Anatase as an alternative application for preventing biodeterioration of mortars: Evaluation and comparison with other biocides

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A B S T R A C T

The aim of this study is the comparison between different treatments (anatase and two conventional biocides: Biotin T and Anios) for preventing biodeterioration of mortars. The treatments were applied both in the laboratory on mortar slabs and in situ on walls of Palácio Nacional da Pena (Sintra, Portugal). Mortar slabs treated with anatase (pure and Fe 3+ doped) applied as a coating or by mixing within the mortar were prepared, and their surfaces characterized by different methodologies. The mortars were inoculated with cyanobacteria and chlorophyta species, incubated for a period of 4 months and the chlorophyll content quantified by extraction method and fluorescence emission. For comparison purposes untreated mortar slabs were inoculated, incubated and finally treated with the biocides. After two weeks the respective chlorophyll contents was quantified. In situ studies in two external walls of Palácio Nacional da Pena covered by organisms were also performed by direct application of aqueous solutions of the three products, and the efficiency of the treatment monitored by spectrophotometry using the CIELAB method. Lichens and other phototrophic microorganisms were identified by direct observation with a microscope and cyanobacteria, green microalgae, bacteria and fungi by DNA-based molecular analysis targeting the 16S and 18S ribosomal RNA genes.

The results show that anatase is a better agent for preventing biodeterioration than the two tested conventional biocides, both in mortars slabs and in situ studies. In fact, photographic and colorimetric records made in two external walls of Palácio Nacional da Pena after two weeks of treatments application showed that lichens and other phototrophic microorganisms disappear from the places where anatase was applied.

1. Introduction

Of all building materials for construction, artificial ones, like mortars, are the most widely used. Biological decay of mortars is a serious problem, as approximately 30% of visible alteration on building materials is due to microbial impact (Kurth, 2008). Effects of microorganisms on building facades are responsible for aesthetic, biogeophysical and biogeochemical deterioration (Saiz-Jimenez, 1999). Due to their photoautotrophic nature, photosynthetic microorganisms, like algae and cyanobacteria, are the pioneering colonizers of building facades, and therefore, the main responsible organisms for a further biological colonization (Tomaselli et al., 2000). The cost of cleaning and treatment
microbial deterioration on buildings is often difficult to estimate. It includes cleaning and repairing procedures, as well as cultural losses due to structural damages, which have been reviewed by Chen and Blume (2002). Thus, the development of successful conservation treatments capable of preventing and inhibiting biodeterioration, rather than the improvement of already existing biocides, is a very important issue in the cultural heritage buildings preservation context. Moreover, the identification of the microorganisms colonizing building materials can give very essential information for the research of new methods capable of avoiding the biodeterioration process.

Procedures for preventing biodeterioration include intervention methods. Chemical methods, like the use of biocides, are frequently applied as a conservation treatment for historic monuments (Canis et al., 1996; Nugari and Salvadori, 2003). Recently, however, the use of biocides is not being well accepted, as these products do not promote a long term protection, (most frequently due to the development of resistance mechanisms by microorganisms and also rain water washing), and therefore need to be repeatedly applied (Russel and Chopra, 1990). Besides the short-time durability of biocides treatment, the application of these products involves other sort of problems as they are toxic and can induce environmental and public health harms (Tiano, 1998). Therefore, new scientific concepts of ecological treatments are needed.

In this investigation, heterogeneous photocatalysis of TiO₂, in the form of nanocrystalline anatase, was used to develop self-cleaning materials that can be applied in cultural heritage building materials.

The incorporation of photocatalysts to construction materials (cement, mortars, exterior tiles, glass) confers anti-microbial and self-cleaning properties, involving no harm to the environment (Maury Ramirez et al., 2010). Promotion of these properties is due to the photocatalytic process that occurs on the surface of the semiconductor. This process is illustrated in Fig. 1.

