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Deep Eutectic Solvents (DESs): first results obtained from their application as biocides in the field of Cultural Heritage

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***Abstract* – In the context of the conservation of cultural heritage, a problem to be solved is the biological degradation of monuments. The biocidal agents used for cleaning biodegraded surfaces are few and must have adequate requirements. One of the main challenges is the development of eco-friendly products for the conservation and restoration of cultural heritage. Deep Eutectic Solvents (DESs) are a class of green solvents, beneficial and safe that could be used as biocides in cleaning and preserving cultural heritage. In this contribution, five different DES were applied for the first time as biocides on a mosaic in the Archaeological Park of Ostia Antica (Rome) and their biocidal action was evaluated by luminescence, bioluminescence and spectrocolorimetry analyses. The first results obtained from this study are very promising for the use of DESs as a new green strategy for the treatment of biodegraded surfaces in the field of cultural heritage.**

I. INTRODUCTION

The problem of biological attack is increasingly widespread in the conservation of cultural heritage and is due to the growth and accumulation of macro- and microorganisms, such as bacteria, algae, fungi, lichens, and mosses which form complex and heterogeneous biostructures of patinas biological, called biofilms [1]. In this way, interactions are triggered between biofilm, substrate, and external environment, which lead to the main processes of biodeterioration of the material with cultural interest, causing an aesthetic alteration. Chemical cleaning methods are used to remove or reduce the biofilms that degrade works of art over time.

In the field of cultural heritage, the cleaning products used are not numerous as they must have specific requirements, such as being effectiveness at low concentrations, not to interfere with the material of the work and to be safe for human and the environment. Among the main products used as biocidal agents in this field, there are quaternary ammonium salts (QASs), such as Preventol RI50. QASs exert their biocidal activity by destabilizing the cell membrane and causing cell lysis. Although they have a good biocide action, they are dangerous for the operator and for the environment [2].

Nowadays, the cultural heritage sector requires new biocide agents with a lower impact on the environment and on the operator. In this regard, DESs (Deep Eutectic Solvents) must be considered. They are a relatively new class of green solvents with many advantages, such as being non-toxic, biodegradable, eco-friendly, non-flammable, stable in the presence of water, low-cost preparation and solvent-free [3]. They are eutectic mixtures obtained by mixing hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) and in a stoichiometric ratio [4], which form a liquid at room temperature.

Some DESs are known in the literature for their antibacterial and antifungal action [5, 6]. It should be considered that the antimicrobial properties of DESs depend on various factors, although the mechanisms are not yet clear. The different biocidal response depends on the nature of the constituent components of DES. Some mixture of DESs are constituted by Choline Chloride, chemical species with a positive charge delocalized that interacts with the negative groups of cell membrane of the bacteria causing distortions e ruptures of the cell wall. On the other hand, antibacterial action is also due to their acidity or alkalinity (pH), because they cause the denaturation of the proteins present in the cell membrane.

Moreover, other studies have demonstrated the antifungal action of DESs against some species such as *Aspergillus Niger*, *Candida cylindracea*, and *Phanerochaete chrysosporium*, also present on the surfaces of cultural heritage.

Thanks to these characteristics, DESs can be judged as an alternative in the cultural heritage sector.

In this study, five specific DESs were used as green solvents with biocidal activity on a mosaic in the Ostia Antica Archaeological Park. To evaluate their biocidal capacity were analysed by luminescence, bioluminometry and spectrocolorimetry, comparing the effectiveness with the results obtained from the application of Preventol RI50.

II. MATERIALS AND METHODS

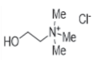
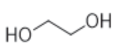
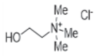
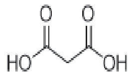
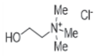
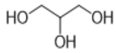
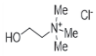
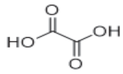
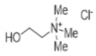
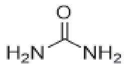
A. DESs preparation

The preparation of mixtures of DESs (DES 1: ChCl/EG, DES 2: ChCl/MalAc, DES 3: ChCl/Gly, DES 4: ChCl/OxAc, DES 5: ChCl/U) consists in weighing the HBA and HBD components at the right molar ratio, mixing them and heating them (80 °C - 100 °C) in times spanning from 1 to 3 hours, until homogeneous liquids are obtained. The chemical structures of the solvent components, their molar ratios and the pH value of each DES is shown in Table 1.

