New synthetic products for the consolidation of wet organic archaeological materials

A. Salvini[a*], A. Papacchini[a], R. Oliva[a], G. Di Giulio[b], B. Perito[c], M. Fioravanti[b]

[a] Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Via della Lastruccia 3/13 - 50019 - Sesto Fiorentino (FI) Italy, E-mail:antonella.salvini@unifi.it
[b] Dipartimento di Gestione dei Sistemi Agrari, Alimentari e Forestali, Università degli Studi di Firenze, Via San Bonaventura 13 - 50145 - Firenze Italy
[c] Dipartimento di Biologia, Università degli Studi di Firenze, Via Madonna del Piano, 6 - 50019 - Sesto Fiorentino (FI) Italy

Abstract

Different compounds and methods are currently applied to the treatment of archaeological waterlogged wood. However, the research in this field is not concluded and there is the need for products able to solve the criticalities shown by these compounds. In this research, new polymers for the treatment of archaeological waterlogged wood have been designed with the objectives of environmental compatibility of the products and affinity for polar materials like wood, paper and natural fibres. Several hydroxylated products (i.e. cellulose derivatives, carbohydrates copolymers and hydroxylated oligoamides) with different molecular weight and different hydrophilic/hydrophobic ratio were obtained and studied as consolidants in the conservation of wet organic archaeological materials. Moreover, to reduce the risk of microbial attack, nanocomposites were studied and used for surface treatment to combine the advantages of inorganic nanomaterials (TiO_2 anatase) in terms of antimicrobial activity and those of polymers obtained from renewable resources in terms of consolidation. Finally, specific diagnostic protocols were designed to investigate positive and negative features of these new products when used in preservation of different wet organic materials.

Keywords: consolidants; renewable resources; water soluble polymers; microbial attack; diagnostic protocol, waterlogged/archaeological wood

Introduction

Recently, the use of renewable resources has been explored to produce new materials useful in many fields of application (Mülhaupt 2013), including cultural heritage conservation (Balliana, 2016). In conservation, sustainability, low environmental impact and generally low toxicity are important topics, as well as respect for the properties of the constituent materials. In this regard, the use of appropriately selected products obtained from natural sources can reduce health, environmental or safety hazards and allow greater compatibility with different substrates.

Traditionally, several compounds and methods have been used for the treatment of wet organic archaeological materials such as waterlogged wood, fibers and leather. For example, polyethylene glycols (PEG) with different molecular weights have been and still are the most used compounds for wood treatment (Kaye 2000; Seborg 1962; Stark 1976; Graves 2004). However, several problems including high hygroscopicity, relatively high cost and chromatic alteration have been observed (Hocker 2012; Kawai 2002; Bardet 2007) suggesting the need for new consolidants.

The study of innovative products for conservation generally requires a full knowledge of the degradation problems that characterize the aging of the natural materials. In the case of wood, for example, several agents can alter the chemical structure of its main components, consequently affecting its mechanical, physical and chemical properties. Water and biological agents can initiate hydrolysis reactions that result in the prevalent loss of hemicelluloses and cellulose, which represent the backbone of the ligneous structure.

Starting from these evaluations, recently our laboratoryhas synthesized several products to obtain a library of molecules suitable to be used as consolidants for wood and other organic archaeological materials. Carbohydrate derivatives (Cipriani 2010) and oligoamides (Cipriani 2013: Oliva 2014) were synthesized and characterized as hydroxylated water-soluble compounds with structural affinity for

cellulosic materials. All the synthesized compounds have a structure that is similar to that of the backbone of the cellulosic materials and, thanks to this feature, they can be used to improve mechanical strength. Furthermore, oligoamides have a structure that is similar to that of the protein contained in the leather so their use appears promising for the conservation of wet leather.
During the study of these new consolidants, it was also taken into account that microbial attack may occur after consolidation treatments, because both synthetic (acrylic and vinyl resins, PEGs) and natural (oils or shellac) products can be used as carbon and energy sources for microbial growth (Sterflinger 2013). Therefore, nanocomposites with TiO_2 were studied to utilize the antimicrobial properties of TiO_2 anatase (Oliva 2015).

