applied sciences

MDPI

Article

From Biodeterioration to Creativity: Bioreceptivity of Spruce Pine 87 Glass Batch by Fungi

Alexandra Rodrigues [1,*], Margarida Alves [1,2], Sara Gutierrez-Patricio [3], Ana Z. Miller [3] and Maria Filomena Macedo [1,4,*]

[1] VICARTE, Research Unit Vidro e Cerâmica para as Artes, NOVA School of Science and Technology Hangar III, Campus da Caparica, 2829-516 Caparica, Portugal; margaridaalves@campus.ul.pt
[2] Faculdade de Belas Artes, Universidade de Lisboa, Largo da Academia Nacional de Belas-Artes, 1249-058 Lisboa, Portugal
[3] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain; sara.gutierrez@irnas.csic.es (S.G.-P.); anamiller@uevora.pt (A.Z.M.)
[4] Department of Conservation and Restoration, FCT-NOVA, Campus da Caparica, 2829-516 Caparica, Portugal
* Correspondence: aj.rodrigues@campus.fct.unl.pt (A.R.); mfmd@fct.unl.pt (M.F.M.)

Abstract: The bioreceptivity, and the consequent biodeterioration of contemporary glass, used by artists worldwide, was studied. The two main objectives were: first, to verify if fungi with some culture media would produce more damages than the same fungi without a nutritional source, and to verify if the two genera of fungi produce the same damage on the same glass. Colourless glass samples with Spruce Pine 87 Batch (SPB-87) composition were inoculated with two distinct fungal species, *Penicillium chrysogenum* and *Aspergillus niger*, separately: *(i)* half with fungal spores (simulating primary bioreceptivity), and *(ii)* half with fungi in a small portion of culture media (simulating organic matter that can be deposited on exposed glassworks, i.e., secondary bioreceptivity). The alteration of glass surfaces were analysed by Optical Microscopy, SEM-EDS and µ-Raman. The mycelium of *Penicillium chrysogenum* generated a higher amount of fingerprints, stains and iridescence, whereas *Aspergillus niger* produced more biopitting and crystals on the glass surface. However, both species damaged the glass to different degrees in 4 and 6 months after the inoculation, producing physico-chemical damage (e.g., iridescence, biopitting), and chemical alterations (e.g., depletion and deposition of elements and crystals). The primary bioreceptivity experiment of glass samples inoculated with *Aspergillus niger* results in less damage than in the case of secondary bioreceptivity, being almost similar for *Penicillium chrysogenum*. The new and in-depth understanding of the bioreceptivity and deterioration of post-modern glass art and cultural heritage provided here is of paramount importance for the scientific, conservation and artistic communities—to protect glass cultural materials, or seen by artists as innovative and inspirational ways of creating glass art in the future.

Keywords: bioreceptivity; biodeterioration; fungi; *Aspergillus niger*; *Penicillium chrysogenum*; soda-lime glass; contemporary glass art; Spruce Pine Batch 87

Citation: Rodrigues, A.; Alves, M.; Gutierrez-Patricio, S.; Miller, A.Z.; Macedo, M.F. From Biodeterioration to Creativity: Bioreceptivity of Spruce Pine 87 Glass Batch by Fungi. *Appl. Sci.* **2022**, *12*, 7672. https://doi.org/10.3390/app12157672

Academic Editor: Gyungsoon Park

Received: 1 March 2022
Accepted: 21 July 2022
Published: 29 July 2022

1. Introduction

The Phoenicians created the concept of glass art, and ever since glass has been used in several forms of creations in our cultural heritage. Glass art and glass blowing can be traced back in history as far as the establishment of the Roman Empire, in the first century BC, the moment when this new technology spread very quickly. More recently, modern glass art blowing owes much of its invention to the rise of the Studio Glass Movement in the early 20th century, and contemporary artists continue to contribute to the glass creations, constituting the heritage of today and of future generations. Concerning the current use of glass as an artistic medium, the Spruce Pine 87 Batch glass (SPB-87) has high importance in contemporary art production. It is used in works of art of many internationally renowned artists, due to its origins in the 1960s United States, where the Modern Art Glass Movement

began and spread to the rest of the world. The SPB-87 glass was, and still is, acquired by glass artists with works indoors (e.g., galleries) and outdoors all over the world. Regardless of their location, all these glassworks are subject to physical, chemical, and biological factors. Concerning the latter, the principal biological agents causing glass biodeterioration are known to be microorganisms, bacteria and fungi being the ones most commonly found on glass [1].

In 1995, Guillite introduced the term bioreceptivity, as 'the ability of a material to be colonised by one or several groups of organisms' [2]. The same author also defines the terms primary and secondary bioreceptivity. The '*Primary Bioreceptivity* ' refers to the susceptibility of the material to be colonised by organisms reliant of its initial state properties. The characteristics of the material can evolve over time under the action of other colonising organisms, dust, or other factors, resulting in a new type of bioreceptivity, called '*Secondary Bioreceptivity* ' [2]. There are many studies reporting that fungi are able to colonise different types of glass, as well as on glass biodeterioration by fungi, demonstrating that these microorganisms can be responsible for irreversible damages (e.g., Carmona et al. [3], Rodrigues et al. [4], Drewello and Weissmann [5], Gorbushina and Palinska [6], Corrêa Pinto et al. [7], Kerner-Gang and Theden [8]). Nevertheless, studies on the bioreceptivity of glass surfaces are scant, despite the fact that the biodeterioration effects in these materials are a consequence of their bioreceptivity.

Two types of fungi over glass are reported in the literature: those identified on the surface but with no information regarding their potential for glass biodeterioration, and those causing damage to glass surfaces [1,9]. Through the most recent literature reviews (see Macedo et al. [1], Macedo et al. [9]), the genera *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp. are presented as those more commonly found on glass. However, within these genera there is not a species that occurs more than twice on glass (see Table S1 from Macedo et al. [1]). Thus, most of the literature in glass biodeterioration refers to fungi genera. Some of the authors studying glass biodeterioration (e.g., Corrêa Pinto et al. [7]) inoculated glass samples with a spore suspension of four distinct fungal species in sterile 0.05% Tween 80 (Panreac) to simulate a real biofilm and a primary bioreceptivity, but the species with major biodeterioration potential could not be identified. Other authors did their biodeterioration experiment by inoculating some fungi with a small amount of culture media, thus simulating secondary bioreceptivity. For instance, Rodrigues et al. [4] used *Penicillium* sp. and *Cladosporium* sp., with a small amount of PDA (Potato Dextrose Agar) on their glass biodeterioration experiment. In this study, large quantities of crystalline substances and prints on the surfaces are attributed to the presence of both fungi, and took place after only 2.5 months of incubation. However, since the effect of *Penicillium* sp. alone could not be separated from the one of *Cladosporium* sp., the bioreceptivity of the glass surfaces to each of the species was out of reach in both studies.

