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Phototrophic Biofilms on Exterior Brick Substrate

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Abstract

La Plata Cathedral is considered a historical monument and the most important and characteristic building in the city. The aims of this work were: to identify the taxa of phototrophic organisms that inhabit on the brick walls of the Cathedral, in order to investigate phototrophic biofilm formation and to assess the risk of biodeterioration, biopitting, and to relate them to the microclimatic conditions that affect the temple and the characteristics of material. Different types of growth of phototropic biofilms sampled were: i) the green one, which is present on the south-east wall, and had moss, genus Henediella, as an external layer and Chlorophyta (Chlorella sp. and Chlorococcum sp.) joined to Cyanobacteria (Synechococcus sp. and Synechocystis sp.); ii) the black one, which was sampled in several areas of the Cathedral. This phototropic biofilm showed pedominant filament forms; iii) the black muddy one combined with a great amount of muddy material which comes from a conduit; here the predominant forms were Chlorophytes (Trentepohlia sp. and Printzina sp.). The great diversity of Pennales Diatoms was a characteristic shared among all the biofilms. Under laboratory assays we observed grooves and biopitting caused by the attack of phototrophic biofilms on the substrate (brick).

Keywords: Biodeterioration; Biopitting; Cultural heritage; Phototrophic biofilms

Introduction

Monuments exposed to the open air over a long period of time deteriorate due to various causes: sun, wind, rain, atmospheric pollution, etc., all these factors contribute to the process of weathering. Sometimes, these pollutants react with materials, affecting their properties; otherwise, they can act as catalysts of the deterioration by other chemical, physical or biological routes [1].

Biofilms are interface micro-habitats formed by microbes that differ from those of the environment. The term 'sub-aerial biofilm' (SAB) was coined for microbial communities that develop on solid mineral surfaces exposed to the atmosphere.

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SAB are ubiquitous, self sufficient, microbial ecosystems that are found on buildings, bare rocks in deserts, mountains, and at all latitudes where direct contact with the atmosphere and solar radiation occurs. Given their characteristic slow and sensitive growth, SAB may also serve as bioindicators of atmospheric and/or climate change. SAB on exposed terrestrial surfaces are characterized by patchy growth that is dominated by associations of fungi, algae, cyanobacteria and heterotrophic bacteria [2-4]. The microorganisms form biofilms and play a key role because of their interactions with physical and chemical agents. The colour texture and appearance of a SAB vary with its microbial composition, which in turn depends on climatic conditions, the nature of the substrate and duration of exposition. Mild, humid conditions allow development of phototrophs, lichens and mosses amongst the microbial consortia.

Prevailing microclimatic conditions of light regime, humidity and temperature have been considered the major factors determining the colonization of building surfaces by microorganisms [5,6]. These factors are influenced by building orientation [7].

The phototrophs organisms, cyanobacteria and algae have been considered the first colonizers of building surfaces [7,8], conditioning the inert surfaces for the growth of heterotrophic organisms, such as fungi. They are commonly found on buildings in humid places, growing on cornices, in holes and crevices or been.

The biofilms formed by phototrophs organisms vary in colour, according to the characteristics of the taxa, from greenish-yellow to dark green and black and from rosy to grey and brown. The morphology and colour of these patinas can change, depending on the physiological cell state and the environmental conditions.

The occurrence of phototrophic microorganisms on stone surfaces does not automatically imply a destructive action. However, considering biodeterioration as any undesirable change in the properties of a material caused by the vital activities of microorganisms [9], the presence of algae on building stones can be considered as biodeteriogenic, simply because of the aesthetic damage they cause. This type of biodeterioration mechanism is called functional degradation or soiling. Many authors have found that local environmental conditions are more important than substrate in determining degree and type of soiling and biofilm formation [8,10,11].

