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INDUCED BIOLOGICAL COLONIZATION ON MODEL HISTORICAL GLASSES AND BIOCIDE TREATMENT EFFECTS FOR ITS ELIMINATION

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Abstract

Biological colonization and later biodeterioration damage are well-known phenomena affecting cultural heritage. Glasses, as those from historical stained glass windows, are frequently biocolonized under certain conditions. There is still limited knowledge about biocolonization growth patterns on glass and safe removing procedures to act before irreversible biodegradation damage occurs. This paper presents the results of an experimental study in which biological colonization of three different model historical glasses, namely soda-lime, lead crystal, and potash-lime silicate glasses, has been naturally induced. Glass samples were exposed for 13-15 months to natural conditions at the mountain region near Madrid (Spain) and monitored over time. After exposition, samples were first observed through fluorescence microscopy (FM), scanning electron microscopy (SEM), and UV-Vis-NIR spectrophotometry. Next, the Acticide® CF biocide was applied on glass samples exposed and were then again observed through the same techniques to evaluate its effect. Biocolonizers detected were algae, fungi, cyanobacteria and heterotrophic bacteria in different combinations and amounts and sometimes forming a biofilm. Soda-lime silicate glasses were the ones that showed the highest biocolonization growth, while lead crystal silicate glass appeared more biocolonized than the potash-lime one. The biofilm formed appeared intensely damaged and was practically no longer visible after the biocide treatment which proves its efficiency for removing glass biocolonization.

Keywords*: Biodeterioration; Biofilm; Glass; Acticide® CF biocide; Removal procedure*

Introduction

Biological colonization and subsequent biodeterioration damage are well-documented phenomena that affect an important part of the built cultural heritage [1, 2]. As other building materials, glasses are also commonly biocolonized under certain conditions [3, 4]. In historical buildings glass plays an important role in stained glass windows, especially in those from Medieval and Renaissance times but also in modern ones [5]. Most common biodiversity found on glass surfaces have been algae, fungi, cyanobacteria and bacteria, which are able to take the compounds needed to grow from the glass itself [6, 7]. Biodeterioration decay causes serious damage on historic stained glasses not only from a structural point of view but also from an aesthetic one, since glass loses its transparency and even its painted iconographic motifs made with grisailles and, consequently, civil or religious buildings may lose most of its internal lighness

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and beauty [3, 8]. Both biodeterioration and biodegradation are sometimes key factors when conservation and restoration work need to be undertaken in historic buildings and architectural cultural heritage. Laser cleaning has been sometimes used to eradicate biogenic layers growth on glass surfaces [9, 10]. However, a biocidal method or a combined procedure of biocide plus some additional instrumental technique has been rarely used, in contrast with those used in other materials such as stone [11] or ceramics [12, 13] in which, more recently, eco-friendly biocides have also been used [14].

Despite glass biological colonization has been widely studied [3, 6-8], there is still limited knowledge about biocolonization growth patterns on glass and about safe removing procedures to promptly act against irreversible biodegradation damage, since it is known that common methods of cleaning of stained glass windows, such as those using water and ethanol solutions, do not fully remove biocolonization [4].

The research presented in this paper is derived from an experimental study in which biological colonization of three different model historical glasses has been naturally and continuously induced. The main aims of this research were to more deeply know biocolonization patterns on historical glasses and the usefulness of a biocidal treatment as a first approach to remove and, if possible, to fully eradicate colonizing species of the glass.

Experimental

Model historical glass samples

To carry out the experiment a set of six glass samples of three different glass compositions (total=18 glass samples) were selected. The three compositional types were: soda-lime silicate (Na_2O-CaO-SiO_2), lead crystal (K_2O-PbO-SiO_2), and potash-lime silicate (K_2O-CaO-SiO_2) glasses. The first and third compositions encompass the most representative glass compositions of historical interest, especially for Medieval and post-Medieval European stained glass windows [15-17], while the second one is representative of high quality ornamentation and tableware items, above all since the late seventeenth century AD. The first glass selected is a common soda-lime silicate glass similar to those currently employed for window glazing. The second glass is a regular lead crystal silicate glass following the Spanish Standard for this type of glasses [18] in which the lead oxide content (PbO) must be $\geq$ 24 wt. %. The third glass is a potash-lime silicate glass which was melted in the laboratory with a Pyrox electric furnace model VL 110 from pure chemical reagents. All the glass samples were prepared in flat pieces of approximately 2x1x0.2-0.5cm in size. All of them where colorless glasses since no chromophores were used to impart them color. Soda-lime glass samples were identified by "Na", lead crystal glass samples by "Pb", and potash-lime glass samples by "K" (Fig. 1a).

The six samples of each glass composition were arranged as follows. The surface of three of them were treated with dilute hydrofluoric (HF) acid (5% by volume) to faster induce and accelerate biological colonization, while the remainder three samples were left untreated. Acid attack corrodes glass surface making it rougher, which can be supposedly more suitable and attractive for microorganisms' colonization than flat and smooth surfaces of untreated glasses. Acid attack firstly causes the leaching of alkaline ions (Na^+, K^+) from the glass surface, a process named dealkalinization, and secondly induces the dissolution of the silica glass network and all the ions bonded to it [19, 20]. Fig. 1b shows the arrangement of samples using potash-lime silicate glasses (K) as an example. Two of the couples (K1 and K2) were exposed in two different places (see next section) while in the third couple one sample was left as witness control and the other was used for chemical analysis. Soda-lime (Na) and lead crystal (Pb) silicate glass samples followed the same arrangement scheme.