DESs are a eutectic mixture obtained starting from natural and cheap components, which occur in a liquid state at room temperature following the union of the solid components and the achievement of the eutectic point. The pH of the DES is due to the solvation water, present in very small amounts, which is closely anchored to the DES.

The DESs obtained were used pure in the present contribution.

Table 1. Composition and pH of DESs

DES code	HBA	HBD	Molar ratio	Ph
DES 1	 Choline Chloride	 Ethylene glycol	1:2	5.6 ± 0.5
DES 2	 Choline Chloride	 Malonic Acid	1:1	2.8 ± 0.5
DES 3	 Choline Chloride	 Glycerol	1:2	5.3 ± 0.5
DES 4	 Choline Chloride	 Oxalic Acid	1:1	3.2 ± 0.5
DES 5	 Choline Chloride	 Urea	1:2	7.2 ± 0.5

B. Experimental and analytical methods

The mixtures of DESs were applied on two areas of a mosaic located in the Archaeological Park of Ostia Antica (Area 1, Area 2). In each area, in the Fig. 1 are indicate the application of Preventol RI50 (red box), the application of DESs (green box, respectively from 1 to 5) and one untreated area and used as a reference (yellow box).

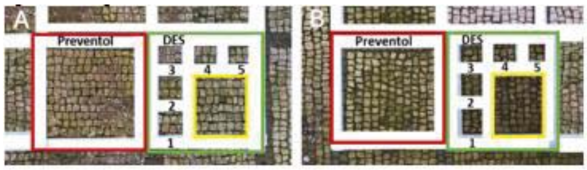


*Fig. 1. Application DESs in two areas of the mosaic in the Archeological Park of Ostia Antica: Area 1 (**A**) and Area 2 (**B**).*

The products were applied by brush, creating an even layer. They were left to act for 3 days, and then they were removed with brushing and washing.

A bioluminometer (KAIROSafe PD30) was used to determine the biological presence on the surfaces treated with products, before and after the treatment [7]. For this purpose, was used the wipe method, repeating the test three times that allowed subsequent statistical analysis. Bioluminescence consists of ability of living organisms to emit light through enzymatic reactions, converting chemical energy into light energy. The amount of light emitted will be directly proportional to the amount of ATP present in the living organisms, expressed by the Relative Light Units value (RLU). So, low RLU values should indicate less organisms present on the surface capable of producing ATP because they have been damaged or killed by biocidal products used.

Ultraviolet fluorescence was used by means of a Madatec multispectral imaging system, to capture the visible light emitted by the microorganisms before and after the application of the studied biocides.

The evaluation of the chromatic variation was observed using a spectrocolorimeter (Y3060 3nh) in the CIE L* a* b* space. Then, chromatic alterations were analysed by studying the ΔE, defined by the equation 1

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Chromatic variation allows to monitor bacterial growth on the surface under examination (negative ΔL* and Δa* and positive Δb* values indicate a bacterial recolonization process, while for ΔL* and Δa* values positive and negative Δb* values indicate a bacterial decolonization process) [8]. The measurements were repeated three times to reduce the uncertainty of the analysis.

The interaction between DES and the substrate (mosaic tesserae) was studied in laboratory through electrical conductivity measurements using a material with low porosity, as marble sample and the most soluble DES 5: Choline Chloride/Urea. Soluble salts are the main degradation problem in porous stone materials, due to the formation of efflorescence. The electrical conductivity allows to evaluate the capillary absorption of the chlorine ions present in the DES by the porosity of the marble.

A HD2156.2 - Delta OHM was used for analysis. 700 mg of DES was applied to the marble sample with a steel spatula. The marble sample was put in an Angelantoni climate chamber for 24 hours at different temperatures: 20, 40, 60 ° C and 70% U.R. These parameters were defined based on the microclimatic conditions of Ostia Antica during the experimentation: 24 ± 2 ° C and 61 ± 5% RH%.

After 24 hours the DES was removed from the marble sample. The marble sample was left in 400 ml of deionized water (EC=7±2 µS/cm) for 3 hours. The electrical conductivity was measured and refereed to the concentration of totally dissolved DES in 400ml of deionized water (750±5.6 µS/cm). The test was repeated three times to reduce the uncertainty of the analysis.