Once UV light is absorbed (\(E \geq E_{\text{bandgap}}\)) promotion of electrons (\(e^-\)) from the valence band to the conduction band occurs, leaving back positive valence band holes (\(h^+\)) (Kelerher et al., 2002; Fu et al., 2005). One of the main paths of this charge carriers (\(e^-/h^+\)) is established on the surface of the semiconductor lattice, where redox reactions occur with some molecules present in the atmosphere (Fu et al., 2005). The following equations illustrate the photocatalytic process, responsible for the degradation of organic matter:

\[
\begin{align*}
\text{TiO}_2 + \text{h} \nu & \rightarrow \text{TiO}_2 + e^- + h^+ \\
e^- + \text{O}_2 & \rightarrow \text{O}_2^- \\
h^+ + \text{H}_2\text{O} & \rightarrow \text{HO}^+ + \text{H}^+ \\
\text{HO}^+ + \text{organic matter} & \rightarrow \text{xC}_2 + y\text{H}_2\text{O}
\end{align*}
\]

Due to its high redox potential and band gap (\(E^0 = 2.8 \text{ V; } E_{\text{gap}} = 3.2 \text{ eV}\)), the anatase variety of titanium dioxide, in the form of nanocrystalline powder, is one of the most widely used semiconductors for photocatalysis processes. The fact that this compound is non-toxic, very photoactive, photoestable, and produces colourless films when applied to materials, is a benefit (Diamanti et al., 2008; Chen and Poon, 2009). Therefore the idea of applying anatase (\(\text{TiO}_2\)) on/in building materials, as an alternative to the use of conventional biocides, is a promising approach that will be developed in this investigation.

2. Materials and methods

2.1. Selection of treatments

Three treatments were selected. Two conventional biocides, Biotin TiO₂(C.T.S España), frequently used in cleaning interventions on monuments, and Anios D.D.S.H® (Laboratories Anios), a biocide used as an antisepetical product for hospital procedures. The first one is a commercial biocide that has alkyl-benzyl-dimethyl-ammonium chloride and isopropyl alcohol as the active principle. The second product is a mixture of \(\text{n,n-didecyl-n-methyl-poly(oxyethyl)}\) ammonium propionate with alkyl-propylene-diaminegualdium acetate.

As an alternative product to biocides, anatase photocatalyst (P25 obtained from Degussa; predominantly nanocrystalline anatase with specific surface area of 50 m² g⁻¹ and a particle size approximately 20 nm) was selected. Additionally, in order to test, in laboratory, the improvement of photocatalytic efficiency, Fe³⁺-doped anatase (0.5 wt %) particles were prepared by wet impregnation of pure anatase on a solution of \(\text{Fe(NO}_3\text{)}_3 \cdot 9\text{H}_2\text{O}\) (Sigma Aldrich®) and fired at 500 °C.

2.2. Application of the treatments

In order to evaluate the anti-microbial effect of the three products previously selected, the experimental work described below was performed following two different lines: one, in which the products were directly applied on mortar covered walls of the Palácio Nacional da Pena (Sintra, Portugal); and other in which the products were applied in mortar samples manufactured in laboratory, following the same composition of the previously mentioned walls mortars. These are mixed binders mortars that some authors (Silva, 2002; Pereira, 2008) consider most suitable for coating walls. The mortars followed the composition of the renders used on the Palácio Nacional da Pena.

Laboratory experiments were made first, and then the in situ treatments were applied.

2.2.1. Laboratory experiments

Two kinds of mortars slabs were manufactured in the laboratory (AC and AQ). Both were composed of two mixed binders (cement and lime), with the same composition ratio, but with different kinds of sand.
The mortars were manufactured using Portland cement, (CEM II/B-L 32.5 R), high calcium hydroxide (Ridel-de Haen 31219) and sand. The mortar AC was prepared using a non-washed yellow river sand, extracted from the sandpit of Corroios, similar to the one used in Palácio da Pena, while the mortar AQz was prepared with washed quartz sand (SiO2 >96%), since silica (SiO2) is considered to be a very efficient support for anatase application due to its high supercritical area (Chen, 2005).

Fig. 2 shows the two kinds of mortars manufactured. The cement: lime: sand proportion was 1:4:12 by volume, respectively. All mortars were executed using wood casts (4.5 x 2 x 2 cm). After 7 days the mortar slabs were removed from the casts, and left curing in a room at 20 ± 2 °C and 50 ± 5% RH, during 50 days.

During the manufacturing process of the mortars, the anatase treatment was applied, following two different methodologies:

On the first one, nanocrystalline anatase powder was applied by direct addition during the manufacturing process of the mortar, at the following proportion, by volume: 12:4:4:1 — sand: lime: anatase: Portland cement.

The second methodology was done having in mind the improvement of the photocatalytic efficiency of the mortars. Therefore an application of iron-doped anatase, containing Fe³⁺ at 0.5 wt%, was prepared (Návio et al., 2008). After fired at 500 °C, the product obtained, Fe–TiO₂, was applied on the mortars slabs by the same proportion of the previous methodology: 12:4:4:1 — sand: lime: Fe–anatase: Portland cement.