One of the aims of this work was also to find diagnostic protocols to perform rapid screening of the properties of the synthesized products. In this way, it is possible to effectively select the most suitable consolidant or nanocomposite for each degraded material (e.g. cellulosic material, or leather, after biotic and/or abiotic attacks), thus optimizing the different specific formulations for cultural heritage preservation.

Material and Methods

Lignin preparation: Klason procedure (TAPPI 222 OM 88)

Oak wood flour (116 mg), obtained by grinding the sample in an agate mortar and filtering it through a 300 μm sieve, was introduced in a Soxhlet extractor using a cellulose thimble. The flask was filled with 200 mL of ethanol (95%), and after 6 hours of extraction process the thimble was placed in oven at 70°C for 24 hours. The pre-extracted wood flour was added to 15 mL of sulphuric acid (72%) in a beaker, and continuously stirred at 0°C for 10 minutes. The beaker was then allowed to reach room temperature, and stirring was continued for 2 hours. The solution was diluted to 3%, brought to boiling point and kept at that temperature for 4 hours, water was added during the process to keep the volume constant. The solution was decanted and the lignin residue was filtered, washed and dried in an oven at 103°C.

Lignin treatments

Oak lignin (15 mg) plus a consolidant (100 mg) were continuously stirred in demineralized water (10 mL) in a flask into a solution for 4 days at room temperature. After filtration or centrifugation, the solid residue was dried in a vacuum and in an oven at 60°C for 24 hours (17 mg), then it was characterized by FT-IR as KBr pellets with a Shimadzu FT-IR-8400S model (resolution 2 cm^{-1}) and elaborated with the software Shimadzu IRsolution 1.04.

Reversibility of lignin treatments

Treated lignin samples were added to demineralized water (10 mL) and magnetically stirred for 24 hours at room temperature. After filtration or centrifugation, the solid residues were dried and analyzed by FT-IR.

Specimen preparation

Oak wood specimens were recovered from an archaeological waterlogged find located in Poggiomarino, Italy. Several cubic specimens were prepared and mass and volume were measured at maximum water content (MWC). The specimens were immersed in demineralized water and kept at room temperature until the beginning of the treatment.

Treatment of the specimens with consolidants

Each specimen was immersed in 7.0 mL of a water solution of a consolidant (300-500 mg) and kept at room temperature for 45 days in a 25 mL Sovirel® tube.

Penetration ability of the consolidant

FT-IR spectra of the wood flours obtained from internal and external sections of each of the treated wooden sample were recorded.

Physical characterization

A protocol similar to the international technical standard UNI ISO 3131 was applied to the physical characterization. The weights of the specimens treated with the different consolidants were evaluated at different relative humidities (R.H.) - 100%, 86%, 65% and 12%. For this work, to evaluate the basic density, the final temperature was 50°C (R.H. 12%), different from the one reported by the standard UNI ISO 3131 (103°C) which instead corresponds to the anhydrous state (R.H. 0%).

The volume of each wood specimen was measured at MWC using the water displacement method. The initial wood moisture content (I.H.) and that at the three

hygroscopic equilibrium values (R.H. 100%, 86%, 65%, 12%) together with the basic density were evaluated using the weights and the volumes measured at each R.H. value.

The volumetric shrinkage β_v of the specimens were also calculated.
$\beta_v = (V_f - V_0)/ V_f \times 100$ (where V_0= ovendry volume and V_f= green volume)

Microorganism and growth conditions
A cell suspension of *Trametes versicolor* strain MB52, purchased from the Austrian Center of Biological Resources and Applied Mycology, was spread on Malt Extract Agar (MEA, OXOID) plates and incubated at 30°C for 4 weeks to obtain a thick mycelium.