In several studies regarding glass biodeterioration by fungi, historical glass (i.e., with more than 100 years) like stained glass windows is usually the main concern [3,4,7,10], as none of these studies relate to contemporary glass art. Therefore, on the one hand, the genera that will be used in this experiment were isolated from stained glass windows. On the other hand, since the SPB-87 glass was a newly developed batch-composition related to the Studio Glass Movement event, it was the natural choice for the present study. It is important to contextualise its origins to understand the relevance of the selected substrate. In 1962, Dominick Labino (the scientist) and Harvey K. Littleton (the artist) brought into the artistic community the use of glass as a medium for expression. Littleton and Labino disseminated the technical knowledge and the glassblowing techniques to work in a portable furnace, and to produce a low melting-point glass, using the batch originally supplied by Dominick Labino. The American Studio Glass Movement took a kick-start with the newly acquired technical skills, and the glass artist as a maker and designer was thus highlighted [11,12]. One year later, at the University of Wisconsin, Littleton established a pioneering glass program in the United States of America. This program attracted student artists that would later be iconic figures, namely Dale Chihuly and Marvin Lipovsky [13]. The name of the Spruce Pine batch comes from the analogous named town in Wisconsin,

to where Littleton moved after retiring from teaching in 1977. There, as an independent artist, he continued his work and founded the Spruce Pine Batch Company in 1988, which up to the present uses the low melting-point soda-lime glass formula created by Dominick Labino. Due to the latter, artists were no longer dependent on factories to manufacture glass artworks, since they possessed this specifically-created formula for use in studio glass creations. This is why glass artists worldwide still use the SPB-87 medium in their artworks today.

As aforementioned, the SPB-87 glass is a soda-lime glass. Regarding the biodeterioration of the soda-lime type, this subject was previously studied in detail solely by two authors: Callot et al. [14] and Gallien et al. [15]. The latter performed biodeterioration experiments using *Aspergillus niger* and *Cladosporium* sp., resulting in the observation acidic glass surfaces' (pH from 4.5 to 1.7 for the *Aspergillus*) in few days of leaching, besides a strongly anchored fungal mycellium on the glass surface [15]. In turn, Callot et al. [14] performed their biodeterioration experiments on glass using *Penicillium notatum*. The results reported in that study exhibited a friable reaction layer at a few isolated spots and hyphae fingerprints on soda-lime glass [14]. The damages are reported, but the glass bioreceptivity is not discussed.

Considering the mentioned results from previous studies, two interesting questions rise: (i) does the primary or the secondary bioreceptivity influence the biodeterioration potential of the fungal species? and (ii) do two different genera produce the same damage on a glass? A third important question can also be added: can the knowledge on the bioreceptivity, and the consequent biodeterioration, of glass substrates influence a better preservation or even the creation of new forms or artistic works? In this study, *Penicillium* and *Aspergillus* were chosen to investigate the bioreceptivity of SPB-87 glass surfaces to each species, and their individual biodeterioration potential. Each fungi, one *Penicillium* and one *Aspergillus*, were individually inoculated on the glass samples, in one experiment only with spores (to study primary bioreceptivity), and in a second experiment with a small amount of PDA. Consequently, the work presented here is focused on two main objectives: to verify (i) if fungi with some culture media (simulating secondary bioreceptivity) would produce more damages on glass than fungi without a strong nutritional source (only spores in Tween), and (ii) if the two fungal species (*Penicillium* and *Aspergillus*) cause the same biodeterioration patterns and/or degree. The inoculated glass used was the Spruce Pine 87 Batch glass, representing the many contemporary glass artworks in our heritage that have never been studied before from this point of view.

2. Materials and Methods

2.1. SPB-87 Glass Production

Spruce Pine Batch 87 (hereafter SPB-87) glass falls within the soda-lime type. It was personally provided to this research by a member of Corning Museum of Glass. The glass samples used in this study were produced in the Vicarte Research UNIT, using the chemical composition of SPB-87. For the production of the glass batch, laboratorial reagents were used, and the mixture was melted in a kiln after stirring (the melting temperature applied was 1300 °C, in a Termolab, Fornos Eléctricos Lda kiln). The produced SPB-87 glass final composition was approximately the following: 71.9 wt% SiO_2, 1.79 wt% Al_2O_3, 14.89 wt% Na_2O, 0.40 wt% K_2O, 1.00 wt% Li_2O, 7.90 wt% CaO, 0.20 wt% MgO, 0.50 wt% BaO, 1.00 wt% ZnO, 0.20 wt% Sb_2O_3, 0.10 wt% Na_3AlF_6, 0.09 wt% Er_2O_3, 0.02 wt% Fe_2O_3. aparafusados

The SPB-87 glass melt obtained was hand-blown into roundels (circular glass plates) using traditional tools and techniques. These roundels were annealed at a temperature of about 500 °C for 4 h, and slowly cooled down to room temperature during approximately one day. For the experiment, square glass samples of 1 × 1 cm^2 and about 2–2.5 mm thickness were cut from the roundels produced.

2.2. Bioreceptivity/Biodeterioration Experiment

2.2.1. Identification of Fungal Strains and Inoculum Preparation

Two fungal strains were considered for this study. They were originally isolated and kept at 4 °C until fresh cultures were prepared for the current experiments. For the inoculum preparation (growth and sporulation), both isolates were transferred to Potate Dextrose Agar (PDA), which is well-known to have the adequate pH for fungal growth (about 6–6.5) [16]. They grew in the laboratory, where further bioreceptivity experiments were conducted (temperature controlled by air conditioning between 22 and 23 °C). The nutritive source, PDA, grants the necessary water activity inside the Petri dish.

DNA from pure cultures was extracted using the DNeasy® PowerLyzer® PowerSoil® kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplification of fungal internal transcribed spacer (ITS) regions was attempted by Polymerase Chain Reaction (PCR) using the ITS1 (5′-TCC GTA GGT GAA CCT GCG G) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers, as described previously [17]. The PCR reactions were performed in a Biometra thermocycler T-Gradient ThermoBlock (Göttingen, Germany) using the following thermal conditions: 94 °C for 2 min; followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min; and a final extension cycle at 72 °C for 5 min. The amplified products were evaluated by electrophoresis on 1% (*w*/*v*) agarose gels, stained with SYBR Safe DNA Gel Strain (Carlsbad, CA, USA) and visualized under UV light. The PCR products were then purified and sequenced by STAB VIDA Sequencing Services (Caparica, Portugal). The two DNA sequences obtained were edited using Bioedit v7.2.5 software (Technelysium, Tewantin, Australia) and compared using the BLASTn (Basic Local Alignment Search Tool) algorithm [18] from the NCBI (National Center for Biotechnology Information), http://www.ncbi.nlm.nih.gov/, accessed on 5 July 2022) for the phylogenetic identification. Both generated ITS sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 5 July 2022) under accession numbers ON926989 and ON926990.

