

ANTI-FOULING ADDITIVES FOR THE CONSOLIDATION OF ARCHAEOLOGICAL MORTARS IN UNDERWATER ENVIRONMENT: EFFICACY TESTS PERFORMED ON THE APSIDAL FISHPOND OF CASTRUM NOVUM (ROME, ITALY)

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Abstract

This paper deals with the formulation of innovative mortars for the consolidation of archaeological structures in underwater environment. The research was conducted within the frame of the MaTaCoS project (Advanced materials and technologies applied to the conservation of underwater cultural heritage) funded by MISE (Italian Ministry of Economic Development). The project concerns the design of advanced tools and methods for the protection of Underwater Cultural Heritage, through specific cleaning and consolidating procedures to be applied directly in situ. The fishpond of the archaeological site of Castrum Novum (Santa Marinella, Rome, Italy) was chosen as a pilot site for experimentation. It is a structure on the coastline, composed of only one tank, with an average immersion of 0.37 m below the sea level, and developing with an NE/SW orientation. The masonry structures reach the maximum thickness at the apex of the fishpond (4.70 m) and consist of a concrete conglomerate composed of slightly rough stones of medium size bound with non-hydraulic mortar. Selected mortars' fragments taken from the fishpond were characterized by means of minero-petrographic technique in order to define their main constituents from a compositional and textural point of view and also to identify the various degradation processes, mainly biological colonization. Starting from the acquired information, innovative mortars were formulated with the addition of two different anti-fouling additives aimed to slow down the above-mentioned degradation. The efficacy of the anti-fouling products was tested on specimens settled in laboratory environment (artificial marine environment). This information could be useful both for the definition of restoration interventions and for planning preservation protocols to be applied in this peculiar environment.

Keywords: Underwater Cultural Heritage; Natural Hydraulic Lime; Anti-fouling agents; SEM-EDS; Biocidal efficacy

Introduction

Marine aggression is a consequence of many factors (physical, chemical and biological damage) that contribute to decay of the submerged materials [1-4]. The principal issue of solid surfaces immersed in seawater is the shallow colonization/corrosion due to marine biofouling. Biofouling is a natural process occurring on submerged materials from colonization and

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overgrowth of epibiotic organisms and represents a major economic problem in both archaeological sites and maritime industries. The first events in biofouling formation are the deposition of a multilayer of organic matter (biofilm) followed by colonization with specific bacterial species (microfouling) [5-7].

This primary microbial film can prepare the surface for subsequent colonization by extracellular polymeric substances production, which are responsible for the adhesion and growth of new microorganisms, plants, algae, and sessile organisms [8-9].

Until a few years ago, the strategy to counter the harmful effect of biofouling on submerged materials was the use of antifouling paint containing biocidal agents that induce general toxic responses in the marine environment associated with heavy metal toxicity and antibiotic toxicity [10].

At present, the development of environmentally safe anti-fouling agent is of remarkable interest, due to the adverse effects of traditional biocides on the marine environment [11-12].

This study was conducted within the frame of the MaTaCoS project (Advanced materials and technologies applied to the conservation of underwater cultural heritage) funded by MISE (Italian Ministry of Economic Development). The project concerns the design of advanced tools and methods for the protection of Underwater Cultural Heritage, through specific cleaning and consolidating procedures to be applied directly in situ. Innovative mortars were specifically formulated in order to be compatible with the ancient mortars constituting the fishpond structures and the anti-fouling effect of two compounds (SiQ and an extract using three between spices and herbs, hereafter B1 and B2) applied by brushing on mortars specimen surfaces, was evaluated after three months of settling in submerged artificial marine environment.

Experimental

Preparation of mortars experimental briquettes

In accordance with what was analytically found by analyzing mortars' fragments taken from the fishpond, that is to say the typical "recipe" used in Roman times to manufacture hydraulic mortars by adding "pozzolana" [13], the experimental mortar briquettes were prepared using two types of commercial natural hydraulic lime (Volteco Microlime Gel and St Astier NHL 3.5) and pozzolana as sandy aggregate, with a binder/aggregate ratio of 1:2 (mainly fine to medium sand sizes between 0.5 and 0.125 mm).

A natural product (Chitosan ®), known for the anti washout and antimicrobial properties, was also added to the formulations [14].

In order to produce a homogeneous mixture, an appropriate amount of deionized water was gradually added to dry sample powder, following the indications reported in the technical sheet (0.88 ml*weight of the binder, in the case of NHL 3.5 and 30% weight of the binder in the case of Volteco Microlime Gel.).