Chemical composition of the three types of glasses is shown in Table 1. Elemental determinations were obtained trough X-ray fluorescence spectrometry (XRF) from pressed pellets prepared by grinding glass samples in an agate mortar, with external surfaces removed to

avoid contaminations. Analyses were carried out by using a PANalytical Axios wavelength dispersed X-ray spectrometer equipped with a tube of rhodium of 4kW and 60kV. Analytical determinations were undertaken through the standard-less analytical software IQ+ (PANalytical) from synthetic oxides and natural minerals.

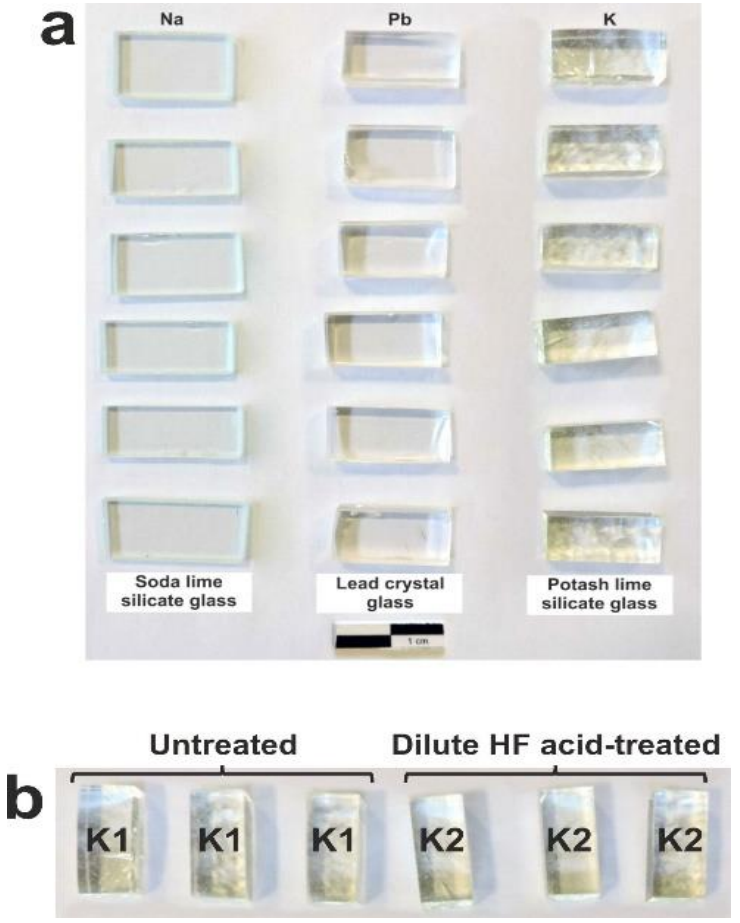

Fig. 1. Glass samples: a) Set of glass samples selected. Scale in cm;
b) Arrangement of samples using potash-lime silicate glasses (K) as an example

Table 1. Chemical composition of glass samples determined by XRF (wt. %).

Oxide	Type of glass		
	Soda-lime silicate (Na)	**Lead crystal (Pb)**	**Potash-lime silicate (K)**
Na_2O	15.72	4.98	1.14
MgO	1.05	---	2.49
Al_2O_3	2.25	0.14	3.21
SiO_2	71.65	54.16	47.83
P_2O_5	0.01	0.01	3.37
K_2O	0.76	8.26	19.18
CaO	8.47	0.06	22.65
Fe_2O_3	0.06	---	0.08
BaO	0.03	2.62	0.05
PbO	---	29.77	---

--- not detected

Experimental setup for induced biological colonization

Each set of six glass samples was mounted on a round plastic dish. Glass samples were attached to the dish with small plastic ties. Once assembled in this way, glass samples were continuously and uninterruptedly exposed to natural weather conditions in the mountain region near Madrid city, which is located in the center of Spain. One of the sets (Dish A) was exposed at Hoyo de Manzanares (1000m altitude) for 15 months (December 2015 to March 2017), and the other one (Dish B) at Bustarviejo (1222m altitude) for 13 months (January 2016 to February 2017) (Fig. 2). Both places are located at an altitude ≥1000m above the sea level and are climatologically very similar. They were selected to find adequate relative humidity and temperature conditions which favour the growth of biological colonization on glass samples, and to mutually confirm biodeterioration results in the two locations. In this mountain region of Madrid summers are short and dry while winters are long and cold with average annual maximum temperatures between 6-8°C in January and 27-30°C in July and August, relative humidity between 37% in summer and 76% in winter, and average precipitation of 55mm in spring/fall and around 10 mm in summer. Dish A was placed at the foot of a juniper and Dish B at a mini oak grove, both in private gardens. Both dishes were secured with a granite stone to prevent the wind and other weather inclemencies would move them.

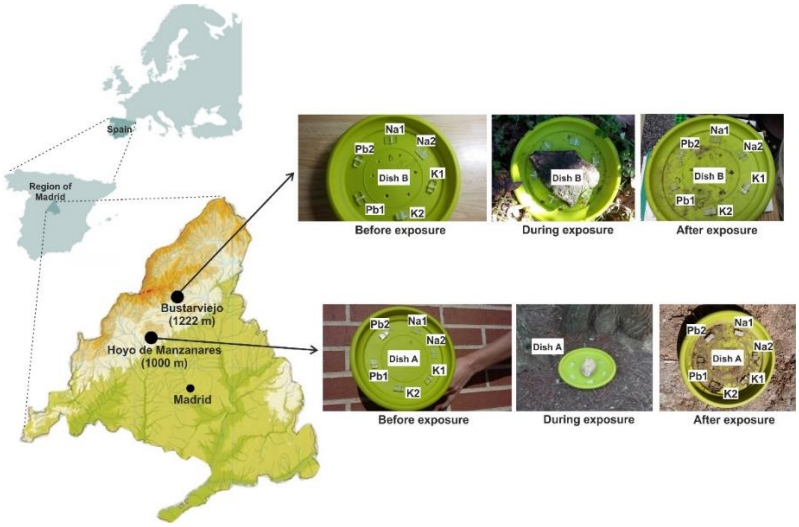

Fig. 2. Map showing locations of exposition and disposition of glass samples in the dishes