For the primary bioreceptivity experiment, an inoculum was prepared with the harvested spores by pipetting sterile 0.05% Tween 80 (Panreac) on the surface of the colonies. These spore suspensions, one for each fungal strain, were determined with a haemocytometer and adjusted to 1×10^6 spores/mL. For the secondary bioreceptivity, a small amount of each fungus with PDA was withdrawal from the 2 Petri dishes.

2.2.2. Inoculation and Tests

In this experiment, two Petri dishes of 150 mm diameter were used, to which distilled water and a stainless steel net were added to accommodate the glass samples. To maintain high relative humidity (RH) for enhancing fungal growth (75–80%), distilled water was added to the bottom; contact with the distilled water was avoided by the net level. Each stainless steel net inside each glass Petri dish was split into three sections–Figure 1a. The Petri dishes with the experimental set were sterilised in an autoclave at 121 °C for 1 h. The isolated and identified fungal strains were inoculated in 12 samples each, separated in 2 different Petri dishes. Besides, 3 samples were added as reference, thus a total of 15 samples were settled on the top of the net inside each Petri dish (one Petri dish per fungal strain). The control samples (i.e., with no contamination by fungal spores, placed in a separate Petri dish), are presented as supplementary material (Appendix A Figure A1) to demonstrate the difference between the fungal colonisation of the surfaces and the environmental alteration on the absence of fungi.

Concerning the inoculation step, 12 samples of each set were inoculated with each fungus separately. Six out of the 12 samples had a small bit of culture medium–Potato Dextrose Agar (PDA)–simulating organic and inorganic matter that can deposit on glassworks, as well as simulating the secondary bioreceptivity of the glass (Figure 1b). The other six glass samples were inoculated with 10 µL of the previously prepared spores suspension (1×10^6 spores/mL in a 0.05% Tween solution, Figure 1c, merely used as guarantee of the same spores concentration in all samples. The use of spores intended to simulate the glass

primary bioreceptivity. Finally, three clean glass samples were placed inside the Petri dishes under the same conditions but without inoculation to be used as a reference of the same material (Figure 1d).

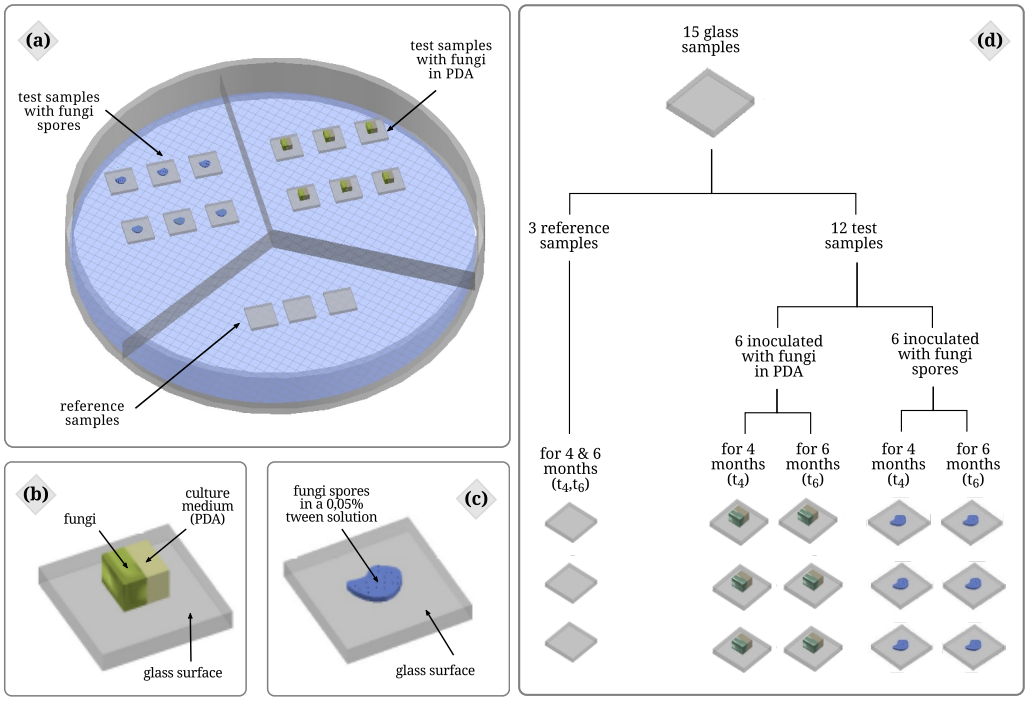

Figure 1. Experimental design (**a**) Petri dish with glass samples (includes reference samples, samples inoculated with fungal spores (**b**) and samples inoculated with PDA (**c**). Glass biodeterioration experiment design and sample number (**d**).

To ensure high relative humidity (75–80% RH) and controlled temperature under laboratory conditions (22–23 °C, controlled by air conditioning), the Petri dishes were kept in an acrylic box one level over the water placed at the bottom. The maximum incubation period lasted for 6 months, but analysis occurred in two different moments: half the samples were analysed after 4 months and the other half after 6 months. Figure 1 shows the experimental design used for each fungal species, replicated for both fungal strains.

2.3. Glass Surface Analyses

To monitor and characterise the morphological and chemical alteration on the glass surfaces during the experiment, a set of analytical techniques was applied: optical microscopy, scanning electron microscopy coupled to energy dispersive X-ray spectroscopy, and μ-Raman. The analyses were performed either before and/or after a cleaning procedure (consisting of the biofilm removal with a cotton swab embedded in a 1:1 water ethanol solution), as indicated in each section.

2.3.1. Optical Microscopy

Optical microscopy observations were carried out on glass samples with biofilm and without biofilm on their surfaces (i.e., before and after the cleaning procedure with 1:1 water ethanol solution) with the use of a Axioplan 2, Zeiss light microscope with a coupled Nikon DMX digital camera.

2.3.2. SEM

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) observations and elemental analysis were performed on the glass surfaces after the cleaning

procedure using a SEM-FIB Zeiss Auriga CrossBeam Workstation at CENIMAT | i3N (NOVA School of Science and Technology).

2.3.3. μ-Raman

Raman micro-spectroscopy (μ-Raman) was performed on the glass surfaces after the cleaning procedure using a Raman Horiba Jobin Yvon–Model LabRaman 300 at NOVA School of Science and Technology, in order to search for and potentially identify corrosion and biocorrosion products [4]. A green-light laser of 532 nm wavelength was focused with a 50× coupled Olympus lens. The analysis were performed with no filter, in a range between 100and 1500 cm^{-1} in 15 cycles of 30 s. A spectra curve fitting was performed and the baseline was subtracted using Labspec (DILOR) software whenever necessary, to reduce background fluorescence and better interpretation of the present biocorrosion products.

