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Development of controlled release systems of biocides for the conservation of cultural heritage

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ABSTRACT

The presence of microorganisms is one of the main causes of monument deterioration. Biocides are usually applied after or before restoration in order to prevent or slow down microbial growth. Frequent applications are necessary leading to increased costs and high risks to humans and the environment. The aim of this study is the design of novel controlled release systems comprising a biocide loaded into a mesoporous silica. Pristine MCM41 as well as MCM41 functionalised with carboxy- (MCM41-COOH) and amino-groups (MCM41-NH2) were used. Biotin T and New Des 50, two commercial formulations, were chosen as biocides. The biocide encapsulation was performed adding the mesoporous silica to an aqueous biocide solution. The release of the biocides in water was investigated by UV-Visible Spectroscopy. A further characterisation of the systems was performed to evaluate their structure and features and to speculate about the mechanisms involved in the release. The effect of both surface modifications on the release of biocides, as well as the biological activity of the systems were tested. The high-performing system was applied on a stone specimen from a fountain in Diamantina (Minas-Gerais, Brazil) to assess the effect on the microbial community up to 12 months.

1. Introduction

Biodeterioration caused by microorganisms (bacteria, archaea and fungi) is one of the main causes of deterioration of archaeological material, monuments and artwork, especially in humid environments (Randazzo et al., 2015). Before a restoration intervention is started, several types of bioremediation treatments, based on various biocides, can be applied to a range of materials in order to eliminate the presence of microorganisms and to prevent their recurrence for short periods of time (Fernandes, 2006; Sterflinger and Pinar, 2013). These methods however, usually work only for limited periods of time, as they can be washed away or they may take part in undesired reactions favoured by humidity. Therefore, treatments with biocides often require frequent applications, resulting in high costs for public administrations, museums and the private sector. In addition, since common biocides carry a risk for humans and the environment, their regular use poses a hazard to the conservators, the environment and eventually even the artwork. Thus, a sensible reduction of the amounts of biocides and the frequency of application would be desirable.

Mesoporous silica-based materials have shown a powerful potential in terms of controlled release of adsorbed compounds in the field of biomedicine. MCM41 shows a large specific pore volume made up of hexagonally ordered regular pores, with a diameter in the nanometer range, which facilitate the controlled delivery of biocides and several types of molecules (Wanyika, 2013; Popat et al., 2012; Koneru et al., 2015; Timin and Rumyantsev, 2015; Zheng et al., 2013). The interface properties can be modified by introducing functional groups which alter the physical and chemical interactions between the pore surface and the embedded molecules. Certain functional groups could improve the retaining of the biocides within the pore for a longer time and favour the controlled release of the biocides in a specific medium. The aim of this study is the design of a system comprising a mesoporous material that releases a biocide in an aqueous medium slowly and

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in a controlled manner and to test the system's performance for up to 12 months on a contaminated stone derived from a fountain in Diamantina (Minas-Gerais, Brazil). Said system could be applied by restorers during the final step of the restoration process to reduce the quantity of biocides and the frequency of their application in order to diminish the aforementioned risks.

2. Materials and methods

Two commercial biocides used in restoration activities are New Des 50 and Biotin T. New Des 50 is the commercial name of a water solution of didecyldimethylammonium chloride, DDAC, a translucent, colourless quaternary ammonium salt surfactant. DDAC is a broadly used molecule for disinfection of medical equipment, facilities, water systems, food handling and storage facilities and for wood preservation (Borokhov and Rothenburger, 2000). Its antibacterial activity is based on its property of disrupting cell membranes (Yoshimatsu and Hiyama, 2007). Biotin T is an aqueous solution of DDAC and 2-octyl-2H-isothiazole (OIT). OIT is used as anti-fouling agent against bacteria, fungi and algae in paints, in products for wood-preservation and stones(Borgioli et al. 2006. Mateus et al., 2013). The molecular structure of DDAC and OIT is reported in Fig. S1 of the Support Information (S.I.). Biotin T and New Des 50 (50%) were supplied by C.T.S. s.r.l. (Altavilla Vicentina (VI), Italy). No information about the purity of the formulation has been provided. Aqueous solutions were prepared by weight, using conductivity grade water. MCM41 mesoporous silica and MCM41 functionalized with carboxyl- (MCM41-COOH) or amino-groups (MCM41-NH_2) were selected as support. The synthesis of the MCM41 mesoporous silica is reported elsewhere (Caponetti et al., 2010).