Fig. 3 illustrates a scheme of the application methodology of the treatments on the mortars slabs. This scheme shows untreated mortars sets, anatase-containing mortars sets and iron-doped anatase-containing mortars sets. After the treatments applied, all mortars were sterilized and placed, in triplicate (in order to assure representative results), inside closed Petri glass dishes (Ø15 cm) with water on the bottom. Afterwards they were inoculated with representative results), inside closed Petri glass dishes (Ø15 cm).

In all mortar slabs, chlorophyll a (Chl a) values were quantified after inoculation and after the four months period of incubation in order to evaluate the biological growth.

Afterwards the untreated mortars slabs were then treated with the two biocides: Anios and Biotin T.

Anios was applied without any dilution and Biotin was applied diluted at 2% (v/v) in distilled water. After 2 weeks, the effect of the application of the biocides was evaluated by chlorophyll a quantification techniques.

### 2.2.2. Inoculation

In order to evaluate the anti-microbial effect of the treatments, the mortars slabs were inoculated with a mixed culture of photosynthetic microorganisms: two green microalgae, Stichococcus bacillaris and Chlorella ellipsoidea, and one cyanobacterium, Gloeocapsa dermochroa in BG-11 liquid culture medium. These photosynthetic microorganisms were selected, because they occur very frequently on stone monuments in European countries of the Mediterranean Basin (Miller et al., 2006; Macedo et al., 2009). All mortars were inoculated with 100 µl of each culture. After inoculation, the mortars slabs were incubated at an exterior terrace, exposed to natural conditions, during 4 months (January, 23rd–May, 23rd, 2009). Moisture levels were maintained by adding sterile water (10 ml), periodically, to the bottom of the Petri dishes.

#### 2.2.3. In situ experiments on the Palácio Nacional da Pena, (Sintra)

In situ experiments were performed on two external walls of the Palácio Nacional da Pena, (Sintra). One of the walls is located on the Arches Yard, facing ENE, not receiving direct sunlight. This wall is extensively colonized by lichenic and algal communities and presents high humidity. The other wall is located on the D. Carlos Terrace, facing east, and receiving direct sunlight during much part of the day. Lichens are scattered distributed in this wall.

Aqueous solutions of the three products (anatase and the two biocides) were applied directly on small areas (50 cm²) of the selected walls on D. Carlos Terrace and on the Arches Yard. These areas were chosen on the basis of homogeneity of substrate and biological growth. Biotin T was applied at 2% (v/v) by brush. Anios was directly applied by spray, without any dilution, and Anatase; at 1% (v/v) in distilled water, was also applied by spray.

#### 2.3. Anatase characterization

The purity and crystallinity of anatase and iron-doped anatase samples were examined by Raman Spectroscopy, with a Labram Laser made by Jobin Yvon, using a 632.8-nm He–Ne ion laser as an excitation source. The laser power on the samples was 2.5 mW.

Surface morphology of iron-doped anatase was analyzed by scanning electron microscope (SEM) with a JEOl Scanning Microscope T330A. Elementary characterization was carried out using energy dispersive X-ray analysis (EDX).

In order to understand the effect of doping anatase on light absorption, samples of pure anatase and iron-doped anatase were analyzed with a Shimadzu UV-2501PC to measure the UV–Visible diffuse reflectance of specimens, using BaSO₄ as a reference sample.

#### 2.4. Analysis of the microbial communities present on the two external walls of Palácio Nacional da Pena

A first survey on the walls was conducted for lichen and algal identification. These organisms represented the bulk of the biomass colonizing the walls. Representative specimens were collected and studied in the laboratory and species were identified or confirmed according to Clauzade and Roux (2002). Abundance was also taken into account. Then, microbial communities...
composed of bacteria and fungi, associated to the lichens and algae, and representing a very minor biomass moiety, were investigated using molecular tools. DNA present in the samples collected from the two external walls of the Palácio were extracted using the Nucleospin Food DNA Extraction Kit (Macherey–Nagel, Düren, Germany). The 16S and 18S rRNA genes were used for the identification of prokaryotes (bacteria and cyanobacteria) and eukaryotes (fungi and microalgae) respectively, as described Miller et al. (2008). Amplification of DNA was carried out by PCR and amplification products were used for two different protocols. The first analysis consisted in obtaining bacterial community fingerprints by DGGE and the second analysis was aimed to obtain 16S gene clone libraries used for sequencing as described Miller et al. (2008).