2.3.4. Damage Measurement Parameters

The surfaces' damage or alteration was attributed as the variable partitioned into components (causes of variation in the ANOVA test). Average damage was classified into a semi-quantitative scale from 0 to 4 – from 0 (non-existent) to 4 (very pronounced), considering parameters such as density, quantity, and dimensions on each type of damage. The following parameters were defined: (i) mycelium growth, (ii) chromatic alteration (stains, iridescence, darkening), (iii) biopitting, (iv) depositions, (v) crystals, and (vi) other corrosion effects (e.g., leaching). The results were retrieved by direct observation and evaluation of the intensity of the latter on the 3 replicated surfaces. The semi-quantitative values represent an hierarchical classification of the observed damage.

2.3.5. ANOVA Test

In order to answer the two main questions of this work, the ANOVA test was employed (see details Appendix A) ANOVA was used to test whether the means of distinct groups are different. The analysis intended to evaluate the variation (general differences) within the groups of results–in this case, of attributed average classifications of damage degree of the glass surfaces. The null hypothesis of ANOVA is that there is no difference among the group means. The alternate hypothesis is that at least one group differs significantly from the overall mean of the dependent variable. ANOVA allows to test the difference between two or more means. This test was made by using Microsoft Excel® software. MS Excel® refers to this test as Single Factor ANOVA. This analysis of variance (ANOVA) was performed, for a Alpha value (p) of 0.05, to evaluate the average variation of the attributed average of the 4 groups (*Penicillium* in PDA; *Penicillium* in Tween and *Aspergillus* in PDA. and *Aspergillus* in Tween).

3. Results

3.1. Taxonomic Identification of Fungal Strains by Molecular Techniques

The taxonomic identification of the two isolated fungi used in this study are shown in Table 1. They were affiliated to the fungal species *Penicillium chrysogenum* and *Aspergillus niger*, both with 100% similarity with the nearest homologues of the ITS region of the database sequences.

Table 1. Taxonomic classification of the fungal strains.

Strain nr.	Nearest Homologue (Accession Number)	Similarity (%)
PS1	*Penicillium chrysogenum* UWR_107 ITS region (KY465761) AN TYPE fungus (NR_077145)	100%
AS1	*Aspergillus niger* OR32F12 ITS region (MT510013)	100%

3.2. Surface Morphology: Optical and Scanning Electron Microscopy

Glass samples were examined by optical and scanning electron microscopy after 4- and 6-month incubation periods (t_4 and t_6, respectively) to assess the glass surfaces' morphology

and to detect the expected biocorrosion products. Optical microscopy allowed us to observe the glass surfaces and the associated mycelium growth, aesthetic and chromatic alterations, and the biocorrosion patterns in comparison with the non-inoculated samples. A summary of the results is presented in Figure 2 (and in more detail Appendix A, Figure A2), and it is shown each type of inoculated surfaces before (left column) and after the cleaning procedure (right column), for t_4 and t_6 periods of incubation.

Over in both PDA and spores in Tween inoculation with *Penicillium chrysogenum* (PS1), biological colonisation was clearly observed on both types of surfaces. Growth and densification of mycelium occurred between 4 (t_4) and 6 months (t_6) after the inoculation. Staining, etching, biopitting, and crystal formation were also observed, even after the cleaning procedure (Figure 2 and Appendix A, Figure A2a, marked as 'B', 'E', and 'F', respectively). The darkening of glass surfaces from deterioration processes is often associated with fungal growth [19].

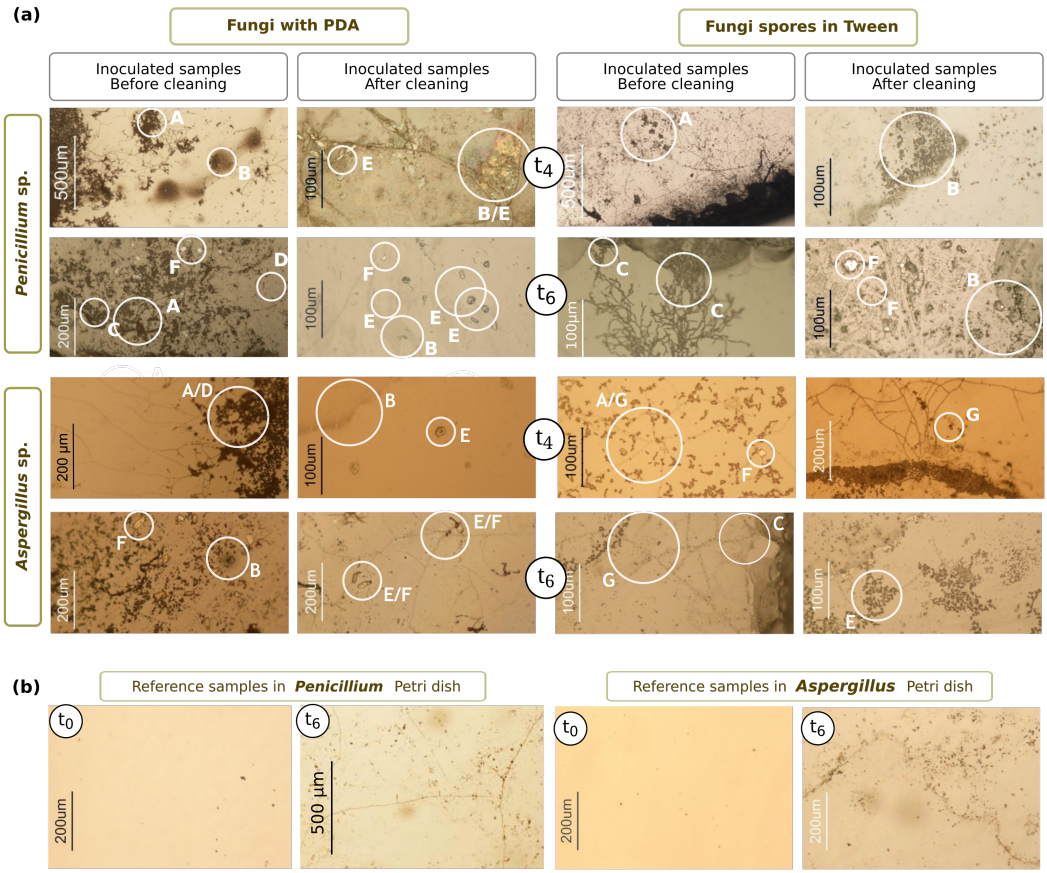

Figure 2. Optical microscopy results from (**a**) the inoculation of SPB-87 with *Penicillium chrysogenum* and *Aspergillus niger*, and of (**b**) the reference samples placed inside the same conditions of the inoculation environment. Legend: (A) clear biological colonisation/mycelium, (B) chromatic alterations (spots, stains, iridescence), (C) mycelium growth up to the edge, (D) denser mycelium near the PDA, (E) Etching/biopitting produced by hyphae, (F) crystal formation, (G) growth of mycelium from the spores.