Preparation of wood specimens for superficial treatment
Sixteen samples were prepared from European beech (*Fagus sylvatica L.*), a wood species that is well known for its low natural durability and high susceptibility to fungi and bacteria. The wood, free of any previous biological alteration (assessed by microscopic observations), was cut with the longitudinal faces parallel to the grain, up to a final size of 10 × 20 × 2 mm. Prior to the treatment the samples were equilibrated at 20 °C and 65% RH inside dryers which contained xylene as an inhibitor for bacterial activity.

Superficial treatment with nanocomposites
A water dispersion of TiO_2-nanocomposite (460 mg in 10 mL) was applied on all faces of 8 samples using a brush (three applications per day for 7 days). The treated and untreated samples were kept at 65% RH and 20°C in a sterile environment (dried with xylene) until the treatment with the fungus. Then the samples were placed on a sterile small net on *T. versicolor* cultures in Petri dishes (2 treated + 2 untreated samples per dish). Half of the dishes were exposed to UV light for three weeks, while the other half was kept in the dark. Exposure to UV radiation was carried out using a Spectroline Lamp, Model ENF-260C/FE, with an emission in UV-A range at wavelength of 365 nm (tube of 6 W). The exposure was carried out to simulate the behavior of the sunlight. The lamp was kept lit for 12 hours and turned off for 12 more using a timer for the whole duration of the test. The samples exposed to the UV light were turned upside down every day in the middle of the illumination period to expose both sides to the radiation during the test. Optical microscope images were recorded with a Dino-Lite Pro Portable Digital Microscope AD413T (1.3 megapixel image sensor, magnification 200x).

Results and discussion
Recently, our laboratory has designed and synthesized a library of molecules suitable for consolidating wet organic archaeological materials (Cipriani 2010; Cipriani 2013; Oliva 2014). What makes these synthetic procedures interesting is the use of renewable resources (cellulose, L-tartaric acid, D(+)-glucose, α,α-trehalose) as starting materials in order to introduce hydroxyl moieties in the polymeric chain. More recently, the library was extended by synthesizing other oligoamides and by using α,α-thehalose and D-cellobiose derivatives as comonomers in the synthesis of vinyl acetate and vinyl alcohol co-polymers. Moreover, many other compounds, synthesized from renewable resources for different industrial applications, can also be applied to cultural heritage preservation. However, diagnostic protocols need to be developed to allow rapid screening of the properties of the various products and to optimize the formulations for each kind of degraded substrate.

Different diagnostic protocols can be designed for wood, leather, fibers and paper taking into account that some properties are common to all of these materials, while other properties are specific for each material. For example, elasticity is an important property for leather samples (van Soest 1984) and studies on the use of polyamides for wet leather recovery are currently underway in our laboratory. These studies have pointed out the need to improve the formulation of the consolidant to achieve better results. However, for all wet organic materials it is important to evaluate shrinkage, the variation of mechanical properties and the effects of attack by microorganisms. In the following section, the protocol developed for small wooden samples will be described in detail.

Consolidants library
Cellulose is one of the most important natural polymers and its synthetic derivatives are of great industrial importance. In fact, in the last decades, a wide range of products having

different chemical and physical properties have been produced. Among these derivatives, cellulose ethers show peculiar features, such as high chemical stability and no toxicity, while their water solubility depends on various factors like an appropriate combination of the different ether groups, the degree of substitution and the distribution of the substituents. In this study, three cellulose ethers were tested: two water soluble cellulose ethers synthesized in our laboratory (lower-DP allyl carboxymethylcellulose and lower-DP allyl n-hydroxypropylcellulose) (Figure 1) and a commercial carboxymethylcellulose. The first two consolidants were obtained starting from a cellulose that was previously hydrolyzed reducing the molecular weight to enhance the wood penetration ability of the final products. Subsequently, two kinds of functional groups were introduced to the cellulose structure: a cross-linkable group (allyl), in order to reduce the mobility of the consolidant after its penetration into the wood and a hydrophilic group (carboxymethyl or n-hydroxypropyl), in order to make the final product partially or totally soluble in water.