It has been functionalised with amino or carboxylic groups (MCM41-NH_2 and MCM41-COOH) following the methods reported elsewhere (Arean et al., 2013; Ho et al., 2003). The properties of used MCM41 are: specific surface area $S_{BET} = 931\ m^2/g$, mean pore width $w_{BJH} = 24$ Å, wall thickness $t = 18$ Å. The structure of the two functionalized MCM41 with functionalization degree of 63% is analogues to the ones of MCM41 (Saladino et al., 2016). The efficiency of the systems were assessed by evaluating the biocide antibacterial activity by means of disc diffusion antibiotic sensitivity assays. The antimicrobial activity was determined against *Kokuria rhizophila* ATCC® 9341™ *(K. rhizophila*) and *Staphylococcus aureus* ATCC® 25923™ *(S. aureus)* as Gram-positive and *Escherichia coli* DH10B (Invitrogen, *E. coli*) as Gram-negative bacteria using a modified disc diffusion antibiotic sensitivity assays as described in literature (Giardina et al., 2010; Lo Grasso et al., 2015; Rubino et al., 2017). Bacteria were prepared as fresh bacterial suspension and stored as frozen cell glycerol stocks as described elsewhere (D'Andrea et al., 2012). Briefly, a dense suspension (~10^7 cells) of each microorganism was spread onto a plate containing Luria Bertani agar (LB-agar; 10 g/L Tryptone, 5 g/L yeast extract, 10 g/L NaCl, 18 g/L Bacto agar, pH 7–7.2). LB-agar is a rich generalist medium for microbial growth. Aqueous suspension aliquots containing different amounts of MCM41 and biocides as references were directly spotted on sterile paper discs with a diameter of 6 mm that were placed on the overlay of bacteria on LB-agar plate. Growth inhibition halos were observed after overnight incubation at 37 °C. H_2O (0.1 mL) was used as negative control for each antibacterial assay. The antimicrobial activity was calculated as a mean of three replicates. To evaluate the Minimal Inhibitory Concentration (MIC) of Biotin T and New Des 50, 10 μl of serially diluted biocide solutions were applied on sterile paper discs. *Viable bacterial count.* For total viable bacterial count, samples of 1 g of untreated and treated stone were weighted, directly put and strongly vortexed in 10 mL of LB broth in order to release bacteria. Six serial dilutions of this bacterial suspension were prepared using LB-broth and 100 μl of each bacterial suspension of each dilution was spread on LB-agar to allow bacterial growth and bacterial counting. Plates were incubated at 37 °C until colonies appeared. Numbers of total viable bacteria are given per g of stone. The experiment was performed in triplicate, the mean values of bacteria were calculated and error bars were evaluated.

The release in water of the two biocides from the mesoporous material was investigated by means of UV-Vis spectroscopy. A detailed characterization of the systems was carried out by using X-ray Diffraction (XRD), Gas adsorption and Infrared Spectroscopy. *UV–vis spectra* were recorded in the range 200–500 nm using a double beam Beckman DU-800 spectrophotometer with a resolution of 1.0 nm. In order to eliminate the effect of instrumental errors and of particle diffusion, the value of absorbance at 500 nm was subtracted to each spectrum. *N_2 absorption-desorption isotherms* were registered at 77 K using a Quantachrome Nova 2200 Multi-Station High Speed Gas Sorption Analyser. Samples were outgassed for 12 h at room temperature in the degas station. Adsorbed nitrogen volumes were normalized to the standard temperature and pressure. The specific surface area (S_{BET}) was calculated according to the standard BET method in the relative absorption pressure (P/P_0) range from 0.045 to 0.250 (Brunauer et al., 1938). The total pore volume (V_t) was obtained from the nitrogen amount adsorbed in correspondence of P/P_0 equal to 0.99. *X-ray Diffraction (XRD)* patterns were recorded with a step of 0.05° and counting time of 5s/step on a PW 1050 Philips diffractometer in the Bragg-Brentano geometry equipped with a Cu Coolidge tube and a scintillation detector beam. The X-ray generator worked at 40 kV and 30 mA; the instrument resolution (divergent and antiscatter slits of 0.5°) was determined using standards free from the effect of reduced crystallite size and lattice defects. The *ATR spectra* were recorded in the 400-4000 cm^{-1} range, with a step of 2 cm^{-1}, by using a Bruker Vertex 70 Advanced Research Fourier Transform Infrared Spectrometer equipped with Platinum ATR. The measurements have been performed at 2 h Pa. Data have been corrected with a scattering-type baseline.

The *critical micelle concentration (cmc)* was obtained from the plots of the specific conductivity (k) as a function of the biocide concentration. k was acquired by using a digital Amel 160 conductimeter (cell constant 0.998 cm^{-1}). The *cmc* values were taken from the intersection of the two straight lines, linear regression, in the pre- and post-micelle k vs. concentration ($\%_{v/v}$) regimes. The measurements were performed in a temperature-controlled double-walled glass cylinder with circulation of water connected to a thermostat at 25.0 ± 0.1 °C. The aqueous solutions at the desired concentration were obtained by adding appropriate aliquots of a stock solution to 15 mL of pure water. Each solution was stirred for 5 min to allow the system to equilibrate and then the conductivity was measured.

2.1. Loading procedure

The loading of the biocides was carried out modifying the procedure reported elsewhere (Saladino et al., 2016). 1 mg/mL of the mesoporous powder was immersed in aqueous solutions of the biocides (0.75, 1.5 and 3.0 $\%_{v/v}$) for 24 h under continuous magnetic stirring. The suspension was centrifuged at appropriate RCF for separation of the powder. The supernatant was thus carefully removed and the loaded samples dried under vacuum overnight. The loaded functionalized powders look similar to the pristine loaded MCM41 but resulted highly dispersible in water. The yield of loading resulted to be 99% for MCM41, 75–95% for MCM41-NH_2 and MCM41-COOH.