Penicillium chrysogenum spores produced biological colonisation and mycelium growth (with hyphae reaching the edges) in all glass samples (Appendix A, Figure A2b). These surface alterations (including crystal formation) increased over 6 months. At t_6, the hyphae fingerprints were visible even after performing the cleaning procedure in all inoculated samples (Figure 2), showing that fungal mycelium was strongly anchored on the glass surface. Therefore, it is not surprising to verify that *Penicillium chrysogenum* spores inoculation produced a mycelium density smaller than the inoculated *Penicillium* in PDA. Hence, in

this case, the nutritional source plays an important role in the fungal growth, although it is not a limiting factor, since fungal growth was observed for all cases.

Observations of the surfaces inoculated with *Aspergillus niger* shown in Figure 2 (and in more detail in Appendix A, Figure A3) also revealed that all samples with PDA showed biological colonisation and mycelium growth after 4 months of incubation (Figure 2a, and Appendix A, Figure A3a for more detail). However, hyphae do not reach the sample edges, in contrast to what occurred with samples inoculated with *Penicillium chrysogenum* (Appendix A, Figure A2a). Biopitting and crystal formation was also observed at t_4 for *Aspergillus niger* in PDA ('E' and 'F' in Figure 2a and Appendix A, Figure A3a), similar to what was observed in the case of *Penicillium chrysogenum* (Appendix A, Figure A2a). After 6 months, all samples inoculated with *Aspergillus niger* samples showed a mycelium density similar to t_4, which was less dense than the one observed for the *Penicillium chrysogenum* one. Nonetheless, an increasing deposition of crystals and other structures, as well as pitting and micro-cracks formation, became more visible after the cleaning procedure. Similar deterioration patterns were reported by other authors–e.g., Kerner-Gang [20] who also reported pits on the glass surface caused by *Aspergillus versicolor* conidia (spores). Bartosik et al. [19] reported the development of conidia of the fungus Aspergillus, together with glass biodeterioration features.

The samples inoculated with *Aspergillus niger* spores revealed that, at t_4, all the inoculated samples showed biological colonisation and mycelium growth (Figure 2, and Appendix A, Figure A3b for more detail). In a similar way to *Penicillium* spores, the mycelium growth did not reach the samples' edges, but crystal formation and hyphae fingerprints were present. At t_6, the density of the mycelium of the samples inoculated with *Aspergillus* spores was similar to t_4. However, by this time of incubation, it already reached the samples' edges, contrarily to the samples with PDA.

Reference samples (with no nutrient medium or inoculation) were kept in the same environment for comparison over time. However, since the reference samples shared the same incubation conditions, some spores from the inoculated samples could be deposited on the reference ones during the incubation period. Indeed Figure 2b shows microbial growth for both set ups. In some cases, hyphae growth was observed after 6 months, despite being much less dense than on the inoculated samples (both PDA and spores in Tween). Additionally, *Penicillium* growth was observed with no nutritional source (Figure 2b). The control samples kept in the prepared environment for the same amount of time, but in a separate Petri dish (Appendix A, Figure A1), show no evidence of the effect from the colonisation such as the observed colour alterations, among others (as in Figure 2).

SEM analysis allowed the observation of physical alterations caused by each fungi on the glass surfaces, complementing the microscopic observations. The SEM results for both species inoculated over the SPB-87 glass are presented in Figure 3a, where the micro-scale morphological alteration is shown on samples after cleaning. The samples inoculated with *Penicillium chrysogenum* in PDA and as spores in Tween solution are shown in Figure 3a on the left side, and the the samples inoculated with *Aspergillus niger* in both media are shown in Figure 3a in the right side. Hyphae and spores fingerprints, crystal formation, and surfaces' alteration are clearly visible in all cases after the cleaning procedure, both at t_4 and t_6. These damages were caused by the two fungi species either in PDA or spores. The crystals over the surfaces often appear close or intertwined with remaining fungal hyphae, likely close-by to other extracellular polymeric substances (e.g., Figure 3a, inoculation with PS1 *Penicillium* in PDA at t_6). The chemical composition of the fungal secretions, surfaces' alteration, and crystalline products are further presented in the next section (EDS microanalysis).

Damages can also be observed over the reference samples contaminated with the two fungal species spores over time in each incubation environments (see Figure 3a right side). The alteration of the pristine surfaces is known to occur over time and in an environment with high relative humidity [21]. However, the presence of fungal strain (verified by optical microscopy) leads us to believe that some of the crystalline structures and other surface

features are formed due to the influence of the air-transported spores and posterior fungal growth over those surfaces.