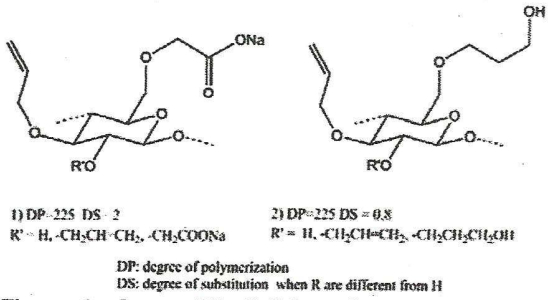

Figure 1: Lower DP Cellulose Ethers: 1) allyl carboxymethylcellulose; 2) allyl n-hydroxypropylcellulose

Hydroxylated oligoamides (m,n) (Figure 2) were obtained as water soluble compounds with high affinity for polar materials and lower molecular weights with respect to cellulose ethers. Natural compounds or their derivatives (L-tartaric acid, D(+)-glucaric acid and α,α-trehaluronic acid) were used as hydroxylated dimethyl esters in polycondensation reactions with ethylenediamine in order to obtain polyethylene-L-tartaramide, polyethylene-D(+)-glucaramide and polyethylene-α,α-trehaluronamide respectively.

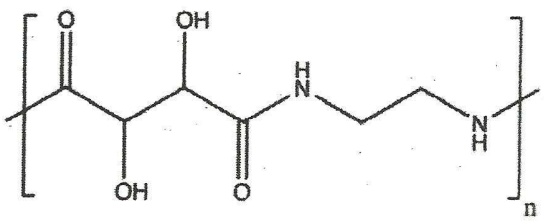

Figure 2: Hydroxylated oligoamides (m,n): polyethylene-L-tartaramide

Finally, vinyl acetate and vinyl alcohol copolymers with α,α-thehalose and D-cellobiose comonomers (Figure 3) were obtained and tested as water soluble consolidants.

Diagnostic protocol

A specific diagnostic protocol was designed to highlight positive and negative features of the new products when applied to archaeological wood samples (Figure 4). The first step of the diagnostic protocol was to evaluate the chemical affinity of the consolidants for partially or strongly degraded substrates through preliminary tests on recent oak wood lignin samples. Even though powdered lignin has some properties that are different from native lignin in the wood matrix, the use of the lignin allowed us to evaluate the affinity of the consolidants, simulating the condition of partially or strongly degraded samples in which holocellulose is largely lost Lignin samples were prepared from wood flours following standard procedures and maintained in aqueous solutions of the consolidants for 24 hours at room temperature.