Biocide treatment

After the exposure periods aforementioned, half of each glass sample exposed was treated with the Acticide® CF biocide supplied by Thor Specialties S.A. Components and details of such biocide are provided among others in this work [12]. The Acticide® CF biocide was applied with a very soft brush. After application, glass samples were left outdoor inside an uncovered Petri cell-culture dish for 1 month to let the biocide work.

Observation and analytical techniques used

Each set of samples, which was exposed at a different geographic location and different microclimatic conditions as previously stated, was firstly observed after the exposure period and was again observed after the effects of applying the Acticide® CF biocide outdoor for 1 month. Observation and analytical techniques used were the following: optical microscopy in transmitted light (OM), fluorescence microscopy (FM), scanning electron microscopy in both secondary electron (SEM-SE) and backscattered electron modes (SEM-BSE) with energy dispersive X-ray spectrometry (EDS), and UV-Vis-NIR spectrophotometry.

Optical (OM) and fluorescence microscopy (FM)

Glass samples were observed using Zeiss Axioimager D1 fluorescence microscope with Plan-Apochromat x63/1.40 oil immersion objective, Carl Zeiss, Germany. Autofluorescence signals of algae were recorded using Rhodamine Zeiss Filter set 20 with excitation/emission: 540-552/567-647nm lengthways. A CCD Axiocam HRc Rev 2 Zeiss camera along with a Carl Zeiss AxioVision 4.7 software were used to capture and record bright field images and autofluorescence signals.

SEM

Either glass surfaces or cross-sections were studied using SEM-SE, SEM-BSE and EDS (elemental distribution maps). Glass surfaces were examined after air drying at least for 24h in SEM chamber at low vacuum with 20kV acceleration potential using FEI Inspect-S SEM equipment, Kyoto, Japan. Cross-sections of the biofilm grown/glass substrate interface were prepared for SEM-BSE using the method developed by Wierzchos and Ascaso [21]. These samples were cut to expose cross-sections of both the biofilm grown and glass substrate. First, the samples were fixed in glutaraldehyde 3.25% in phosphate buffer and osmium tetroxide 1% in the same buffer, dehydrated in an ethanol series and embedded in LR-White resin. After polymerization at 60°C for 48h, the samples were transversally cut with diamond saw and surfaces were fine-polished and carbon coated. Cross-sections obtained were examined using FEI Inspect-S SEM equipment with a solid-state four-diode BSE detector operated at 20-25kV acceleration potentials.

UV-Vis-NIR spectrophotometry

An Ocean Optics HR 4000 CG equipment was used. Transmittance spectra were acquired and recorded in the range of 200 to 1100nm.

Results and discussion

Macroscopic observations and loss of transparency

Images of figure 3 shows glass samples before and after exposition. Although visible spectra were acquired from all the samples, next to images some selected spectra derived from untreated and dilute HF acid-treated glass samples before and after continuous exposition in both sites are also shown in figure 3. Regarding the glasses before being exposed and in comparison, with untreated ones, dilute HF-treated K2 samples were the glasses that lost the most transparency (almost 60% approximately), while Pb2 samples lost about 42% and Na2 samples around 20%.

As expected, dilute HF acid-treated glass samples were relatively more biocolonized after exposition than those untreated. This is particularly visible above all in glass sample Na2. In addition, Na2 samples from both exposition sites were the glasses that lost the most transparency (more than 80%). Pb2 samples lost a little less than 80%, while the loss of transparency of K2 samples was around 60 %. These data therefore confirm that the synergic effect of both chemical alteration and later biocolonization cause a high loss of transparency, especially in soda-lime (Na) and potash-lime (K) silicate glasses, which may have an important negative influence on indoor lighting of buildings, as well as on reading of iconographic motifs if glasses belonged to historical stained glass windows.

Biological colonization of glasses after exposition

There was an important biocolonization difference in the case of K glasses, between those that were treated with dilute HF acid and those untreated. Untreated glass showed a very smooth surface in which only some algae were observed, both when its surface was observed (Fig. 4a), or when a cross section of the glass was made, as in Fig. 4b. However, after treatment with dilute HF acid, the glass surface becomes rougher and houses numerous fungal hyphae as well-established biocolonizers in the substrate, as can be seen in Fig. 4c. In this figure, algae are also observed in a quite dehydrated state. It may be algae trapped by the network of fungi that colonize these glass samples treated with dilute HF acid.