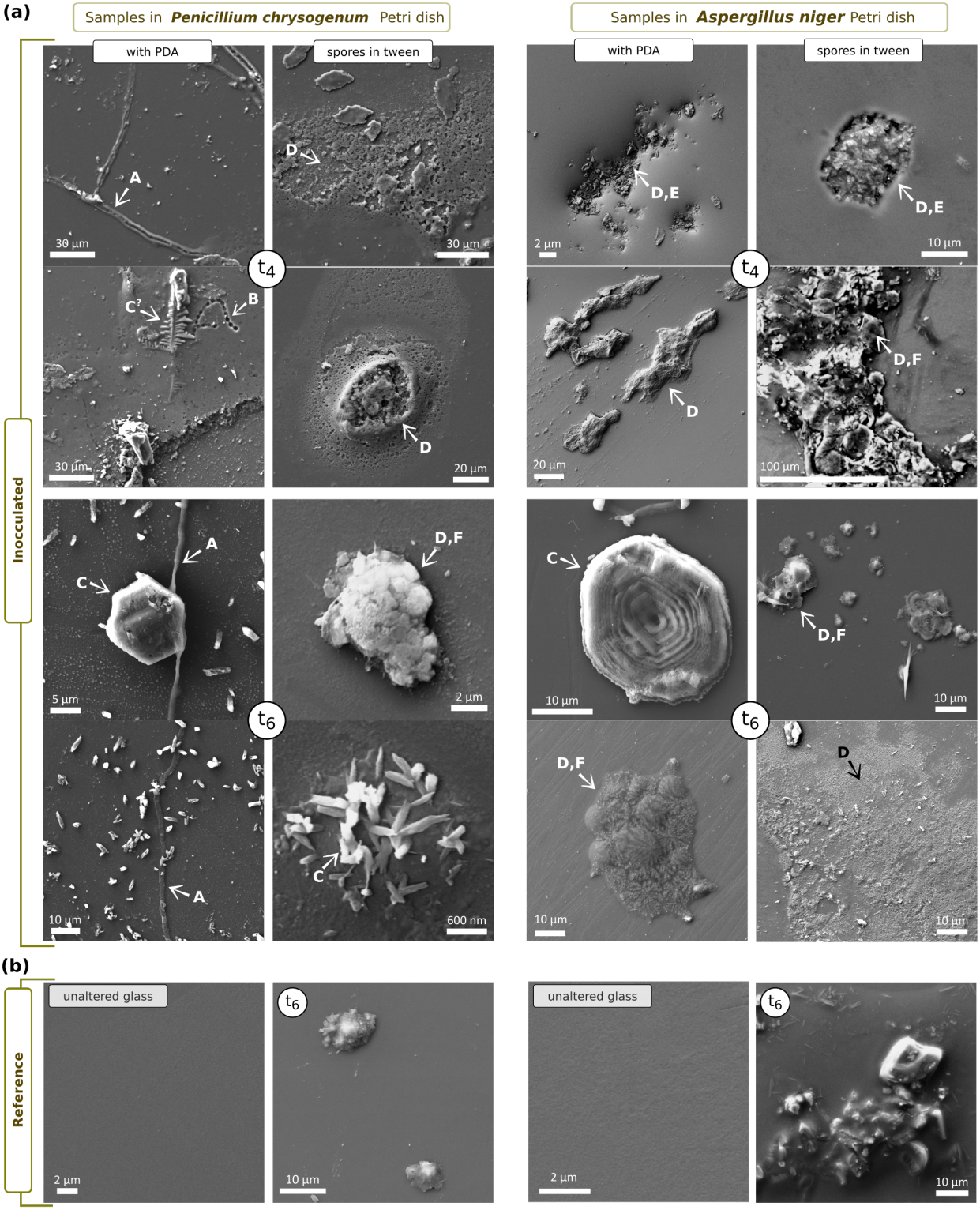

Figure 3. SEM images of samples inoculated with (**a**) *Penicillium chrysogenum* and *Aspergillus niger*, in PDA or as spores in Tween solution, at t_4 and t_6 periods of incubation; and (**b**) of the reference samples incubated in the same Petri dishes after 6 months (t_6). Legend: (A) hyphae fingerprints; (B) spores fingerprints; (C) crystal formation; (D) depletion/deposition of elements; (E) biopitting; (F) exometabolites.

3.3. Chemical Alteration: SEM-EDS and μ-Raman

SEM-EDS microanalyses also allowed the evaluation of chemical alterations caused by *Penicillium* and *Aspergillus* on the glass surfaces. This information was complemented by the identification of crystalline products on glass by μ-Raman. The results from the elemental alterations is shown in the EDS quantification results in Table 2.

Table 2. Elemental composition (atomic %, at%) on the glass surface after 4 and 6 months inoculation with *Penicillium chrysogenum* and *Aspergillus niger*–spectra presented in Figure 4. Mean values correspond to the average of 3 samples analysed prior to inoculation. Legend: Sp = spot or area of SEM analysis where the EDS results were retrieved (see Figure 4); BQL = below quantification limit; '–' = not present.

		Penicillium chrysogenum						***Aspergillus niger***					**Reference**	
	Glass	**PDA**			**Spores in Tween**			**PDA**		**Spores in Tween**			***Pen.***	***Asp***
		Sp1	**Sp2**	**Sp3**	**Sp1**	**Sp2**	**Sp3**	**Sp1**	**Sp2**	**Sp1**	**Sp2**	**Sp3**	**Sp2**	**Sp3**
at%	$\bar{x} \pm \sigma$	**t4**	**t4**	**t6**	**t4**	**t4**	**t6**	**t4**	**t6**	**t4**	**t4**	**t4**	**t6**	**t6**
C	–	26.0	25.3	22.3	19.9	–	–	BQL	20.3	19.0	74.2	16.2	27.3	60.2
O	55.9 ± 0.9	30.7	53.1	58.5	58.9	59.6	66.9	62.6	57.5	48.2	20.0	58.8	49.5	25.2
Na	9.0 ± 0.5	15.2	0.6	–	0.4	1.7	3.7	1.7	–	2.5	1.0	2.1	1.8	6.3
Mg	–	1.8	1.2	–	1.7	1.8	1.7	–	0.2	–	–	0.6	0.2	0.3
Al	1.0 ± 0.1	–	–	–	–	0.4	0.5	19.1	–	13.9	0.2	0.3	0.3	0.5
Si	30.3 ± 1.0	9.5	8.8	0.3	0.8	15.7	11.3	15.6	0.6	15.6	3.6	5.9	9.2	2.6
Cl	–	15.3	–	0.4	–	–	–	–	–	0.2	0.2	0.5	–	0.9
K	0.3 ± 0.0	0.4	–	–	–	–	0.2	0.4	–	0.5	0.2	0.3	–	0.2
Ca	3.3 ± 0.1	1.1	11.1	18.6	18.7	21.4	15.6	0.5	21.4	0.1	0.6	15.2	11.6	0.8
Zn	0.3 ± 0.1	–	–	–	–	–	–	–	–	–	0.1	–	–	–
Mn	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–
Ti	–	–	–	–	–	–	–	0.2	–	–	–	0.1	–	–
S	–	–	–	–	–	–	–	–	–	–	–	–	–	1.2
Fe	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5

Additionally, Figure 4a,b present the SEM-EDS results, and Figure 5a (plus Appendix A, Figure A4a) present the μ-Raman results of the samples inoculated with PS1 in PDA and as spores in Tween solution. Figure 4c,d present the SEM-EDS results, and Figure 5 (plus Appendix A, Figure A4b) present the μ-Raman results of the samples inoculated with AS1 (with PDA or Tween).