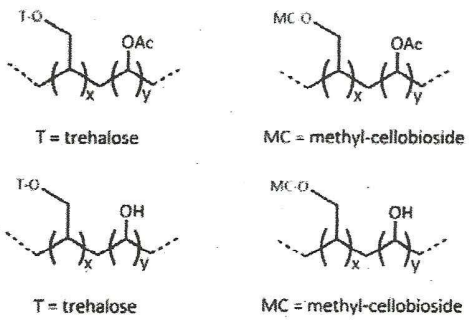

Figure 3: Carbohydrate /vinyl alcohol or vinyl acetate co-polymers

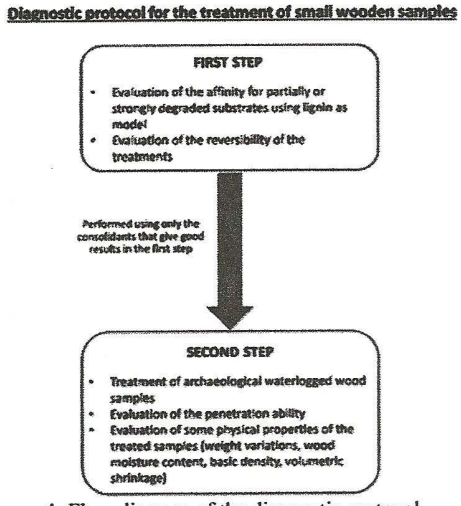

Figure 4: Flow diagram of the diagnostic protocol

The FT-IR spectra recorded for each treated sample was compared with the spectra of untreated lignin and with that of the respective consolidant to evaluate the effectiveness of the treatment. The absorption of the consolidant can be confirmed by the variation in the shape and in the intensity of some lignin signals due to the overlapping with the signals of the consolidant. All products described in the library were tested on wood lignin samples. As an example, FT-IR spectrum of the lignin treated with polyethylene-L-tartaramide is reported (Figure 5).

Then, the reversibility of the treatments was evaluated. Reversibility is generally one of the most important requirements in the field of conservation of cultural heritage even if, as in the case of wood conservation, full reversibility is not always achievable. The reversibility of the treatment was evaluated adding demineralized water to treated lignin samples treated. After

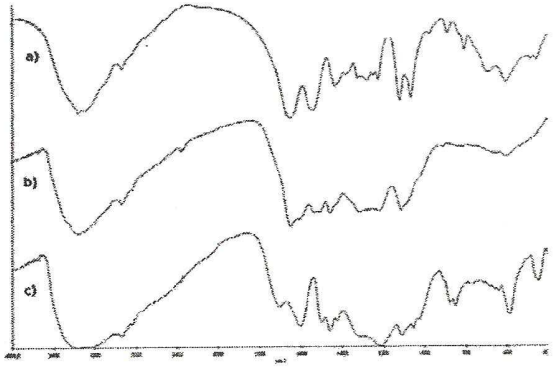

Figure 5: FT-IR spectra of: a) polyethylene-L-tartaramide; b) beech wood lignin treated with polyethylene-L-tartaramide; c) beech wood lignin

centrifugation, dried samples were analyzed and the FT-IR spectra were compared with those related to the treated lignin samples. This comparison showed that products without allyl groups (oligoamides, cellulose ether) were completely removed while those containing allyl groups were only partially removed. This behavior agrees with the reactivity of allyl groups, which can cross-link and create a less soluble reticulated structure in the cellulose network.

The products that showed the best results in the affinity tests were used in the second step of the protocol, in which their ability to penetrate inside the cellular walls of waterlogged wood was evaluated using small archaeological wood cubic specimens (volume 1 cm^3). After the treatment, the chemical affinity for wood was studied and some physical wood properties, like hygroscopicity, volume shrinkage, density and basic density, were evaluated before and after different treatments. The treatments were carried out for a shorter time than that used in the standard procedure. After 45 days, FT-IR spectra of wood flours obtained from internal and external sections of all the treated samples were produced and compared. As an example, the FT-IR study of the penetration of polyethylene-L-tartaramide is reported (Figure 6).

At the same time, other specimens were subjected to gravimetric (UNI ISO 3131) and volumetric analyses to determine their physical properties at different R.H. (100%, 86%, 65% and 12%). For this work, the final temperature was 50°C (R.H. 12%), different from the one reported for the basic density by the standard UNI ISO 3131 (103°C), in order to keep the consolidants far from their transition glass temperature, T_g (Table 1). Generally, archaeological wood has a higher moisture content compared to recent wood because of the partial or total loss of the constitutive polysaccharides, which leads to an increase in the sites available to create bonds with water molecules. In the treated specimens the consolidants limited this phenomenon saturating most of the hydroxyl groups of the wood. For this reason, in all the treated

specimens the moisture content appeared to be lower than that of the untreated wood. The determination of volumic mass was carried out by weighing every specimen and calculating the volume through the water displacement method. In general, archaeological wood has a volumic mass lower than that of recent wood, due to the loss of material, which can vary depending on its degradation state. When comparing recent wood with archaeological wood this difference can be clearly noticed. The comparison between the volumic mass values of the samples treated with different consolidants showed, instead, a slight variability.