The aggressive action of cyanobacteria and algae in relation to the substratum where they develop has been considered negligible by some authors. These biofilms contribute to the deterioration of building materials [5]. Apart from the unaesthetic appearance evident in most of the reports on algae on historic buildings, there are references in the literature that point to direct decay mechanisms. In fact, it has been reported that epilithic cyanobacteria may play a role in rock surface weathering in nature through various effects upon the carbonate dissolution system. These problems reach further economic and social dimensions when the colonized substrata belong to cultural properties.

In recent decades, biodeterioration processes have increased significantly, especially in urban areas due to increasing environmental pollution. The chemical compounds in the atmosphere as SO_4, NO_3 and NH_3, are nutrients for algae.

La Plata Cathedral is located in the center city, Buenos Aires province, Argentina. It is a neo-gothic temple. The construction of the cathedral started in 1884, when the first stone was placed and after an interruptive construction, the temple was opened in 1932.

The aim of this work is: i) to identify the taxa of phototrophic organisms that inhabit on the brick walls of La Plata Cathedral, in order to investigate phototrophic biofilm formation, and biopitting and to assess the risk of biodeterioration and ii) to relate them to the microclimatic conditions that affect the temple and the characteristics of material such as composition, texture, slope, etc.

Experimental Section

Study building and visual inspection

La Plata has an average annual temperature of around 16.3°C and the average annual rainfall is 1023 mm. As the city is close to the La Plata River, it tends to be rather humid, with an average annual relative humidity of about 77 %. The air temperature during the sampling period was approximately 20°C and the relative humidity 50-55%.

Its walls are made predominantly of brick and mortar; concrete and iron reinforced structures complete the list of main building materials [12].

Visual inspection of crypts of historical and architectural interest showed clear evidence of significant phototrophic biofilms. Three zones were selected for the study with different biofilms in the Southwestern (SW) wall: a, b and c. The wall a) was green with a moss physiognomy b) was black and c) black muddy too and was receptor of a drain conduit. The front of the cathedral (14th St. NE) was not sampled as it did not present serious problems and damage; therefore there were no biofilms (Figure 1).

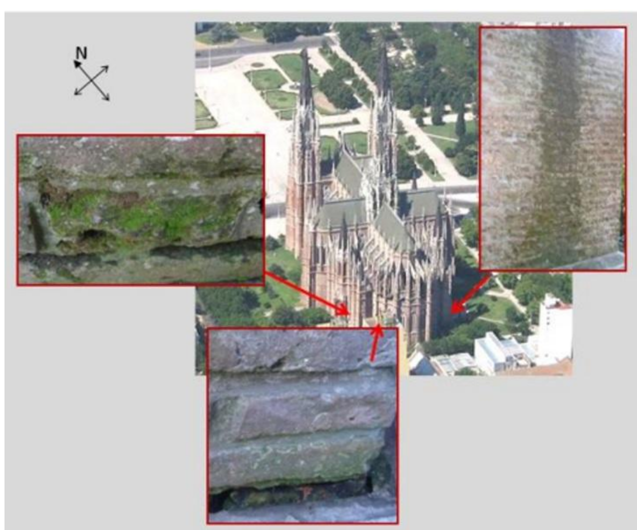

Figure 1: Different phototrophic biofilms were sampled at the cathedral: Biofilm green (left), Biofilm black (bottom), Biofilm black muddy (right).

Sampling strategy

Phototrophic biofilms (1 cm^2) were gently scrapped, using a sterile scalped. Scrapping samples were placed into sterile saline solution and then taken to the laboratory. In the laboratory, the same scraping samples were inoculated into BG11 liquid media [13] and the other samples were preserved in formaldehyde 4%, examined microscopically with an inverted microscope Olympus BX51 and placed on clean slides and then sprayed with sterile water for taxonomically identification using as keys Prescott [14].