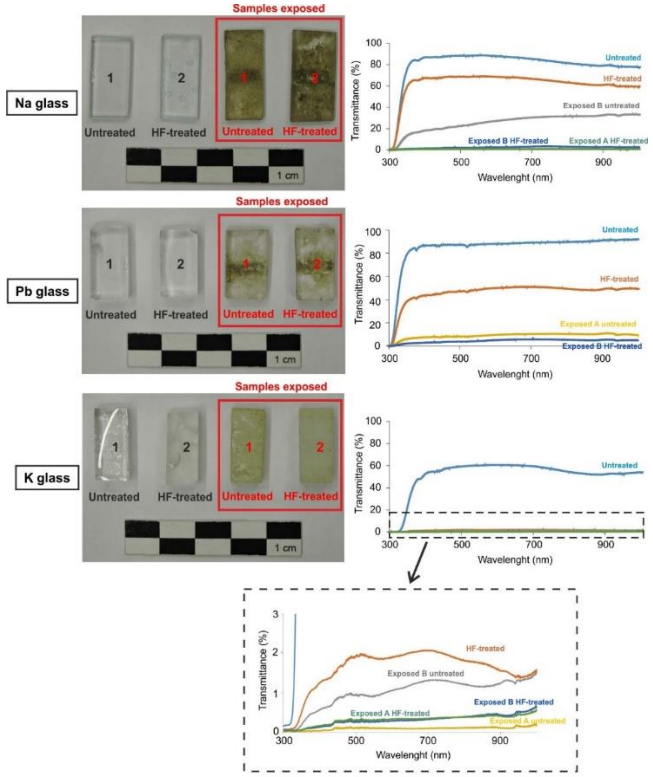

Fig. 3. Images of glass samples before and after exposition and visible transmittance spectra of glass samples from both locations

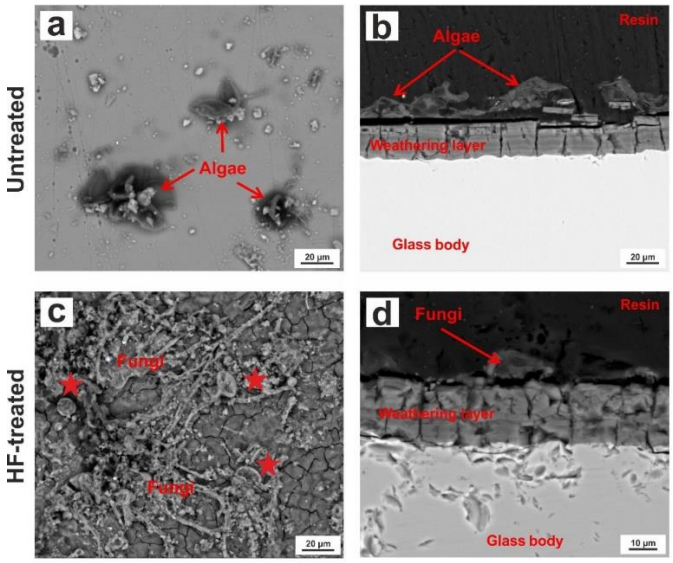

Fig. 4. SEM-BSE micrographs of biological colonization from K glass samples:
a) Surface of untreated glass showing little colonization, fundamentally algae;
b) Cross-section of untreated glass showing algae in the upper part;
c) Surface of dilute HF acid-treated glass showing colonization by fungi and some algae (stars);
d) Cross-section of dilute HF acid-treated glass showing fungi in the upper part

A fragment of fungi can be seen in the cross-section of Fig. 4d. Thickness of the weathering layer, around 20µm compared to that of Fig. 4b, is remarkable and was obviously favored in part by the treatment with dilute HF acid. K glass is the type of glass that showed the least chemical durability. This weathering layer is characterized by the superficial dealkalinization of glass produced by the leaching of K_2O and CaO and the relative enrichment of SiO_2 which forms a silica gel layer [19].

Biological colonization of Pb glass samples, untreated (Pb1) and dilute HF acid treated (Pb2), are shown in figure 5. In general, Pb glasses exhibited higher biocolonization than K glasses, showing a biofilm of about 10µm of average thickness. Fungal cells with collapsed aspect and some algae present in both samples (Pb1 and Pb2) were observed by OM (Fig. 5b and f) as well as by SEM-SE (Fig. 5a and e). Images of FM (Fig. 5c and g) showed the algae chlorophyll autofluorescence signal. This chlorophyll signal in algae could indicate good physiological status or at least good chlorophyll preservation.

In the case of Pb glass samples, when observations were made from cross-sections, it was possible to better observe the intense induced biocolonization occurred during the exposure time. Micrographs of Figs. 5d and 5h were obtained by SEM using detector in the backscattered electron mode (BSE). They were taken on a sample transversely cut and surface polished, after fixation and corresponding preparation of the microbiota, to provide the cells with the required contrast. With this procedure an increase in the contrast of the membranes is achieved, which allows a better observation in the BSE mode, both in untreated and dilute HF acid treated Pb glasses. In acid treated glasses, algae seem to have a greater facility to form and maintain colonies, even though they did not appear to be much more colonizable than untreated glass samples.