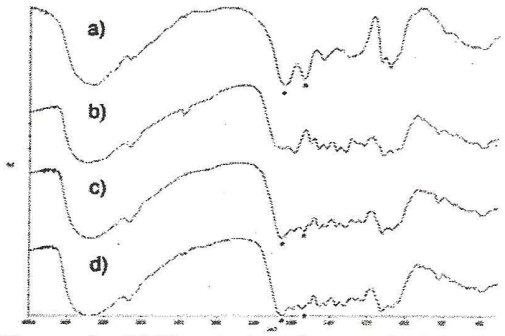

Figure 6: FT-IR spectra of: a) polyethylene-L-tartaramide; b) untreated archeological oak wood; c) external section of the wood specimen treated with polyethylene-L-tartaramide; d) internal section of the wood specimen treated with polyethylene-L-tartaramide.

The results related to the basic density showed that the consolidants penetrated the wood leading to an increase of the density compared to that of the untreated wood. The volumetric shrinkages, instead, are clearly reduced in the treated specimens respect to that of the untreated ones (Table 2).

As a general result of this study, when samples were treated with polyamides they showed better dimensional stability, according to the higher adsorption of the consolidant, demonstrated by the increment in the values of basic density.

When organic compounds are used as wood consolidants, their resistance to microbial attacks during treatment in solution must be evaluated, as well as the susceptibility of the treated wood to the microbial attack when exposed to air after the treatment. To test the susceptibility of the consolidants to biodegradation, during the treatment of archeological wood samples, microbial growth in treatment solutions was monitored by the viable count method. The data obtained in the presence of polyethylene-L-tartaramide are reported (Table 3). The viable count of the bacteria in the consolidant solution was one order of magnitude larger than that of the bacteria in the control solution, nevertheless the presence of the commonly used biocide Preventol RI 80 was effective in completely controlling microbial growth until the end of the treatment.

Antimicrobial treatments:

To prevent fungal attack on wooden finds during excavation or prolonged exposure to aerobic environments, antimicrobial products for superficial treatments were studied. The nanocomposite TiO_2-polyethylenetartaramide, obtained with a controlled growth of oligoamide on TiO_2 nanoparticles (Figure 7) (Oliva 2015), was studied. It showed a good efficacy against fungal attack by *Trametes versicolor* on wood specimens (*Fagus sylvatica*). By microscopic analysis, white rot fungi growth was observed on the untreated samples exposed to UV. The absence of fungal growth was confirmed on samples treated with the nanocomposite and exposed to UV light (Figure 8) (Oliva 2015).

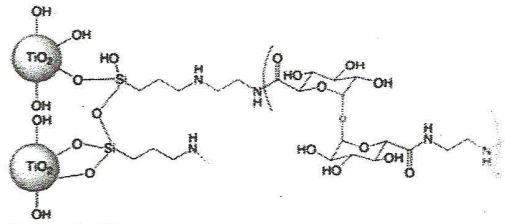

Figure 7: TiO_2 nanocomposites.

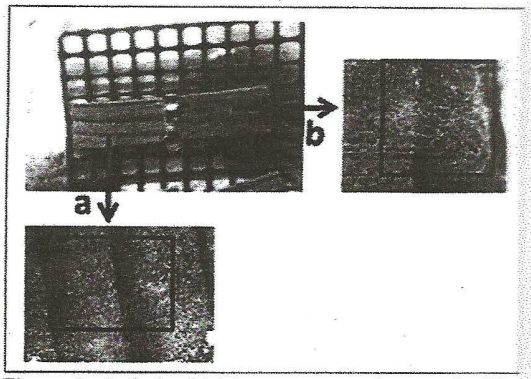

Figure 8: Antimicrobial treatment: sample treated with nanocomposite, a) exposed to the UV light, b) kept in the dark