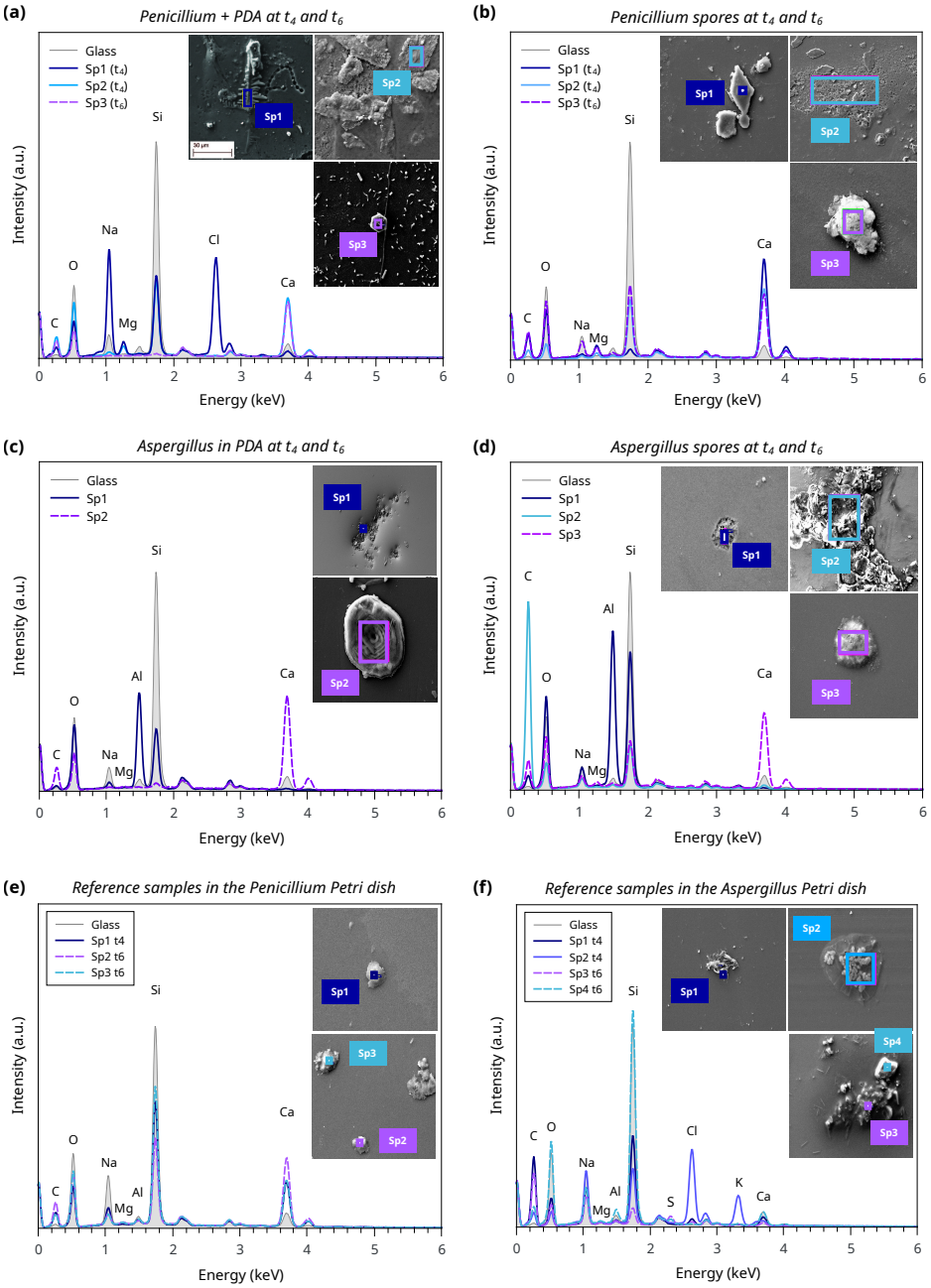

Figure 4. SEM-EDS spectra of crystals and surface alterations of the SPB-87 glass samples (analysis spots marked in the SEM images presented), inoculated with (**a**) *Penicillium chrysogenum* (PS1) in PDA or (**b**) PS1 spores in Tween; (**c**) *Aspergillus niger* (AS1) in PDA or (**d**) spores in Tween; (**e**) reference samples in the PS1 and (**f**) AS1 environments at t_4 and t_4.

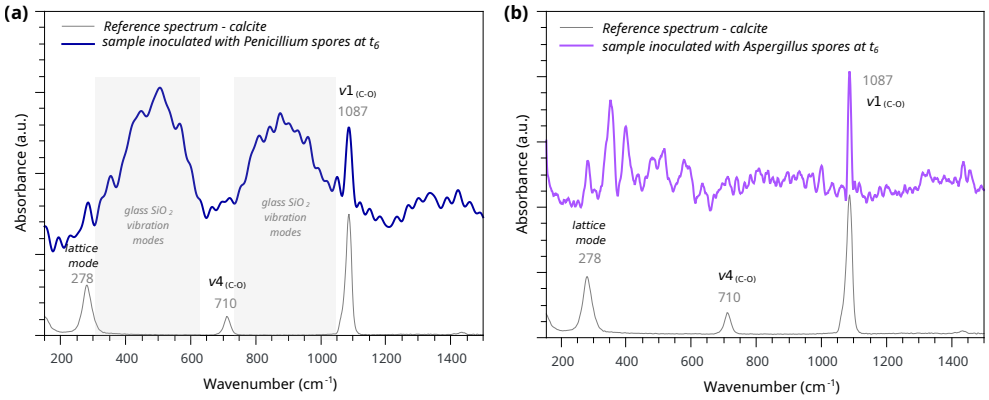

Figure 5. Raman spectra of the idetified crystalline products on the glass surfaces inoculated with (**a**) weddelite ($CaC_2O_4 \cdot H_2O$) identified in PS1 in PDA at t_6 identified according to literature data [22,23], and (**b**) calcium carbonate ($CaCO_3$) identified in AS1 in PDA at t_6, identified according to literature data [23].

As can be observed from the results in Table 2, elements deposition or enrichment over the glass surfaces were also noticed after the inoculation. In the inoculation of SPB-87 with PS1 in PDA, fungal fingerprints together with the probable formation of NaCl are observed in the first EDS spectrum (Sp1) in Figure 4a. Those elements appear in a 1:1 proportion, as can be seen in Table 2. Enrichment in Ca, C, and O can be observed for the same genus in Sp2, likely related to fungal excretions and possibly leached elements. Clear crystalline surfaces are also present at t_6, as can be observed in Sp3 in the same figure (most likely calcite/aragonite–$CaCO_3$–well related by the proportion at% of the constituents being 1:1:3, *c.f.* Table 2), and in the Raman spectrum in Figure 5a, where likely weddellite ($CaC_2O_4 \cdot 2\,H_2O$) is present. In the latter, the calcium oxalate mineral can be identified by the $\nu_{(C-O)}$ antisymmetric stretching band at around 1475 cm^{-1}, the $\nu_{(C-C)}$ stretching mode around 907–909 cm^{-1}, and finally the low wavenumber bands observed around 188 cm^{-1}, which can possibly be assigned to the CaO stretching and bending vibrations [22,24]. More difficult to observe is the band around 505 cm^{-1}, which may be attributed to the symmetric bending mode $\delta_{(O-C-O)}$ in the calcium oxalates [22], due to the glass matrix vibration modes overlapping [25].

Similar observations can be made for the inoculation with PS1 spores, where at t_4 it is possible to observe the formation of likely calcite/aragonite crystals (see Figure 4b Sp1 and the corresponding column in Table 2). Both at t_4 and t_6, fungal fingerprints can be found associated with Ca and O enrichment (Sp2 and Sp3 in Figure 4b and Table 2). At t_6, the marks of inoculation with PS1 spores are clearly identifiable by the presence of crystalline $CaCO_3$ by Raman spectroscopy (Appendix A, Figure A4a): (i) the 'internal vibration modes', originating from vibrations between the C and O of carbonate (CO_3, are present as a strong $\nu 1$ peak around 1087 cm^{-1} (symmetric stretching of C–O bonds), and the in-plane bend ($\nu 4$) in the 700–720 cm^{-1} region of C–O bonds (slightly overlapped by glass matrix vibration modes) [26], and also (ii) the crystalline 'lattice' or 'external modes' in the <400 cm^{-1} region that result from vibrations between molecules in the lattice, being mineral-specific, and in this case appearing around 278 cm^{-1} [23].

For the inoculation of SPB-87 with AS1 in PDA, fungal fingerprints together with an enrichment in Al and O is observed in the first EDS spectrum (Sp1) in Figure 4c. A similar behaviour is observed in the samples inoculated with AS1 spores also at t_4, in Sp1 in Figure 4d. In this case, a slight increase in C was quantified by EDS (Table 2), contrary to the possibly very small increase below the quantification limit of the EDS system in the first case. These enrichments are possibly associated with AS1 oxalate and other acid excretions, since this ligand is known to be able to form strong complexes with Al and enhance silicate

dissolution [27,28] and extraction from mineral lattice by fungal exometabolites [29,30], which could likely be extended to the current scenario.