The experimental briquettes have been shaped and sized 10x5x0.8 cm in specifically designed wood frames (Fig. 1). Before the application of the biocide additives, a curing time of 28 days was achieved.

Anti-fouling additives features

SiQ (B1) is a hybrid organic-inorganic compound carried out to remain biologically active when bonded to inorganic surfaces. It has a non-leaching behavior due to its reactive surface, which allows the regulation of surface microbial contamination without the continuous release of toxic components into the environment, which can favour the formation of resistant organisms.

From the mid-1960's, several researchers noticed that antimicrobial organofunctional silanes could be chemically bound to responsive substrates from what was believed a Si-O linkage. The method has been described as orienting the organofunctional silane in such a way

that the hydrolyzable groups on the silicon atom were hydrolyzed into silanol. After that the silanols formed chemical bonds with each other and the substrate [15-17].

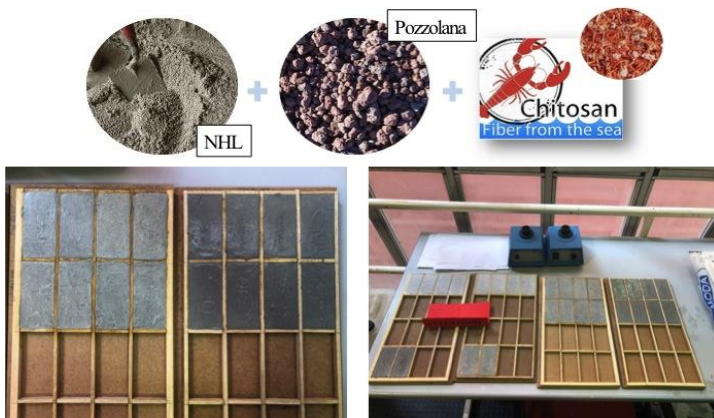


Fig. 1. Mix design of the innovative mortars and wood mould used to realize the experimental briquettes.

The modification of the resulting surface, when an antimicrobial portion such as quaternary nitrogen was included, provided that the antimicrobial was oriented away from the surface.

It has been shown that this covalently linked siloxane polymer produces broad-spectrum antimicrobial activity while providing a strong water-repellent characteristic directly on the surface of the treated product. Further studies have suggested that the use of a 3-trimethoxysilyl propyldimethyl octadecyl ammonium chloride (Si-Quat), an immobilized antimicrobial agent, can also eliminate some costs, difficulty, loss of lifestyle and additional worries imposed by the traditional solutions of monitoring microbial infestation.

The surfaces were then transformed into active control devices by the antimicrobial agent, destroying the organism on contact and also providing a water repellent barrier (Fig. 2).

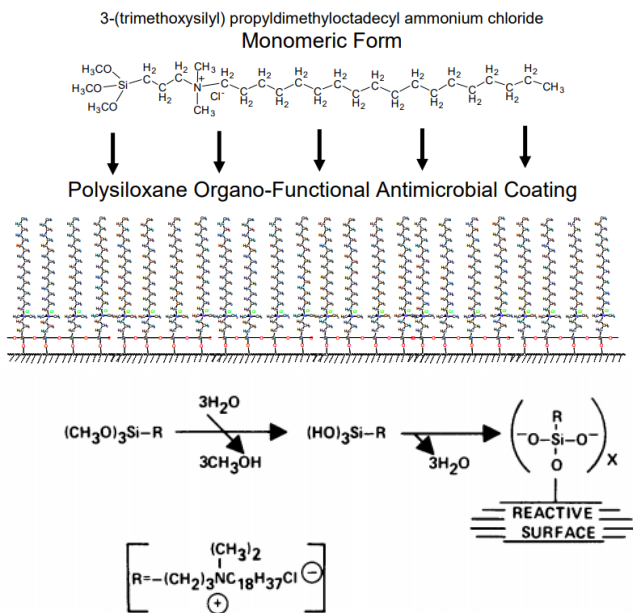


Fig. 2. Covalently bound siloxane network that is formed starting from the silane-Quat monomer

In this paper the SiQ were prepared as ethanol solution containing 30% solids (w/v) of form 3-tri-(2-hydroxyethoxy) silylpropyldimethylocta decyl ammonium chloride (synthesized as described in Patent US20080026156A1 United States) and TEOS (produced by CTS).

Biocide 2 (B2) is an extract using three between spices and herbs. Antimicrobial properties of spices have been documented in ancient literature and the interest continues to the present [18-21]. In the present study, we carried out an extract using three between spices and herbs very known for their antimicrobial effects (Table 1): chili peper (spice), Thyme (herb), oregano (herb).