Phototrophic biofilm formation

Biofilms were grown in the laboratory in liquid medium BG11 for multiplying the biomass of microorganisms investigated. After 30 days of culture, the biofilms were inoculated from each culture (5 mL) on brick samples of about 2 cm^2 previously sterilized. Brick inoculated were grown in petri dishes under light conditions, humidity, temperature controlled, 16/8 (light/dark) and saturated atmosphere of humidity at 28°C and was sprayed with BG11 liquid to promote the metabolic activity of the microorganisms. The cultivation time is approximately 2 months.

Studies of phototropic biofilms by microscopic techniques

The brick samples and small pieces of biofilms brick were observed by optical microscope (OM) Olympus BX51, stereoscopic microscope (SM) Nikon SMZ-10, and the samples were metalized with Au/Pd and observed by scanning electron microscope (SEM) Jeol 6360Lv.

Results and Discussion

Characterization of the phototrophic biofilms

The distribution of biofilms throughout the cathedral suggests major problems of biofouling in the back (15^{th} St. SW) and the southeastern walls, (53^{rd} St. SE) of the building, where the light intensity is low and humidity is high. These two ecological factors make these areas conducive to the development of epilithic communities visible to the naked eye as biofilms places (Figure 1).

The Green biofilm (Figures 1 and 2) has a main macrophyte a moss of the genus *Hennediella* (Pottiaceae), where are present between its thalli great diversity of microorganisms such as bacteria, cyanobacteria, algae and fungi. A large quantity and diversity of diatoms pennales were found, and together with the unicellular Chlorophyta from the genus *Chlorococcum* and *Chlorella* there are abundant forms of microflora, the genus *Apatococcus*, and Cyanobacteria Chroococales (*Synechocystis* sp.) which has well known species that cause biofouling upon walls were observed. It is interesting to note the presence of hypnozygote, which are resistance forms of some Chlorophyta (Ie: *Chlorococcum* sp.) and the result of the sexual reproduction, that occur during stress cycles, for example lack of water, corresponding to the drought in the last months. This shows the capacity of these algae to survive unfavorable periods of time, reappearing when the environment conditions are better. Further is evident the amount of dead diatoms, their own frustules are the proof of it, that could be the result of the water stress that previously mentioned.

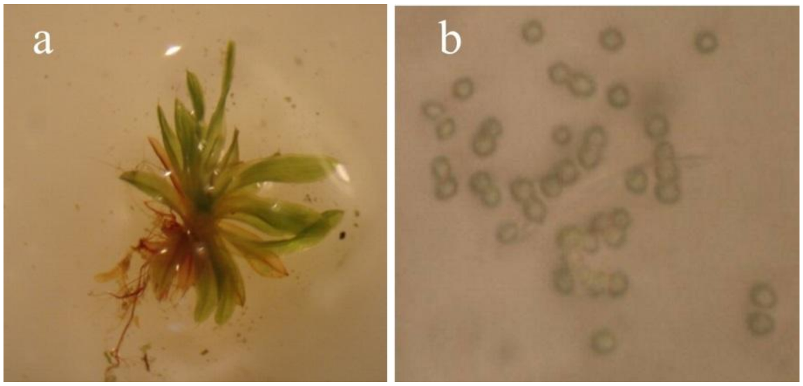

Figure 2: OM image of the green biofilm samples. a) *Hennediella* sp.; b) *Synechocystis* sp.

The Black biofilm (Figures 1 and 3) the main taxa are from *Klebsormidium* cf *flaccidum*, a typical Charophyta of this enviroment, joined to filament cyanobacteria, such as *Oscillatoria* sp. and *Pseudoanabaena* sp. A filament Chlorophyta, *Stichococcus bacillaris*, is also present in this biofilm.