2.5. Evaluation of efficacy of the treatments

In the laboratory experiments, evaluation of the treatments efficacy was based on the assessment of microbial growth, by quantification of chlorophyll a content on the mortars slabs, relating it to the different treatments applied. Quantification of chlorophyll a content was estimated by its chlorophyll a fluorescence emission, using an optical fiber, and upon chlorophyll a extraction method.

Three fluorescence emission measures were performed before, immediately after inoculation, and after the period of incubation, using a spectro-fluorometer (SPEX Fluorolog-3 Model FL3-22), fitted with an optical fiber (Horiva-Jove-Yvon Model F3000). The optical fiber was placed perpendicular to the mortar surface, and to assure always the same distance of irradiation, an “O-ring” was placed between the optical fiber and the mortar slab. All samples were excited at 430 nm.

Chlorophyll a content was also quantified, immediately after inoculation and after the period of incubation, upon extraction method, using dimethyl sulfoxide (DMSO) as a solvent (Wollenweider, 1979). For both chlorophyll a quantification techniques, biologic growth ratio was calculated, meaning the ratio between after incubation chlorophyll a values and after inoculation chlorophyll a values.

Regarding the in situ experiments on Palácio Nacional da Pena, evaluation of efficacy of the treatments was carried out by colour measurements and photograph records before and after two weeks of application of the treatments.

Colour measurements were performed using a portable spectrophotometer (Minolta CM-508i). The results are the mean value of ten measures per area. CIELAB method was used in order to characterize the surface colour by three parameters: L* (lightness), a* and b*(chromatic coordinates), defined by CIE (Commission Internationale de l’Eclairage). Total colour variation (ΔE*) was calculated on the same spot, as a spatial difference between two points, corresponding to the initial colour, before the treatment applied and to the colour after treatment applied:

\[
\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2},
\]

being ΔL* = L*(after treatment) − L*(before treatment); Δa* = a*(after treatment) − a* (before treatment); Δb* = b* (after treatment) − b* (before treatment).

3. Experimental results

3.1. Anatase characteristics

Pure anatase (P25-Degussa) and iron-doped anatase films show the anatase crystal phase confirmed in the Raman spectrum by five peaks at 144, 197, 397, 518 and 640 cm⁻¹, originated by anatase tetragonal structure \((3E_g + 2B_{1g} + 1A_{2g})\). The anatase structure is maintained upon doping with iron.

SEM image of an iron-doped anatase sample is shown in Fig. 4. In this figure is observed aggregates and discrete particles of mixed Fe–TiO₂ oxides. EDX analysis determined the elementary composition of the iron-doped anatase. It confirmed the presence of a Fe–Ti phase \((Fe:Ti = 23.63\%:76.37\%)\), suggesting that the doping process was efficient.

The doping effect on light absorption is shown in Fig. 5. After the doping process has been completed, the colour of the sample turned from white to yellow, as the absorption of the iron-doped films shifted more to the visible region (400–700 nm), when compared with the un-doped TiO₂ film. The shift to the visible
3.2. Microbial communities present on the two external walls of Palácio Nacional da Pena

3.2.1. Lichen and algal communities

The distribution of lichens and algae in Palácio Nacional da Pena is closely related to the microclimatic conditions of the walls. These are determined by the orientation of each wall, as the level of exposure to sunlight seems to be a primary factor, influencing both the likelihood of lichen colonization and the thallus type. The community present in the Arches Yard has a dark colour and covered most part of the walls. This was composed of a community present in the Arches Yard has a dark colour and the likelihood of lichen colonization and the thallus type. The community present in the Arches Yard has a dark colour and covered most part of the walls. This was composed of a majority member, followed by Opegrapha calcarea Sm., Bacidia cf. scopulicola (Nyl.) A.L.Sm., Ramalina sp., and cf. Cystocoleus cf. lentigera, as a majoritary member, followed by Opegrapha calcarea Sm., while in the area with some more light and wind was settled Ramalina sp. Bacidia cf. scopulicola appeared close to Opegrapha calcarea, while Cystocoleus appeared in semiexposed areas, next to Ramalina sp. Trentepohlia occupy most part of the wall, no matter their exposure and water availability. The occurrence of Trentepohlia indicates a high degree of atmospheric humidity. If the atmospheric conditions are drier (for example by wind action) then lichens are dominating. In these case Cystocoleus sp., lichen that has Trentepohlia as photobiont, is the dominant. The lichenization of Trentepohlia as Cystocoleus allows these algae to colonize a new and more arid microhabitat (Chapman and Waters, 2002). In fact, the filamentous Trentepohlia is one of the most common green algae phycobionts (Nash, 1996).