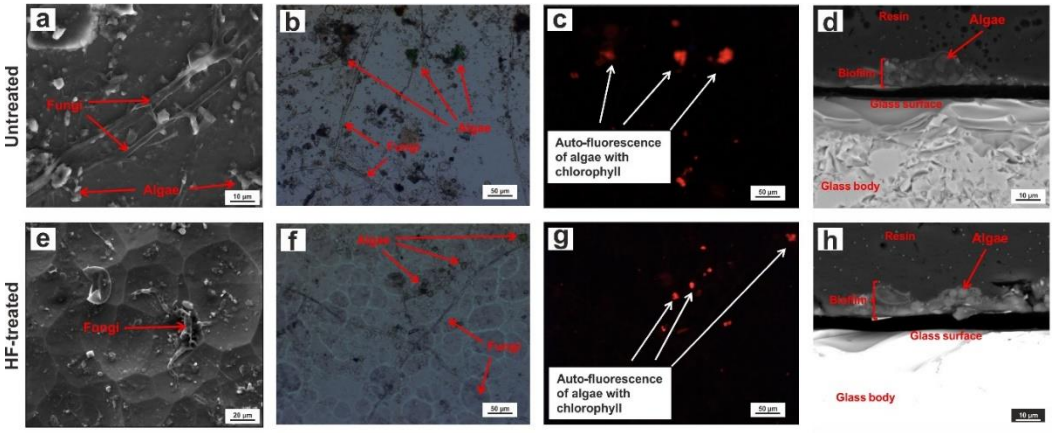

Fig. 5. SEM micrographs, OM and FM images of biological colonization from Pb glass samples:
a) Surface of untreated glass showing fungal cells collapsed and some algae, SEM-SE;
b) Surface of untreated glass showing fungi and algae, OM;
c) Surface of untreated glass showing algae chlorophyll autofluorescence signal, FM from zone in "b" image;
d) Cross-section of untreated glass showing algae, SEM-BSE;
e) Surface of dilute HF acid-treated glass showing fungi and some algae, SEM-SE;
f) Surface of dilute HF acid-treated glass showing fungi and algae, OM;
g) Surface of dilute HF acid-treated glass showing algae chlorophyll autofluorescence signal, FM from zone in "f" image;
h) Cross-section of dilute HF acid-treated glass in which algae that come together in colonies are easily distinguishable, SEM-BSE

Among the three types of glasses exposed, Na glasses were the samples that showed the most severe biocolonization as demonstrated by their well-developed biofilm. Fungi, algae, and bacteria were observed in both untreated and dilute HF acid-treated glass samples. Fungal cells

with collapsed aspect and algae were observed by OM (Fig. 6a and b). These latter were unequivocally distinguished by the chlorophyll preservation signals (Fig. 6b). Algae and bacteria colonies were also observed in a cross-section of the biofilm (Fig. 6c).

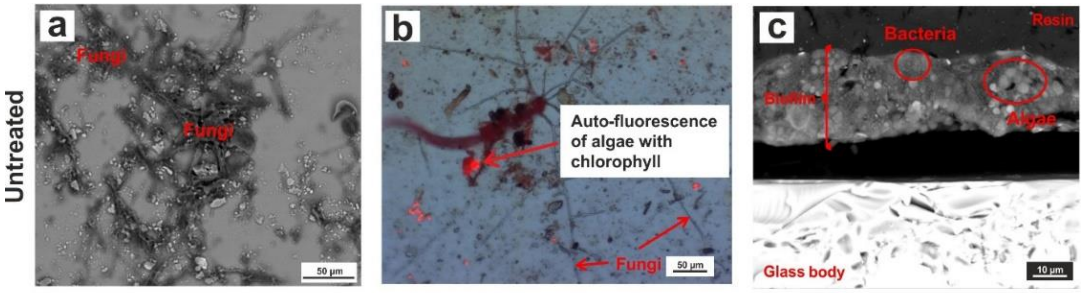

Fig. 6. SEM micrographs, OM and FM images of biological colonization from untreated Na glass samples:
a) Surface of glass showing fungal cells collapsed, SEM-BSE;
b) Composed image (OM and FM) of glass surface showing fungi and algae (OM) and algae chlorophyll autofluorescence signal (FM);
c) Cross-section of glass, SEM-BSE. Algae and bacteria colonies are easily distinguishable (circles) and there is also probable presence of fungi

Dilute HF acid treatment firstly produces surface dealkalinization and then gradually weakens the glass network, causing the spread of small craters and pits throughout all glass surfaces. Collapsed algae, or what is the same dehydrated, were observed by SEM-SE in most of these irregular craters and pits on the surface of samples (Fig. 7a and b). However, SEM-BSE observations of cross-sections through the interface shown in figure. 7c and d demonstrated well-developed biofilm with abundant algae inside possibly due to a higher local humidity concentration.

In terms of chemical durability and according to the DIN ISO 719 standard of hydrolytic resistance of glasses [22], soda-lime is a highly resistant glass, lead crystal is a medium resistant glass, while potash-lime is a low resistant glass. This classification is in agreement with the data obtained after continuous exposure of the model glasses since the "Na" soda-lime glass showed the least corroded surface (Fig. 6c), whereas the "K" potash-lime glass displayed the most corroded one with even a well-developed weathering layer of around 20µm in thickness (Fig. 4b). Dilute HF treated counterparts were always obviously more degraded (Fig. 7c and d).

However, the growth of the biofilm colonization follows an opposite pattern, the higher the resistant of glass the higher is the biocolonization growth (Na glass), and also, the lowest the resistant of glass the lowest is the biocolonization growth observed (K glass) with the Pb glass showing an intermediate pattern in terms of both resistant and biocolonization. That is, the more attacked the glass surface the less biocolonization was observed.

Biofilm in Na glass samples was on average about 25 µm in thickness, which represent something more than double that the thickness observed in Pb glass samples. It is known that soda-lime silicate glasses absorb more surface water than lead crystal and potash-lime silicate glasses [23]. The presence of higher amount of water on soda-lime (Na) glass surfaces can make the glass more bioreceptive and, consequently, more attractive for biocolonizing species [10]. This fact may explain why the surface of Na glasses were more intense biocolonized than the surfaces of Pb and K glasses, respectively.

Algae, fungi, cyanobacteria and heterotrophic bacteria are otherwise some of the microorganisms most commonly identified in historical glasses, including stained glass windows. The literature mentions that they always result in a dense biocolonization of the material which forms a biofilm on the glass surface [4, 8, 24-26], just in the same way as what has been here obtained in the continuous exposure experiment. In all cases microorganisms play the role of

enhancing and catalyzing the chemical-physical processes involved in glass degradation and is the main reason why an early intervention is strongly recommended, since as they grow over time, they will have more capacity to go through the interstices of the glass substrate causing an ever-deeper alteration.