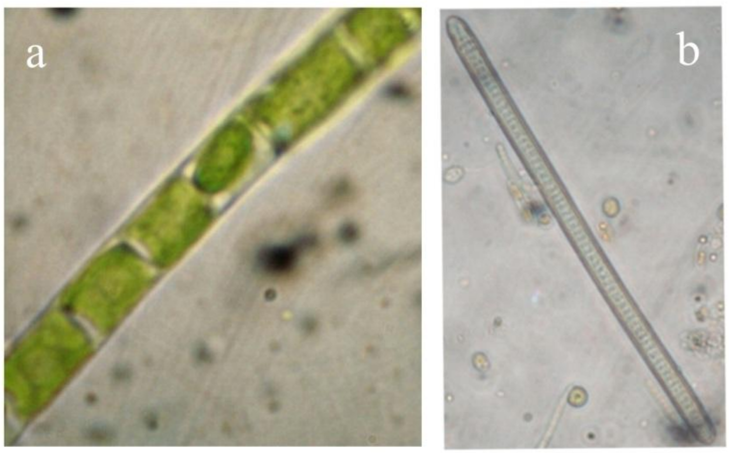

Figure 3: OM image of the black biofilm samples. a) *Klebsormidium* sp.; b) *Oscillatoria* sp.

The Black muddy biofilm (Figures 1 and 4) characterized by the presence of branched Chlorophyta, of the order Trentepholiales. *Trentepohlia* and *Printzina* genera were found. In this biofilm the cyanobacteria were almost absent; there were only a few specimen of the genus *Cyanothece*. All the species, filament and colonial ones were found attached to the mud.

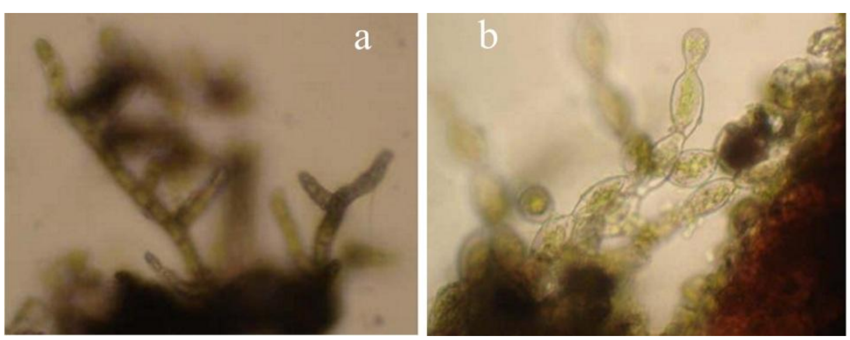

Figure 4: OM image of the black muddy biofilm samples. a) *Trentepohlia* sp.; b) *Printzina* sp.

In the front of the cathedral (14$^{th.}$St. NE) no was observed phototrophic biofilms formation probably due to the intensity of light recorded in this area leading to low relative humidity (extreme environment).

The walls facing the southeast (53rd St. SE) presented greenish biofilms (Figures 1 and 4) covering much of the surface of bricks and joints moreover, a considerable growth of vascular plants was observed. The presence of biological growth on the walls oriented towards the southeast is due the shortest period of exposure to direct sunlight and was verified that the area is hardest hit by the winds and rains during the winter.

The walls facing the NW, (51st St. NW) did not present greenish biofilms covering the surface of bricks; moreover, a considerable amount of guano of pigeons was observed.

Stone surfaces and other inorganic materials are used as substrates by a wide variety of microorganisms, chemoorganotrophs, chemolithotrophs and phototrophs, actinomycetes, fungi and lichens [15].

Complex microbial communities immersed in extracellular polymeric substances (EPS) that constitute biofilms and biofouling on the surfaces of structural materials lead to biodeterioration. The major compounds of the EPS are polysaccharides [16]. Biodeterioration effects include (a) chromatic changes on the stone substrate produced by biogenic pigments, which, depending on the size of area they occupy, can alter the aesthetic appearance and thus the interpretation of the work of art; (b) the mechanical stresses on the mineral structure caused by EPS, which increase with successive hydration and drying cycles; and (c) of atmospheric pollutants adsorbed by the biofilm [17].