The wall of D. Carlos Terrace was covered by Caloplaca group cirrina; Fulgensia sp.; Squamarina cf. lentigera; Toninia candida; and cf. Pyrenocollema sp. irregularly distributed as patches. In addition, the cyanobacterium Scytonema sp. and the moss Tortula sp. were found. In this terrace, the areas subjected to water run-off or soaked by water developed cf. Pyrenocollema sp., and Scytonema sp. In the exposed areas with a stable surface appeared Caloplaca group cirrina. In the places with fissures, where some dust was accumulated, the presence of squamulose thalli of Fulgensia sp., Squamarina cf. lentigera, T. candida and the moss Tortula sp. were observed.

3.2.2. Molecular biology

The purpose of a molecular study was to know the microorganisms associated to the lichenic and algal communities. These associated microorganisms were a very minor proportion in biomass with respect to the presence of lichenic thalli and Trentepohlia filaments. Table 1 presents the algae, identified by molecular biology methods, from the two external walls of Palácio Nacional da Pena. The microalgae detected in this study were mainly Trentepohlia and other Chlorophyta. Trentepohlia occurred only in the Arches Yard. However, Chlorophyta chloroplasts appeared in D. Carlos Terrace. This is because oligonucleotide primers originally developed for the specific amplification of 16S rRNA gene segments from cyanobacteria not only targeted cyanobacterium sequences, but also sequences derived from phototrophic eukaryotes (Burja et al., 2006).

Trentepohlia can be considered a terrestrial alga and usually presents an orange or red-brown colouration (Graham and Wilcox, 2000). Regarding D. Carlos Terrace, the DNA analysis only allows us to determine Chlorophyta chloroplast, which probably belongs to lichen phycobionts.

Table 2 presents the results of the prokaryotic microorganisms identified. The bacteria biodiversity is significantly higher in the Arches Yard than in D. Carlos Terrace. This can be explained by the fact that the Arches Yard wall receives less sunlight and it is usually more humid than the D. Carlos Terrace wall, which is subjected to

![Fig. 4. SEM image of an iron-doped Anatase sample.](image)

![Fig. 5. Diffuse reflectance spectra of Anatase and Fe-doped Anatase films.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Phyllogenetic affiliation</th>
<th>Similarity (%)</th>
<th>Primer</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trentepohlia sp. (DQ399592)</td>
<td>99</td>
<td>EukA–EukB</td>
<td>Arches Yard</td>
</tr>
<tr>
<td>Trentepohlia sp. (DQ399592)</td>
<td>98</td>
<td>EukA–EukB</td>
<td>Arches Yard</td>
</tr>
<tr>
<td>Trentepohlia sp. (DQ399592)</td>
<td>97</td>
<td>EukA–EukB</td>
<td>Arches Yard</td>
</tr>
<tr>
<td>Chlorophyta chloroplast (AB374385)</td>
<td>93</td>
<td>616F–515R</td>
<td>D. Carlos Terrace</td>
</tr>
<tr>
<td>Chlorophyta chloroplast (FJ028695)</td>
<td>99</td>
<td>Cya 106F-Cya</td>
<td>D. Carlos Terrace</td>
</tr>
<tr>
<td>Chlorophyta chloroplast (EU175192)</td>
<td>98</td>
<td>Cya 106F-Cya</td>
<td>D. Carlos Terrace</td>
</tr>
</tbody>
</table>

* Closest relatives obtained by comparison with the NCBI database. Accession numbers of the closest related database entries are given between brackets.
the sunlight almost all day. Also because the abundant biomass in the Arches Yard represents a better substratum than the scattered lichen colonies found in D. Carlos Terrace wall. Bacteria need high moisture values (or substratum water activity) and organic matter from exudates of lichen hyphae to develop (Chen and Blume, 2002).

Regarding Bacteria domain, biodiversity is significantly higher in the Arches Yard than in D. Carlos Terrace, as correspond to a more humid microclimate and higher organic matter availability. Members of the Sphingomonadaceae, Flexibacteraceae, Bacteroidetes and Hymenobacter were retrieved. However, the similarities of these bacteria are enough low to ascribed the sequences to a determined phylogenetic affiliation.

Table 3 are presented the fungi identified. Most of the fungi were identified in the Arches Yard while in D. Carlos Terrace it was possible to retrieve some lichens.