Table 1. Composition of Biocide 2

Common name	Scientific Name	Components present	% in Extract
Thyme	Thymus vulgaris	Thymol	2-4
Oregano	Origanum Vulgare	Carvacrol	2-4
Cayenne pepper	Capsicum annuum	Capsaicin (8-methyl-N-vanillyl-6-nonenamide)	4-6

The leaves (in total 10 g) of the various herbs were overnight soaked in 100 ml alcoholic/water =30/70 solution + 5NaOH. The sample was then centrifuged (5000 rpm for 10 minutes) and the supernatant obtained was used as an extract.

Both additives have been applied on mortar specimens (10x5x0.8 cm) by brushing until they were getting saturation. In all steps of the laboratory experimentation, untreated samples were used to make a comparison with treated specimens.

Methods

A rectangular glass tank of 100 L capacity (100 cm long, 45 cm deep, 45 cm wide) has been filled with 80L of natural seawater taken at a depth of about 2 meters and filtered to remove large metazoans and detritus. A sandy substrate inside the aquarium was collected from a clean site nearby the same area.

The aquarium was left to stabilize for about a month with constant monitoring of chemical-physical parameters via a multiparametric probe (YSI 556 MPS). After stabilization period, to simulate the submarine degradation conditions, mortars samples in multiples of three, both treated with biocides and untreated (control groups), were immersed in the aquarium for about 3 months (Fig. 3).

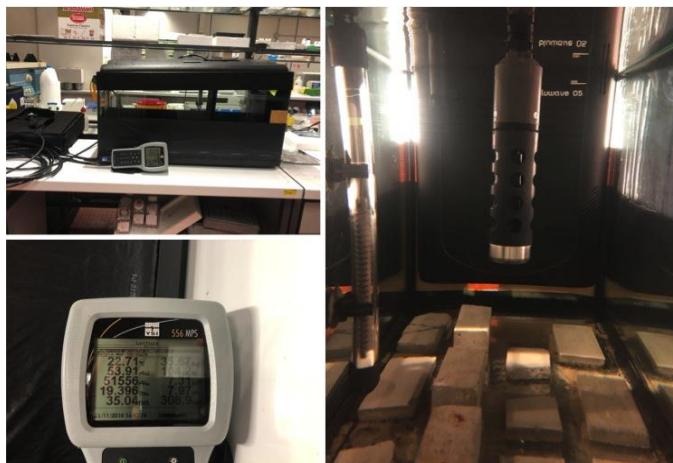


Fig. 3. Arrangement of experimental specimens inside the aquarium and monitoring system of seawater with the multiparametric probe

During this time, physical and chemical parameters (temperature, pH, oxygen level, salinity, resistivity, etc) were constantly monitored at regular intervals (weekly).

After 3 months, mortars samples have been “scraped” with sterile little pests (using both non-invasive or minimally invasive sampling methods for substrates and non-destructive for the biofilm) and then crawled on solid culture medium (agar) or immersed in liquid culture medium (broth). Bacterial cultures were incubated for a maximum of 72 hours at 23 °C (to simulate the aquarium temperature).

The bacterial growth (agar) was constantly monitored by optical microscopy observation (data not shown). On the culture broths two microbiological parameters were detected: the pH value and the turbidity measured respectively by using a pH-meter and a spectrophotometer. At the end of the 72h, broth aliquots were centrifuged at 2000 g for 5 minutes at 4°C in order to obtain a cellular pellet, which was subsequently fixed in paraformaldehyde for electron microscopy observation (SEM FEG QUANTA 200 FEI).

Results and discussion

The microfouling formation on mortars samples after 3 months in the marine aquarium was analyzed by both electron microscopy observation and microbial biomass evaluation (units of optical density). In the control samples (Fig. 4A, D), the newly formed microfouling are organized in mixed communities of microorganisms in which bacterial forms predominate. Indeed, as is known from literature data, bacteria are the initial colonizers of the immersed surfaces [22].

It is interesting to note that differences in the intensity of colonization between the two types of commercial mortar used, St Astier NHL3.5 (ST) and Volteco Microlime Gel (V) are not estimable (Fig. 4A, D). Analyzing the first results on the biocidal activity of the used compounds, encouraging results could emerge from treatments with B2 additive that, in this evaluation phase, showed a slowdown in bacterial growth (Fig. 4C, F). Regarding the biocidal efficacy of the compound B1, there are no significant effects on either of the two mortar used (Fig. 4B, E).