In recent decades, biodeterioration processes have increased dramatically, especially in urban areas due to increased air pollution. Chemical compounds in the atmosphere as SO_4, NO_3 and NH_3, are nutrients for algae. According to information obtained from 6 different weather stations Argentina, La Plata has one of the most corrosive atmospheres in the country with high concentrations of SO_2 deposition (values from 6.20 to 8.21 mg/m^2/d.) [18].

The presence of cyanobacteria and algae and their metabolic activity contributes to different accelerating and catalytic effects on the formation of crust and also to a biogenic coloring of the stone with a consequent loss of aesthetic value. Darkly colored biofilms on natural stone or buildings of monuments enhance the physical stress of the structural material by its increase in specific heat uptake as well as it alteration of thermalhydric expansion behavior and moisture retaining capacity [19].

Coccoid-shaped cyanobacterial cells like *Gloeocapsa* sp. have been shown to be involved in degradation and boring into limestone [20,21].

Trentepohlia sp. algae present in the walls of cathedral, has long been recognized as biodeteriogenic on buildings and is well known for its production of pink/brown discoloration [22] caused by biogenic pigments. It has also been associated with mechanical degradation of monuments in Spain [23] and Mexico [24]. Pigment production in these biofilms is an adaptation that increases resistance against environmental stress. The stone surface showed surface erosion in the areas [6,25].

All diatoms found (Figure 2) belong to the order Pennales with rafe; this structure is associated with the biosynthesis of mucopolisaccharides, for the fastening and movement. This material contributes to the biofouling in its capacity to catch materials in suspension and consequently in the damaging the substrate (Figure 5).

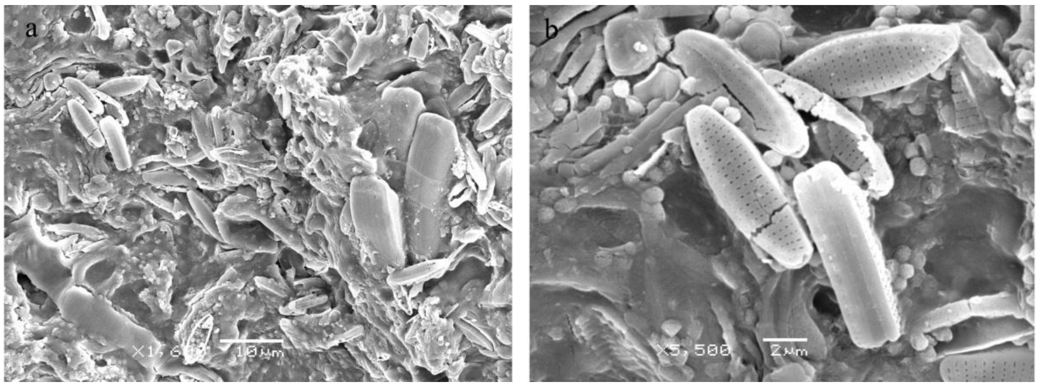

Figure 5: SEM image of a) biofilm forming to brick substrate (1600X); b) in more detail can be observed diatoms and signs of degradation of brick caused by phototrophs (5500X).

We can observe "biopitting" (Figure 5) caused by the endolithic algae attack on the substrate (brick) known as micropitting [26].

Under optimal laboratory conditions, the growth of phototrophic organisms (Figure 6a), the extracellular polymeric substances (EPS), and colour intensity (Figure 6b) were observed. These biofilms contribute to deterioration of building. The brick shows signs of degradation caused by phototrophic organisms (Figure 7).