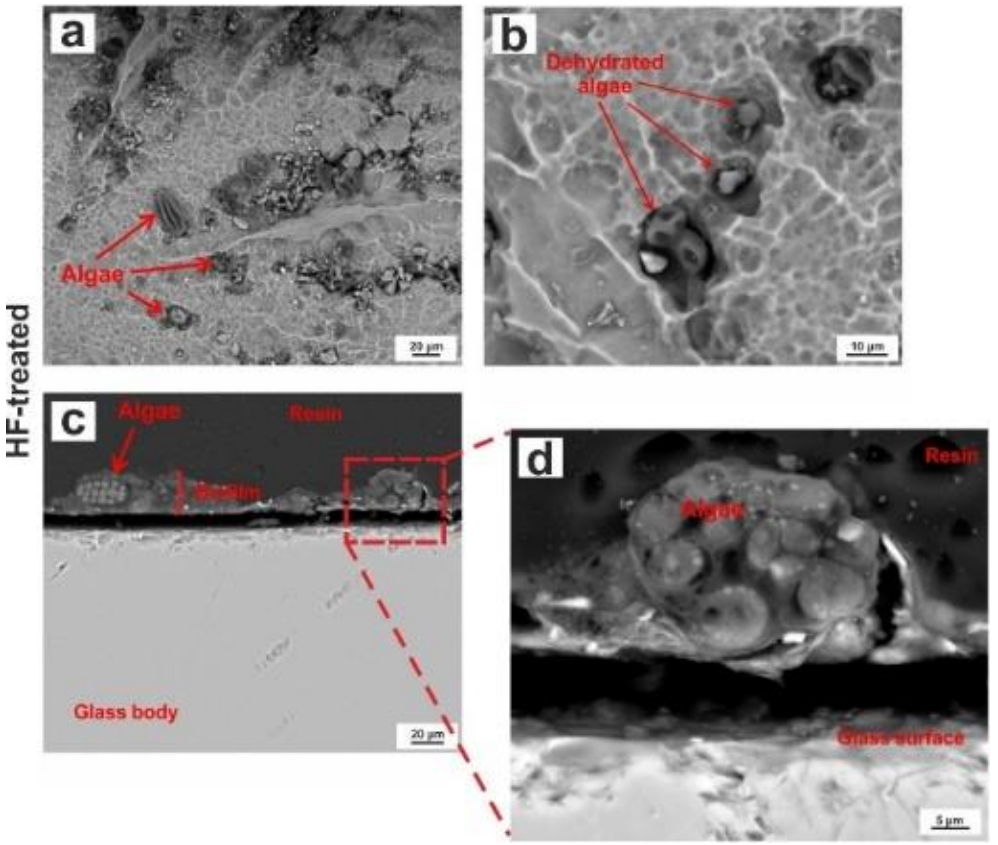

Fig. 7. SEM-BSE micrographs of biological colonization from dilute HF acid-treated Na glass samples:
a-b) Surface of glass showing dehydrated algae inside craters and pits;
c-d) Cross-section of glass showing the biofilm in which algae are easily distinguishable

Effects of the biocide treatment

As mentioned above Na glasses, no matter if acid-treated or untreated, were the samples that showed the most severe biocolonization after exposure outdoors for continuous induced biological colonization, as can be seen in thick and well-developed biofilms (up to 35µm in thickness) displayed in figure 8a and c. However, once these samples were treated with the Acticide® CF biocide and left outdoor for 1 month to let the biocide work, such biofilms and their corresponding internal microbiota appeared intensely damaged and were practically no longer visible through observation techniques. Comparison between samples before and after the biocide treatment clearly shows completely damage of the biofilms until their practical disappearance (Fig. 8b and d, see stars).