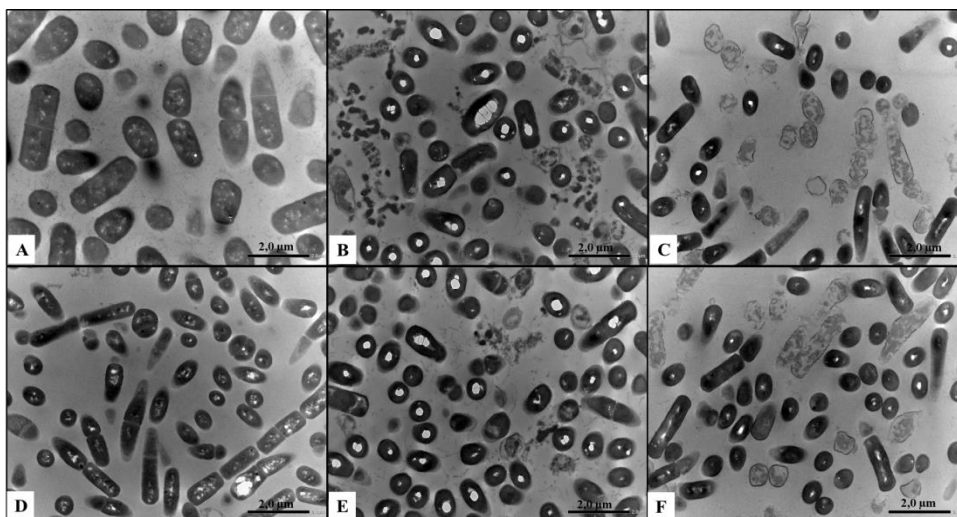


Fig. 4. Biocidal efficacy of treatments with B1 and B2 additives on liquid culture medium pellet fixed and observed by electron microscopy: (A) St Astier untreated; (B) St Astier+B1; (C) St Astier +B2; (D) Volteco untreated; (E) Volteco+B1; (F) Volteco+B2.

To evaluate the microbial biomass growth in presence of biocidal compounds, measurement of the optical density of bacterial cultures grown in liquid medium were also carried out. The obtained results confirm that the compound B2 exerts an important biocidal effect in both mortar types, statistically significant on the Voltco (V) mortar (Fig. 5). Also in this case the compound B1 did not show biocidal efficacy, even on the mortar St Astier (ST) there is an increase in bacterial growth (Fig. 5).

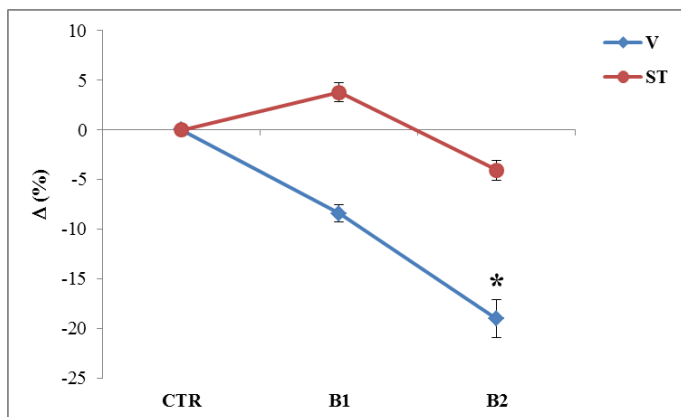


Fig. 5. Biocidal efficacy expressed in % of turbidity on liquid culture medium (n= 9; *= $p < 0.05$).

These results, even if preliminary, underline the importance of analyzing the efficacy of biocidal compounds from the very early stages, i.e. the formation of microfouling, as bacterial adhesion, is the most critical step to target in the search for an efficient antifouling.

Conclusions

Mortar-based biocide coatings to apply in the conservation of underwater cultural architectures were designed. The mortars were realized using nano-agents based on colloidal hydroxide dispersions, an antimicrobial silicone quaternary ammonium (B1) and an extract using three between spices and herbs *Origanum vulgare*, *Rosmarinus officinalis* and *Thymus* (B2).

The relationship between the chemical and physical characteristics of mortars surfaces and their ability to have an enhanced resistance to algal growth was studied through an innovative methodology in laboratory simulating seawater real conditions.

Between the two additives, the treatment with B2 additive showed a slowdown in bacterial growth with respect the B1 one, providing a better protection against biofouling.

In conclusion, this initial study provides useful informations for the development of new restoration mortars to be applied and tested in situ to consolidate the wall structures of underwater archaeological sites, having antifouling activity with lower toxic substances.

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