4. Evaluation of efficacy of the applied treatments

4.1. Laboratory experiments

As shown in Fig. 6, fluorescence emission spectra of the mortars slabs before inoculation assure that no photosynthetic microorganisms were present on the slabs, as no emission on the correspondent spectra region of Chla was detected (650–700 nm). Immediately after inoculation of the mortars slabs, fluorescence emission was once again measured. Chl a is present, as a strong peak was detected at 683 nm, proving that all mortars were inoculated with success.

After the period of incubation (4 months), fluorescence spectra and quantification of Chla concentration upon extraction method were once again measured. Quantification of chlorophyll a content techniques (spectro-fluorescence and upon extraction method) allowed the evaluation of efficacy of the treatments. However, it must be stressed that the results obtained with the extraction method and the spectro-fluorescence method give different kinds of information, as the first one allows the quantification of the chlorophyll a content on the total mortars slabs volume and not just of its surface, as the spectro-fluorescence technique does. Sometimes, this fact has strong consequences on the data interpretation, as referred by Miller et al. (2010), namely when there is endolithic growth.

Tables 4 and 5 show the chlorophyll a values obtained by spectro-fluorescence and by extraction method, immediately after inoculation, after incubation and after application of the biocides, for AC mortar and AQz mortar, respectively. In general, all treatments were efficient, as all treated mortars present a lower chlorophyll a content than the untreated mortars for both types of mortars (Tables 4 and 5). However, the best results were obtained for the mortars slabs treated with TiO2 and Biotin T. As observed on Tables 4 and 5, these treatments present the lowest photosynthetic growth ratio of all treatments. However, these results show that only mortars slabs treated with anastase were able to mineralize organic matter, as the chlorophyll a content obtained after treatment was lower than the one estimated immediately after inoculation (Tables 4 and 5) and the growth ratio was approximately nule for AC and AQz mortars. These results prove the efficient photocatalysis power of TiO2 on the degradation of organic matter. Although the mechanism of biological inactivation is yet not very well understood, Wu et al. (2009), suggested that the anti-bacterial
effect of TiO₂ is attributed to the destruction of the bacterial cell wall and membrane by the photocatalytic oxidation process of TiO₂.

There is yet a lack of data regarding the speciation of the biocides. The growth ratio values are always referent to the untreated slabs of each mortar. Being the growth ratio of untreated mortars a maximum value – 100%.

% growth ratio values are always referent to the untreated slabs of each mortar. Being the growth ratio of untreated mortars a maximum value – 100%.

5. Discussion

The Palácio Nacional da Pena stands on the top of a hill above the town of Sintra, surrounded by a vast forested area and is a UNESCO World Heritage. This monument constitutes one of the major expressions of 19th century Romanticism in the world. The walls of the Palace were colonized by lichens and algae and the species distribution depends on the orientation, water availability, building material, etc. The identification of the lichens was based on morphological, anatomical and chemical data and follow Clauzade and Roux (2002). However, when using molecular tools we found identities that they do not correspond exactly with the conventional identifications. This is because, apparently, database of lichens are far from complete and, therefore molecular identifications, are in some cases incorrect. Crespo and Pérez-Ortega (2008) reported that there is a lack of correlation between phylogenetic and morphological data, at least for characters normally used in lichen systematics.

### Table 4

<table>
<thead>
<tr>
<th>AC mortar</th>
<th>Spectro-Fluorescence (cps at 683 nm)</th>
<th>% growth ratio</th>
<th>Extraction Method (µg Chla)</th>
<th>% growth ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>After Inoculation: 5.6 × 10⁸</td>
<td>100</td>
<td>6.9 × 10⁻⁵</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 5.5 × 10⁸</td>
<td>0</td>
<td>6.5 × 10⁻⁴</td>
<td>0</td>
</tr>
<tr>
<td>Anatas</td>
<td>After Inoculation: 5.5 × 10⁸</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 7.7 × 10⁴</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Fe–Anatas</td>
<td>After Inoculation: 5.6 × 10⁸</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 6.4 × 10⁴</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Feio D.D.S.H</td>
<td>After Inoculation: 5.6 × 10⁸</td>
<td>11</td>
<td>5.0 × 10⁻⁵</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 6.4 × 10⁴</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Biotin</td>
<td>After Inoculation: 5.6 × 10⁸</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After 2 weeks: 7.5 × 10⁴</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
</tbody>
</table>

% growth ratio values are always referent to the untreated slabs of each mortar. Being the growth ratio of untreated mortars a maximum value – 100%.

