples (initial concentration was 500 mg/L). Namely, after one day of application, the nitrate concentration was 100 mg/L, which is 350 mg/L lower compared with the initial concentration. Moreover, after six days of the bioactive poultice application, the final nitrate concentration was only 50 mg/L. According to the obtained results, it may be assumed that the bioactive poultice presented in this work has a great potential in the decreasing procedure of nitrate content in stone materials, even up to 90%. The results have a good correlation with our previous research of the application of bioactive poultices for the removal of nitrate from brick samples [8].
The efficiency of the designed biocleaning has been proved in laboratory and already tested in situ on two case study objects in Serbia (Bač Fortress and calcite stone monument in urban area of Novi Sad). The long-term monitoring is being performed, while the possible side-effects of residual bacteria cultures within the stone pores should also be investigated.

3.2. Protection: nanotechnology approach

The total colour change of the investigated stone samples is given in table 2.

| Table 2. Total colour change of the protected stone samples (ΔE*) | Table 3. Contact angle values [°] |
|---|---|
| non-contaminated and protected sample | blank sample |
| NC+P | B | 101.00 |
| contaminated and protected sample | non-contaminated and protected sample |
| C+P | NC+P | 80.55 |
| contaminated, cleaned and protected sample | contaminated and protected sample |
| C+CL+P | C+P | 86.50 |
| | contaminated, cleaned and protected sample |
| | C+CL+P | 82.64 |

The results presented in table 2, show that there is no significant colour change for the stone samples after the application of the developed photocatalytic suspension. Namely, the values of ΔE* are very low (< 5), signifying that the TiO₂/ZnAl LDHs coating deposited onto surfaces of the stone samples does not affect their aesthetic appearance. This is a necessary criterion for the application of protective suspensions both in cultural heritage buildings and modern ones.

![Figure 2](image.png)

**Figure 2.** Nitrate concentration in contaminated stone substrates before and after cleaning procedure

*a new fresh layer of poultice was applied after 48 h (two-stage application)

| Figure 3. The contact angle measurements for samples a) B, b) NC+P, c) C+P and d) C+CL+P |

The results of the contact angle measurements, table 3 and figure 3, indicate that the application of the developed coating leads to a decrease of the contact angle. These results proved the hydrophilic effect of the developed material. Moreover, the identified decrease is evident in all the examined
cases. Nevertheless, the contact angle values are lower in the case of previously cleaned samples and converge to the non-contaminated and protected samples, the fact that speaks strongly in favour of the biocleaning method used. The results of the Rhodamine B colorimetric test are shown in table 4 and figure 4. In the first 3.5 h of irradiation no significant difference can be observed among the photocatalytic activities of the samples NC+P (34%), C+P (35%) and C+CL+P (39%). This result implies that the presence of the nitrate salts does not negatively influence the photocatalytic function of the coating. Additionally, the described cleaning procedure has no negative impact to the photocatalytic properties of the coating either.

Table 4. Results of photocatalytic activity measurements (Rhodamine B colorimetric test)

| Irradiation time (h) | B  | NC+P | C+P | C+CL+P |
|----------------------|----|------|-----|--------|
| 0.5                  | 0.5| -4.43| 21.36| 21.95  | 19.39  |
| 1.5                  | 1.5| -8.33| 28.00| 34.55  | 26.97  |
| 2.5                  | 2.5| -6.69| 33.68| 28.49  | 31.23  |
| 3.5                  | 3.5| -5.27| 34.10| 35.16  | 39.29  |
| 4.5                  | 4.5| -3.06| 36.70| 37.01  | 51.44  |
| 24                   | 24 | 4.85 | 42.35| 51.50  | 57.71  |

*blank (B); non-contaminated + protected (NC+P); contaminated + protected (C+P); contaminated + cleaned + protected (C+CL+P);

The photocatalytic activity results obtained after 24 h of UV-irradiation, figure 4, suggest that the photocatalytic function of the coating is more expressed on the cleaned and protected surface. Moreover, the high photocatalytic activity values for the protective coating were gained in the case of previously cleaned samples (C+CL+P: contaminated + cleaned + protected), which leads to the conclusion that there are no negative effect due to the application of the proposed procedures.

4. Conclusion

In this study, a two-step methodology for the cleaning of soluble salts and protection with photocatalytic coating is presented. The already confirmed methodology for the development and application of biocleaning poultices for the reduction of nitrate salts on the brick substrate is also confirmed on stone substrate. Moreover, the necessity to design the most appropriate bioactive poultice (a combination of the traditional poultice as a support for bacterial culture) is once more emphasized. Namely, the comparative study of the pore sizes and distribution of the contaminated stone substrates and bioactive poultice already promised the optimal conditions for advection mechanism of desalination and high desalination efficacy. The obtained results confirmed this hypothesis - after the application of bioactive poultice, the measured reduction of nitrate salts was very high. After one day of bioactive poultice application, the quantity of nitrates fell from 500 mg/L to 100 mg/L, while after six days of two-stage application, the nitrates content was only 50 mg/L, which approximately presents 90% efficiency of the applied cleaning (desalination) procedure.

Following the successful cleaning procedure (nitrate salts reduction), the protection of the stone substrates was performed with the photocatalytic material designed in the FP7 project HEROMAT. A TiO₂-layer double hydroxide (TiO₂-LDH) suspension was applied onto the surface of stone samples. In order to examine the coupling effect of the performed biocleaning and protective treatments, the contact angle measurements and photocatalytic activity test were performed. The results indicate good photocatalytic activity and hydrophilicity of the examined samples, emphasizing better values of photocatalytic activity in the case when the protective suspension was applied on previously contaminated and cleaned samples (C+CL+P).
The combination of this newly designed desalination method based on bio-cleaning and the application of developed protective coating could replace a group of traditionally used procedures in the maintenance of built cultural heritage. The proposed innovative techniques (bulk desalination and surface protection based on self-cleaning phenomenon) could have highly positive environmental and economic impacts: the salt crystallization process and damaging microbiological growth will slow down. As a consequence, the time between the two interventions will increase leading to the reduction of long-term maintenance costs. The outcomes of this study could be tested on other mineral substrates further increasing the value of the obtained results and possibilities of their utilization in practice.

Acknowledgments
The authors acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia within the Project III 45008.

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