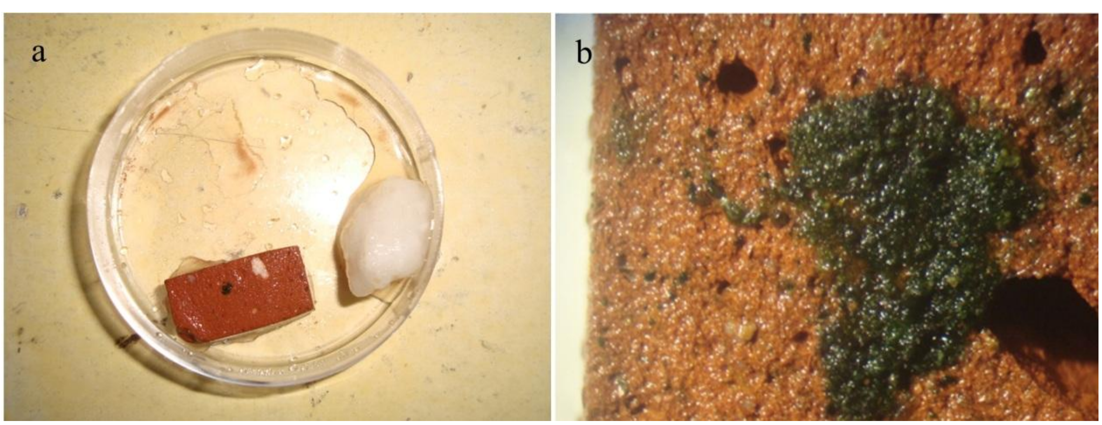

Figure 6: a) Image of inoculated of mixed cultures consisting of phototrophic algae on brick; b) Detail of grow of phototrophic biofilm on brick after two month.

SABs also excrete multifunctional mucilage comprising acidic polysaccharides, organic acids, proteins and complex-forming agents. This gel-like matrix serves to anchor the SAB to the solid surface, to capture moisture as well as substances from the air but also as the interface with the underlying substrate. Organic acids released from the mucilage help solubilise essential minerals but also cause pitting of the substrate which allows material penetration by filamentous SAB consortium members [13].

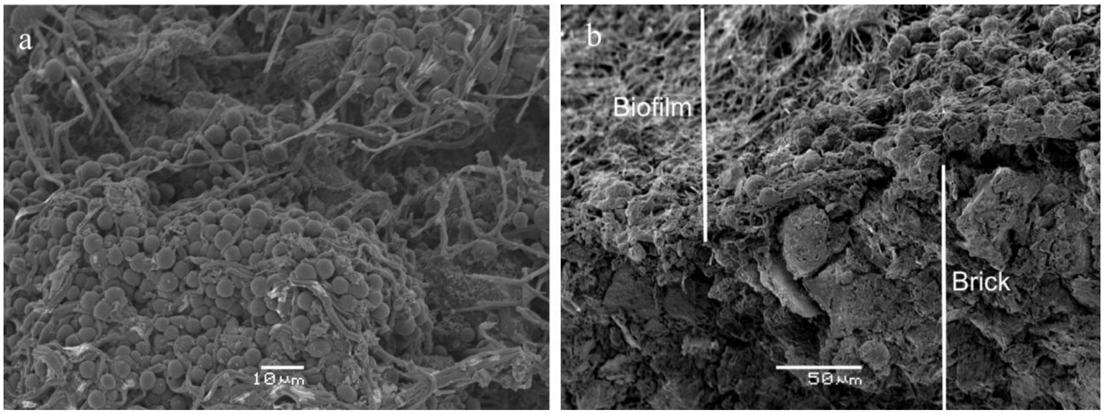

Figure 7: SEM image of a) phototrophic biofilm under laboratory conditions after two months (1000 X); b) lateral view of phototrophic biofilm formed on brick substrate (400X).

Conclusions

The diversity of algae specifies showed variations depending on the environment in which the biofilms were developed.

In this research, we can see biopitting and grooves caused by the attack on the substrate (brick) due to algae, endolithic algae forming phototrophic biofilms that excrete acid metabolites that contributed to the geochemical processes.

This type of biodeterioration can be markedly potentiated for the effects of environmental factors such as humidity and temperature, in addition to natural and anthropogenic pollution. An important role of atmospherically pollutions in the structural materials would be occurring. The Cathedral is located in a mixed urban environment with aggressive characteristics from industry.

These studies contribute to the understanding of material transformations under environmental influences and different associated causes.

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