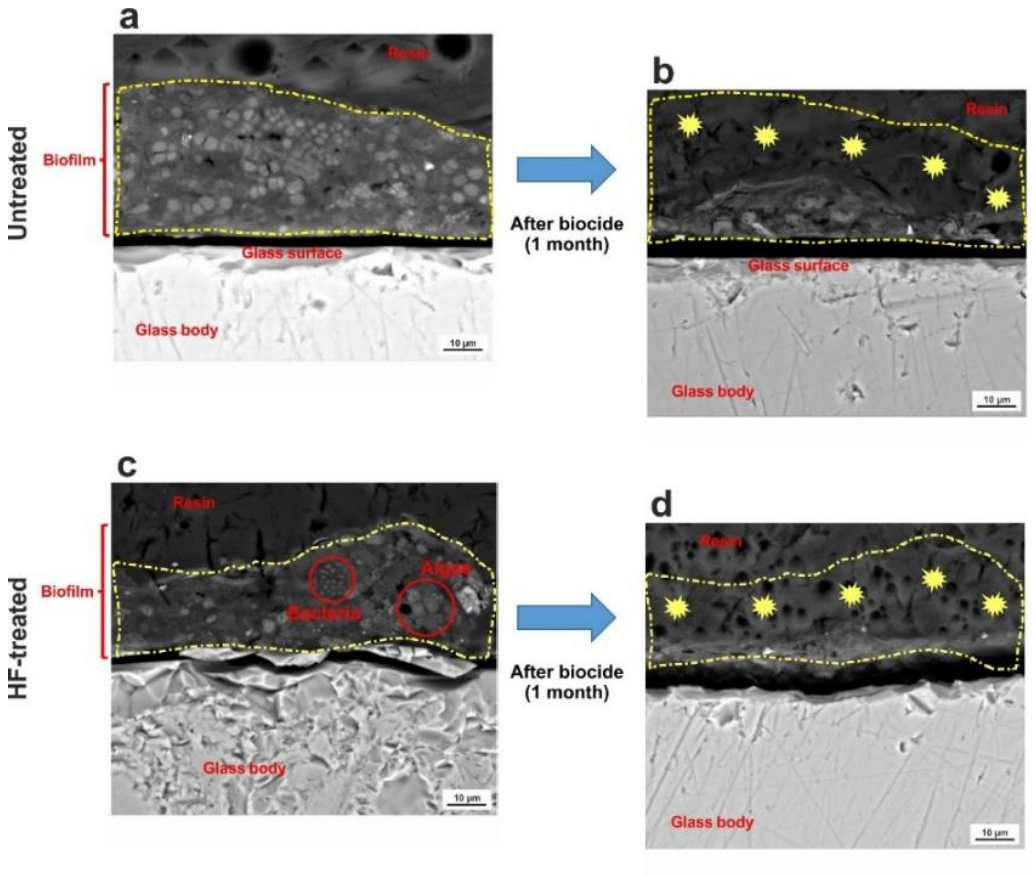

Fig. 8. SEM-BSE micrographs of cross-sections from Na glass samples untreated (Na1) and dilute HF acid-treated (Na2): a) Very complex biofilm formed on untreated glass surface in which cyanobacteria and heterotrophic bacteria can be observed; b) Biofilm destroyed on untreated glass surface after the Acticide® CF biocide treatment; c) Biofilm formed on dilute HF acid-treated glass surface in which clearly distinguisible colonies of algae and bacteria (circles) can be observed; d) Remains of the biofilm on dilute HF acid-treated glass surface after the Acticide® CF biocide treatment. Biofilms or the area they occupied are indicated by dot and dash yellow lines in all micrographs. Stars in b and d indicate the area where the biofilm was present before the biocide treatment

The same behavior with respect to the biocide treatment was observed as far as K and Pb glasses are concerned in those examples with considerable biocolonization growth (Fig. 9a and c). In these cases, biofilms and their corresponding internal microbiota also appeared intensely damaged and were practically no longer visible through SEM-BSE observations (Fig. 9b and d, see stars).

The Acticide® CF biocide is therefore effective since it fully removes the biofilms and their composing internal microorganisms, as well as their structures and water supply routes, which means in short that biofilms are no longer functionality viable. Furthermore, the biocide treatment has the advantage that it does not produce any additional damage on glass substrate. It only directly targets biofilms and their microbiota. Due to the biofilm appears seriously and structurally altered and damaged it seems unlikely that it could recover over time. Nonetheless, further research is certainly needed to evaluate longterm bioweathering behavior of glasses after having been treated with the Acticide® CF biocide.

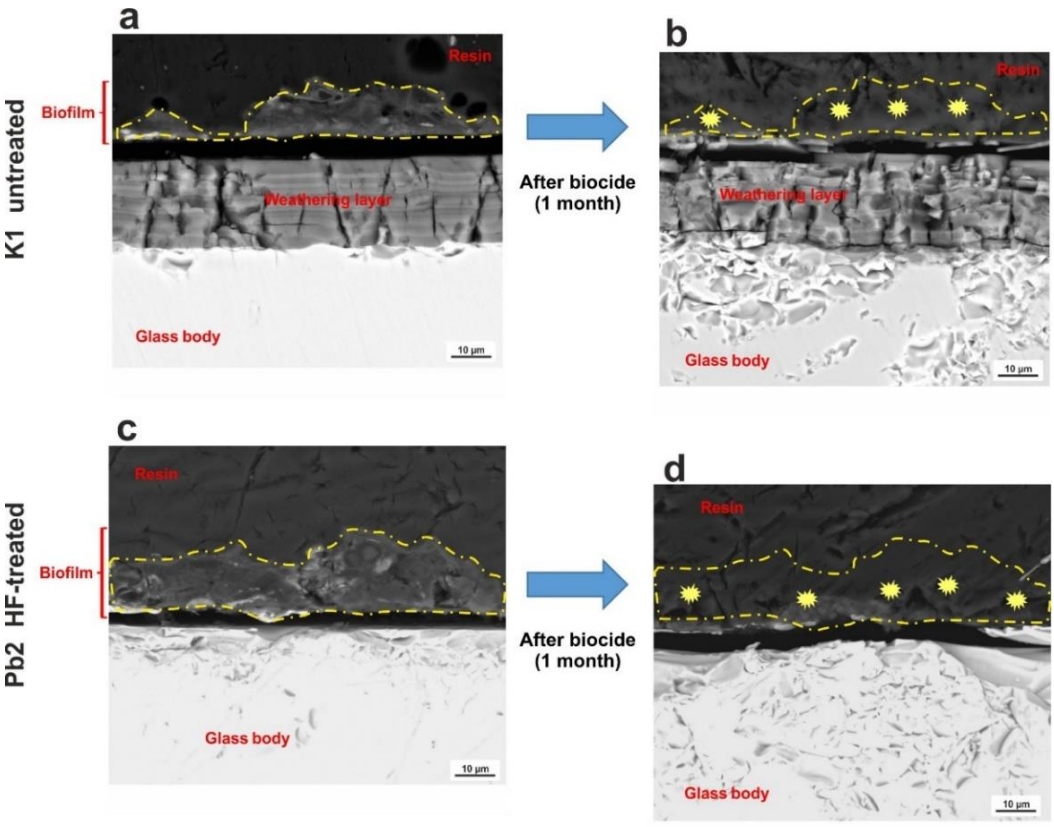

Fig. 9. SEM-BSE micrographs of cross-sections from K1 and Pb2 glasses:
a) Biofilm formed on untreated K1 glass surface;
b) Biofilm destroyed on untreated K1 glass surface after the Acticide® CF biocide treatment;
c) Biofilm formed on dilute HF acid treated Pb2 glass surface;
d) Biofilm destroyed on dilute HF acid-treated glass surface after the Acticide® CF biocide treatment. Biofilms or the area they occupied are indicated by dot and dash yellow lines in all micrographs. Stars in b and d indicate the area where the biofilm was present before the biocide treatment