III. RESULTS AND DISCUSSION

From ultraviolet fluorescence analysis is possible to know the presence and the distribution of the microorganisms on the surface in the examination. Before treatment, a red fluorescence is observed, which is faded after the application of the products, following the devitalization of the photosynthetic microorganisms (Fig. 2).

In Area 1 a good biocidal response is observed with DES 5, 4 and 2, followed by DES 3 and 1; in Area 2 with DES 4, 2 and 5, followed by DES 3 and 1. DES 2 and 4 have a more acidic pH, therefore they have a greater biocidal capacity.

Despite the good performance obtained, DESs do not reach the standards obtained by using Preventol RI50.

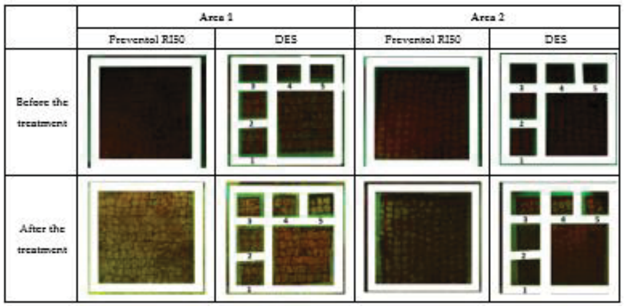


Fig. 2. Ultraviolet fluorescence imaging of Areas 1 and 2, before and after treatment.

The values of RLU and standard deviation (SD) determinated with bioluminometer are reported in Fig. 3 indicting the average and the SD of the different areas. The low values reported indicating a lower production of ATP due to the death or damage of the cells microorganisms, indicating their good biocidal action.

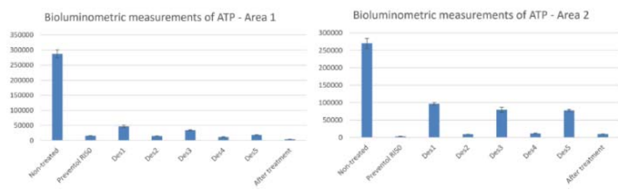


Fig. 3. Bioluminometric measurements of ATP of the areas of interest.

The best results were obtained for Area 1 and Area 2 applying DES 2, DES 4 and Preventol RI50.

Table 2 shows the variations of the colorimetric values (L*, a*, b*) after the application and removal of the biocides in the two areas.

Table 2. Colorimetric parameters of Area 1 and Area 2

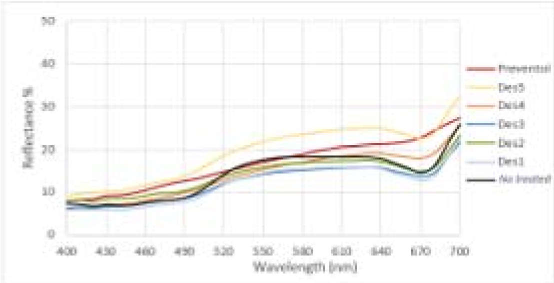
Area 1				
Biocide	ΔL*	Δa*	Δb*	ΔE
Preventol RI50	-3.96	2.05	0.15	4.46
DES 1	0.15	-1.13	-9.63	9.70
DES 2	6.78	-1.97	-7.52	10.3
DES 3	5.03	-0.67	-1.81	5.39
DES 4	9.37	-1.52	-6.75	11.6
DES 5	1.69	-0.34	-1.73	2.44

Area 2				
Biocide	ΔL*	Δa*	Δb*	ΔE
Preventol RI50	0.18	2.78	-6.17	6.77
DES 1	-4.01	0.96	-2.64	4.89

DES 2	-0.29	0.55	-6.96	6.99
DES 3	-2.89	0.88	-4.93	5.78
DES 4	-0.63	2.85	-3.86	4.84
DES 5	6.62	1.40	-2.17	7.10

The average colorimetric values of the parameters L*, a*, b* before treatment in Area 1 correspond to L* 41.01; a* 2.56; b* 19.87; in Area 2 correspond to L* 45.87; a* 0.78; b* 23.61. For Area 1, positive ΔL* values and negative Δa* values are observed for each area treated by the DES, while for Preventol Rl50 there is a negative ΔL* value and Δa* positive. As regards Δb*, there are negative values, except for Preventol Rl50. This indicates that the areas treated with DESs show a greening (Δa* negative), although not perceptible as the decrease in terms of absolute value is minimal. The increase in brightness (ΔL* positive) is due to the removal of the biological patina, which allows the colour of the mosaic tiles to re-emerge. In Area 2 shows negative ΔL* values for most DESs, except for DES5 and Preventol Rl50. Positive and negative Δa* and Δb* values are observed for each treated area, indicating less greening and yellowing. Therefore, the best results are obtained by DES 2 and 4 for Area 1 and DES 5 for Area 2.