### Table 5

<table>
<thead>
<tr>
<th>ACQz mortar</th>
<th>Spectro-Fluorescence (cps at 683 nm)</th>
<th>% growth ratio</th>
<th>Extraction Method (µg Chla)</th>
<th>% growth ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>After Inoculation: 2.6 × 10⁵</td>
<td>100</td>
<td>6.9 × 10⁻⁵</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 5.8 × 10⁶</td>
<td>0</td>
<td>6.5 × 10⁻⁴</td>
<td>0</td>
</tr>
<tr>
<td>Anatas</td>
<td>After Inoculation: 8.5 × 10⁶</td>
<td>0</td>
<td>6.2 × 10⁻⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 9.0 × 10⁴</td>
<td>11</td>
<td>5.0 × 10⁻⁵</td>
<td>12.5</td>
</tr>
<tr>
<td>Fe–Anatas</td>
<td>After Inoculation: 5.5 × 10⁸</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After 4 months: 4.7 × 10⁵</td>
<td>0</td>
<td>6.2 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Feio D.D.S.H</td>
<td>After Inoculation: 2.6 × 10⁸</td>
<td>19</td>
<td>6.9 × 10⁻⁵</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>After the biocide: 2.6 × 10⁸</td>
<td>0</td>
<td>6.9 × 10⁻⁵</td>
<td>2.3</td>
</tr>
<tr>
<td>Biotin</td>
<td>After Inoculation: 1.1 × 10⁸</td>
<td>0</td>
<td>6.9 × 10⁻⁵</td>
<td>2.3</td>
</tr>
</tbody>
</table>

% growth ratio values are always referent to the untreated slabs of each mortar. Being the growth ratio of untreated mortars a maximum value – 100%.
and the synthesis of UV sunscreen compounds.

...solar radiation since they present distinct mechanisms to prevent that many cyanobacteria can inhabit environments with intense places exposed to sunlight, at 50 m height (the case of D. Carlos Terrace). Some authors (Garcia-Pichel and Castenholz, 1993) state that many cyanobacteria has been found in the walls of Arches Yard. Ortega-Calvo et al. (1993) surveyed the cyanobacteria and chlorophyta colonizing the terraces. This cyanobacterium is capable of fixing nitrogen directly from the air and it can transform molecular nitrogen gas into ammonia, which can then be assimilated into amino acids, proteins and other nitrogen-containing cellular constituents. This nitrogen-fixation capacity allows this cyanobacterium to live under severe environmental conditions or extreme habitats. For instance, deserts and grasslands lichens are typically associated with an array of soil cyanobacteria such as Nostoc species to form desert crust consortia (Graham and Wilcox, 2000). This might explain why N. punctiforme grow well in the walls of D. Carlos Terrace which are dryer and receive more sun than the Arches Yard. The others cyanobacteria also appear to prefer D. Carlos Terrace, in fact, only one cyanobacteria has been found in the walls of Arches Yard. Ortega-Calvo et al. (1993) surveyed the cyanobacteria and chlorophyta colonizing the walls of Salamanca and Toledo cathedrals. They found that samples taken near ground level (the case of Arches Yard) are characterized by the absence of cyanobacteria, which were however present in places exposed to sunlight, at 50 m height (the case of D. Carlos Terrace). Some authors (Garcia-Pichel and Castenholz, 1993) state that many cyanobacteria can inhabit environments with intense solar radiation since they present distinct mechanisms to prevent UV photodamage among which are the negative photomovements and the synthesis of UV sunscreen compounds.

Table 6
Colour variation measured on D. Carlos Terrace wall.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatase</td>
<td>5.36</td>
<td>5.00</td>
<td>-2.57</td>
<td>7.77</td>
</tr>
<tr>
<td>Biotin T</td>
<td>3.26</td>
<td>3.99</td>
<td>-1.67</td>
<td>5.42</td>
</tr>
<tr>
<td>Anisos</td>
<td>0.45</td>
<td>3.16</td>
<td>-1.88</td>
<td>3.70</td>
</tr>
</tbody>
</table>

Table 7
Colour Variation measured on the Arches Yard wall.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatase</td>
<td>10.49</td>
<td>3.31</td>
<td>-5.25</td>
<td>12.19</td>
</tr>
<tr>
<td>Biotin T</td>
<td>6.68</td>
<td>3.08</td>
<td>-2.46</td>
<td>7.76</td>
</tr>
<tr>
<td>Anisos</td>
<td>-0.59</td>
<td>1.34</td>
<td>-4.86</td>
<td>5.08</td>
</tr>
</tbody>
</table>