Table 1: Moisture Content of the Specimens at Different R.H. Values

Oak wood samples	MWC	RH 100%	RH 86%	RH 65%
Untreated wood	332%	244%	--	17%
PEG 400 and 3350	167%	167%	--	10%
Carboxymethylcellulose	220%	215%	--	18%
Polytartaramide	228%	190%	19%	13%
Polyglucaramide	237%	119%	19%	12%
Polytrealuronamide	274%	132%	21%	13%
Recent wood	80%	30%	18%	12%

Table 2: Basic density (BD) and volumetric shrinkages (βvol)

Oak samples	BD (g/cm³)	βvol
Untreated wood	0.230	53%
Carboxymethylcellulose	0.361	55%
Polyethylenetartaramide	0.346	43%
Polyetheyleneglucaramide	0.334	39%
Polyethylenetrealuronamide	0.301	49%
Polyhexamethylenetartaramide	0.232	43%
Recent wood	0.690	18%

Table 3: Bacterial Viability Count

Samples	Bacterial growth (v.m.)		
	t = 0	t = 4 weeks	t = 6weeks
Water	$1{,}4 \times 10^{3}$	$3{,}4 \times 10^{6}$	$1{,}3 \times 10^{7}$
Consolidant and water	$1{,}1 \times 10^{2}$	$1{,}4 \times 10^{8}$	$1{,}7 \times 10^{8}$
Consolidant, water and biocide	0	0	0

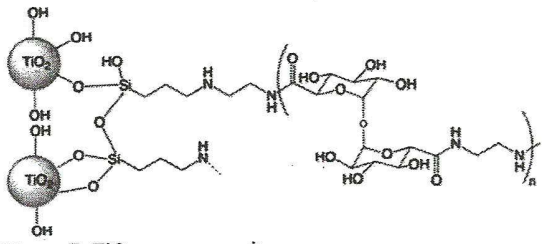

Figure 7: TiO_2 nanocomposites

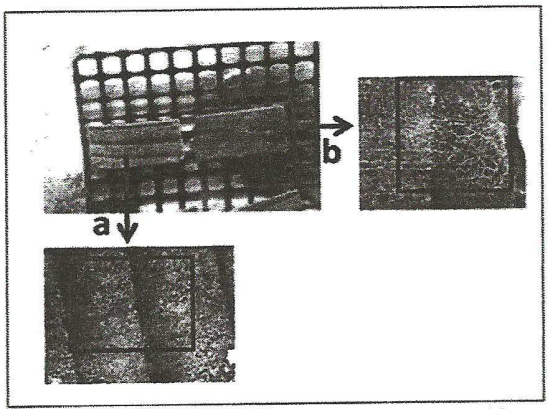

Figure 8: Antimicrobial treatment: sample treated with nanocomposite, a) exposed to the UV light, b) kept in the dark

Conclusions

In this study, a library of hydroxylated derivatives was designed for wet organic archaeological materials and they were tested for waterlogged wood consolidation. These compounds have a chemical structure similar to cellulose and hemicelluloses and a high affinity for lignin was observed for all of them. In addition, partial retention of the consolidants was observed and it was attributable to the formation of secondary interactions between the consolidant and the wood. In the case of cellulose ethers, even partial crosslinking can further reduce the mobility of the consolidants.

The amount of consolidant in the internal section of the wood specimens was low for cellulose ethers, probably because of their higher molecular weight. In agreement with this behavior, the penetration was high for the hydroxylated oligoamides, characterized by low molecular weights while it was variable with molecular weight and monomer ratio for carbohydrate/vinyl acetate (or alcohol) copolymers. Finally, the data related to the physical properties of the degraded wood specimens treated with the hydroxylated oligoamides showed an increase of the basic density and a decrease of the volumetric shrinkage with respect to untreated wood. This behavior demonstrates the effectiveness of these compounds in the consolidation process of waterlogged wood. However, these results should be confirmed on larger scale application (i.e. full-sized objects) and in long-term tests. Inhibition of fungal growth was obtained in

samples treated with the TiO_2-polyethylenetartaramide nanocomposite and irradiated by UV, as confirmed by microscopic analysis.

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