The full spectrum colour measurements of Area 1 and Area 2 with the respectively untreated areas are shown in Fig. 4. The absorption of chlorophyll is observed, indicated around 670 nm induced by ultraviolet (UV) light at 365 nm, for all the biocidal products used. But there is a greater decrease of the absorption of chlorophyll in Area 1 and Area 2 where the Preventol RI50 has been employed, and to follow in the areas treated with DES 2 and 5. There is a reduction of chlorophyll, and therefore of the green colour, because there is a reduction in the number of living photosynthetic microorganisms.



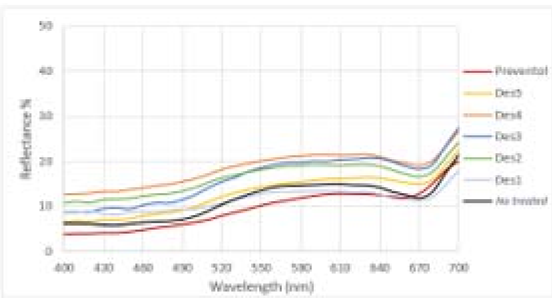


Fig. 4. Full spectrum colour measurements of Area 1(above) and Area 2 (below).

Electrical conductivity measurements, showed in Table 3, allowed to define the solvent's interference with the material sample using the ratio between the EC measured on the DES residue present in sample and the EC of the totally dissolved DES in water and the relative error (expressed in %).

Table 3. Electrical conductivity measurements (EC)

Applied DES (mg)	Temperature (C°)	DES in sample (mg)	EC (µS/cm)	DES in water (%)
523±5	60	304±2	414±6.4	55%
512±7	40	55±2	73±5.7	9%
520±4	25	34±5	41±5.3	6%

A low capillary absorption of ions chlorine by the substrate there is in the same microclimatic conditions of the experimentation. The absorption of DES by the marble sample increases as a function of the temperature due to the deliquescence of the salts used in the DES.

IV. CONCLUSIONS

In this work, for the first time, five DESs with different compositions based on choline chloride with ethylene glycol, malonic acid, glycerol, oxalic acid and urea, were tested pure, without dilution with water or other solvents, as biocides in the field of cultural heritage. The results obtained have been compared with the results obtained with the traditional biocide in water solution Preventol RI50. They were applied in-situ on a surface biodegraded of mosaic in the Ostia Antica Archaeological Park and after application the biological patina were removed by mechanical action. To evaluate the biocidal action of the products various analytical investigations were used: ULV imaging, spectrocolorimetry, and bioluminometry. The best results among the DESs tested were obtained by DES 2, 4 and 5, but their biocidal action is lower than that of the traditional Preventol Rl50.

Acid-based DESs (malonic acid = DES 2; oxalic acid = DES 4) have a higher biocidal action than DES 5 (Choline Chloride/Urea) showing good efficacy at a more basic pH (7.2 ± 0.5).

The use of DESs compared to the traditional Preventol RI50 biocide commonly used in the field of cultural heritage, is convenient because they are stable, non-volatile, eco-friendly and are obtained starting from two natural components, not harmful to the operator who applies the product on the stone artefact and for the environment, and do not require solvents for the dilution.

The use of these green solvents in the conservation of cultural heritage is a new horizon. Following their recent introduction in the field of restoration, their effectiveness over time has not yet been clarified, but the preliminary results obtained from this first step of research are very promising in their possible use in the cleaning and conservation of cultural heritage, both as solvents and as biocides. For the continuation of the experimentation of DESs in the restoration sector, further studies of the properties of DES, the combination, modulation, and variation of the molar ratios of the components and the water content, are foreseen, in order to make them more effective and easier to apply on different types of materials.

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