Although cyanobacteria appear in both walls, *Nostoc punctiforme* was only detected in D. Carlos Terrace. *Nostoc* primarily occurs in terrestrial habitats, frequently in association with fungi in lichens. This cyanobacterium is capable of fixing nitrogen directly from the air and it can transform molecular nitrogen gas into ammonia, which can then be assimilated into amino acids, proteins and other nitrogen-containing cellular constituents. This nitrogen-fixation capacity allows this cyanobacterium to live under severe environmental conditions or extreme habitats. For instance, deserts and grasslands lichens are typically associated with an array of soil cyanobacteria such as *Nostoc* species to form desert crust consortia (Graham and Wilcox, 2000). This might explain why *N. punctiforme* grow well in the walls of D. Carlos Terrace which are dryer and receive more sun than the Arches Yard. The others cyanobacteria also appear to prefer D. Carlos Terrace, in fact, only one cyanobacteria has been found in the walls of Arches Yard. Ortega-Calvo et al. (1993) surveyed the cyanobacteria and chlorophyta colonizing the walls of Salamanca and Toledo cathedrals. They found that samples taken near ground level (the case of Arches Yard) are characterized by the absence of cyanobacteria, which were however present in places exposed to sunlight, at 50 m height (the case of D. Carlos Terrace). Some authors (Garcia-Pichel and Castenholz, 1993) state that many cyanobacteria can inhabit environments with intense solar radiation since they present distinct mechanisms to prevent UV photodamage among which are the negative photomovements and the synthesis of UV sunscreen compounds.

Among the bacteria identified, the most abundant are sphingomonads, which are widely distributed in nature, having been isolated from many different soil and water habitats, as well as from plant root systems, clinical specimens, and other polluted environments.

Some of the fungi identified were previously related with building deterioration. Recently, Sert et al. (2007) identified new species of the genus *Capnobotryella* on monument surfaces. Their occurrence on marble monuments is associated with aesthetic degradation due to the colour changes and black spots. Others fungi are plant pathogens, such as *Mycosphaerella* and *Cercospora* (Goodwin et al., 2001).

Concerning the experiments performed in the laboratory and in Palácio Nacional da Pena walls, both demonstrate the high efficacy of anatase treatments for preventing (and treating) biodeterioration of mortars. The use of anatase photocatalysis, on or in building materials will be able to spare financial costs for cleaning and repairing procedures, as it is a more preventive and cheaper treatment than biocides. The microorganisms present in the Palácio Nacional da Pena walls should be monitored by molecular biology methods and also by colorimetric method for at least 12 months, on a monthly basis, in order to make sure that the Anatas treatrnent has a long lasting effect.

However, for the lichens and mosses colonizing mortars, a previous mechanical removal can facilitate the Anatas action. We found different morphologies of lichen thalli; fruticose like in *Ramalina* sp., squamulose like in *Squamarina* sp. and crustose like *Coloplaca* sp. All of them have stratified thalli, with an external cortex layer constituted by hyphal cells. The cortex protect the algal body. Furthermore, the rhizines that penetrate into the mortars are protected by the lichen thallus against Anatase and these hyphal cells, if airborne algal cells are captured, can generate a new lichen thallus. A similar process can be assumed for mosses, and rhizines can be protected in deeper mortar layers from the Anatas action.

6. Conclusions

Nowadays, from the conservation viewpoint it is important to control biodeterioration process with new environmentally friendly technologies. Therefore, this research allowed the authors to conclude that anatas photocatalyst is a better agent for preventing biodeterioration than the conventional biocides, conferring an excellent protective coating and self-cleaning properties to...
building materials. Moreover it is non-toxic and by consequence a good alternative to conventional biocides.

Although, it will be necessary to develop a further scientific research on anatase photocatalysis self-cleaning applications, the potential of combined nanotechnology and preventive conservation will allow a positive compromise for an environmental method of preventing biodeterioration of building materials.

Acknowledgements

The authors wish to thank Parques de Sintra – Monte da Lua for the possibility to study this beautiful monument that is Palácio Nacional da Pena. We also wish to thank the Departamento de Quimica - REQUIMTE for technical and experimental support. Acknowledgements are also due to the CICEGE-FCT/UNL, for SEM-EDX facilities, and to the DCT-FCT/UNL, for the use of the terrace for external conditions experiments. This is a TCP CSD2007-00058 paper.

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