Comparison with a real case

Some years ago, some of the authors of the present paper conducted the study of biodeteriorated and biodegraded stained glass windows from the Royal Charterhouse of Santa María de Miraflores (Burgos, Spain) [3]. They were Flemish stained glass windows made in the late fifteenth century, in the Medieval-Renaissance transitional period, from potash-lime silicate glass of type 5 (medium stability glass) according to *Müller et al.* [27] classification. As can be observed in figure 10 (left), some of the Royal Charterhouse glasses showed a deeply and extensively advanced biocolonization process that had caused in them an irreversible damage. Such glasses can be considered very similar in chemical composition to K glasses studied in the present work. Although after experimental exposition K glasses were the least biocolonized of the three types of glasses exposed, they show a quite similar biocolonization pattern (Fig. 10, right) in comparison to that found in the Miraflores glasses. Accordingly, they could represent a first stage in the biodegradation process compared to the more advanced stage shown by the real case glasses. This fact can certainly illustrate what might happen with a historical glass if proper conservation measures are not taken in the earliest stages of the process. Early cleaning with a biocide such as that here tested may be a suitable preventive conservation strategy to be

undertaken before a more advanced and irreversible biodeterioration takes place, since prevention is always much better than cure.

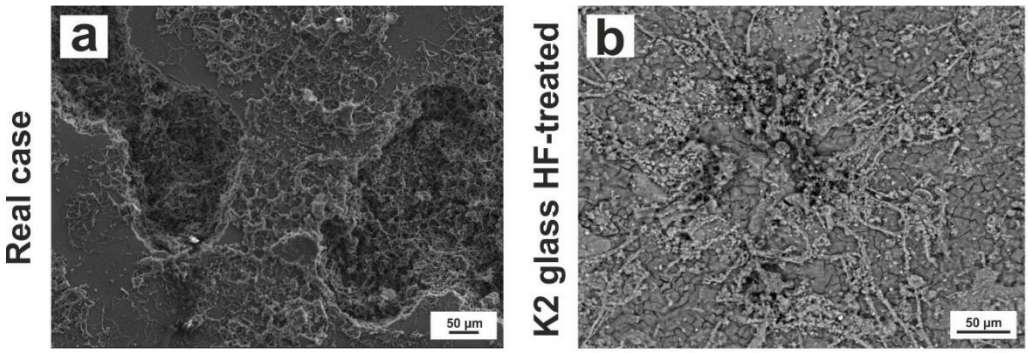

Fig. 10. SEM micrographs showing a real case of biodeterioration from a potash-lime silicate glass coming from the Royal Charterhouse of Santa María de Miraflores (Burgos, Spain) [3], and the surface of K2 dilute HF acid-treated glass after exposition

Conclusions

Induced biological colonization on three types of model historical glasses, namely soda-lime (Na), lead crystal (Pb), and potash-lime (K) silicate glasses, has been studied through a continuous exposition to natural weathering conditions at the mountain region near Madrid (Spain). Half of the glass samples were treated with dilute HF acid to faster induce and accelerate biological colonization while the remaining half was left untreated. After exposure algae, fungi, cyanobacteria and heterotrophic bacteria were the biocolonizers detected. Depending on the type of glass, the biocolonizers sometimes formed a well-developed biofilm, especially on Na and Pb glasses. The type of glass that showed the highest biocolonization was Na glass, while the least biocolonized was K glass. Although biocolonization grew in both untreated and dilute HF acid-treated glass samples, biocolonizers seem to have a greater facility to form and maintain colonies in the latter, the acid-treated ones. The state of the surface therefore influences biocolonization. However, biocolonization was also strongly influenced by the chemical composition of glass since the more attacked the glass surface the less biocolonization was observed. That is, the higher the resistant of glass the higher was the biocolonization growth (Na glass). Lead crystal glass (Pb) showed an intermediate pattern in terms of both resistant and biocolonization. Synergic effect of both chemical alteration and later biocolonization cause a high loss of glass transparency. Soda-lime (Na) lost more than 80%, lead crystal (Pb) a little less than 80%, while potash-lime (K) lost around 60%.

Removal of the biofilm formed on model historical glasses were tested by using a treatment with the Acticide® CF biocide. Once the samples were treated and left 1 month to let the biocide work, biofilms and their corresponding internal microbiota were intensely damaged and were practically no longer visible through observation techniques. The Acticide® CF biocide has proved its effectiveness since it fully removes biofilms and seriously decreases its thickness, its structure and its water supply route, which in short means that they are no longer functionality viable. The biocide treatment also presents the advantage that it does not produce any additional damage on glass substrates since it only directly targets biofilms and their internal microbiota.

The Acticide® CF biocide treatment may be therefore an adequate preventive conservation strategy when biocolonization is at a first stage of development and, consequently, cannot be removed through conventional methods of cleaning by using, for instance, a solution of water and ethanol. At this first stage an early intervention should be advisable because when biodeterioration in cultural heritage glasses is in deep progress it then becomes an irreversible damage.

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