The fungal exometabolites can also be present in the features analysed by EDS, corresponding to the Sp2 spectrum in Figure 4d–t_4–, to which the respective composition in Table 2 evidences a clear increase in C and O at%. Additionally, there are similar observations to the samples inoculated with PS1 spores at t_6 and the ones inoculated with AS1 in PDA and spores concerning the likely formation calcite/aragonite crystals (see Sp3 spectrum in Figure 4d corresponding column in Table 2, and see also Figure 5b and Appendix A, Figure A4b). Besides $CaCO_3$, also weddellite ($CaC_2O_4 \cdot H_2O$) was identified by μ-Raman in samples inoculated with AS1 spores.

Once again, damages of the reference samples–where two fungal species spores landed during the incubation period–were observed in the alteration of the surface composition for both fungal species (see Figure 4e,f and Table 2). It was not possible to identify the small crystalline structures by Raman spectroscopy, but the consequences of the fungal growth over those surfaces can easily be detected by the increasing C and O contents and the features rich in Cl, S, and Fe that should relate with the excretions of these microorganisms–e.g., exopolymers containing ionic groups and/or elements chelated or exchanged from the glass substrate [31]. This will be discussed further in the next section. A summary of the chemical surface alterations analysed by EDS and μ-Raman is presented in Table 3.

Table 3. Summary of the analysed chemical alterations analysed by Raman and EDS. (↑) enrichment; (↓) depletion.

		***Penicillium chrysogenum* (Incubation Environment)**			***Aspergillus niger* (Incubation Environment)**		
		with PDA	**Spores**	**Reference**	**with PDA**	**Spores**	**Reference**
Raman	t_4	–	–	–	–	–	–
	t_6	$CaC_2O_4 \cdot H_2O$	$CaCO_3$	–	$CaCO_3$	$CaCO_3$ $CaC_2O_4 \cdot H_2O$	–
EDS	t_4	NaCl (?) (↑) Ca, Mg, S, Cl, C, O (↓) Na	$CaCO_3$ (↑) Ca, Mg, Cl, Zn, Al, C, O (↓) Na	(↑) Ca, C (↓) Na	(↑) Al, O	(↑) Al, C, O	(↑) Ca, Cl, K, C, O (↓) Na
	t_6	$CaCO_3$ (↑) Ca, Mg, S, Cl, C, O (↓) Na	(↑) Ca, Mg, Zn, C, O (↓) Na	(↑) Ca, C, O (↓) Na	(↑) Ca, Mg, C, O	(↑) Ca, Cl, Mg, Zn, C, O (↓) Na	(↑) S, Fe, Cl, C, O (↓) Na

Additionally, a summary of the observations of the damages caused to the SPB-87 glass by the two fungal genera is presented in Figure 6–a colour scale is used to simplify the observations made on the glass samples. To aid data interpretation, ANOVA statistical analysis was applied to Figure 6 in all columns except references–these do not have a direct comparison due to the fact that these samples were not colonised at the same time as others.

Regarding the overall damage degree, the results of the four groups (columns) showed that $F > F_{crit}$ (and $p < 0.05$), which indicates that the mean values of the damages occurred on glass were significantly different, or that at least one mean is different (Appendix B, Table A1). *Aspergillus* spores presented the lower average value, producing lower damage on glass than the other fungi, since the ANOVA results for the three remaining groups (Appendix B, Table A2) presents a $F < F_{crit}$ (and a p value > 0.05)–i.e., lower differences among these groups. However, by the analysis of Tables A1 and A2 in Appendix B, the high variance of the group *Penicillium chrysogenum* spores may have been responsible for this result, and careful interpretation should follow.

Parameters		PS1			AS1		
		Fungi with PDA	Fungi Spores	Reference	Fungi and PDA	Fungi Spores	Reference
Mycelium growth	t_4	3	3	1	3	3	0
	t_6	4	4	1	3	3	0
Chromatic Alteration (stains, iridescence, darkening)	t_4	4	3	0	2	1	0
	t_6	3	3	0	2	2	0
Biopitting	t_4	3	2	0	3	2	0
	t_6	3	3	0	4	3	0
Depositions	t_4	3	3	1	2	2	1
	t_6	3	3	1	3	2	2
Crystals	t_4	2	0	0	3	2	0
	t_6	3	2	0	4	2	0
Corrosion	t_4	3	2	0	2	2	0
	t_6	4	3	1	3	3	1

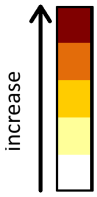

Figure 6. Summary of the damages observed in all samples after 4 and 6 months incubation–comparative analysis. Average damage is classified into a semi-quantitative scale from 0 to 4 (from non-existent to very pronounced, considering parameters such as density, quantity, and dimensions). A chromatic scale is used for better visualisation of the data.

Regarding our main question (i) concerning primary versus secondary bioreceptivity, when comparing *Aspergillus niger* with PDA and spores (Appendix B, Table A3), the results show $F > F_{crit}$ and $p < 0.05$, and that the mean values of the damages occurred on glass were significantly different–i.e., secondary bioreceptivity (PDA) produced more damages then primary bioreceptivity (Tween). On the contrary (see Appendix B, Table A4), in the case of PS1, the damage degree is closer between the two different media. The perception of these differences is enhanced to the observer by the use of different colours in the scale of damage shown in Figure 6.

Considering our main question (ii) related to comparing the damage degree between the two species, due to the above mentioned variance in the second group, the differences are better illustrated by the colour scale in Figure 6.

4. Discussion

Considering the main questions of this study, the authors selected two fungal genera based on two main following criteria: (i) most common fungi encountered on glasses with different composition (merely identified on glass); (ii) top two fungi that were found more often as glass biodeterioration agents. This selection was performed based on recent literature reviews [1], which allowed for the identification of AS1 and PS1 as the two genera corresponding to both criteria (i) and (ii). These are filamentous airborne fungi that produce spores which are easily released and dispersed into the air, are very abundant saprophytes, prevailing in soils, as well as in both indoor and outdoor environments [32,33]. They can stimulate the dust fallen from the air and deposits on the glass, as primary bioreceptivity.

4.1. Bioreceptivity: From a Nutritive Environment to Nutrient-Free Glass Surfaces

Three different nutrient levels were studied in this work—(i) clean glass samples, (ii) spores with Tween and (iii) the two fungi in PDA medium—and all present colonisation potential, showing fungal growth after 4 and 6 months. It has therefore been observed that: (i) reference samples could be a typical example of indoor artworks without cleaning after 6 months; (ii) samples with spores in Tween could represent outdoor artworks, where organic depositions naturally occur; (iii) and, finally, samples with fungi and PDA could represent a community that is already processing organic matter (e.g., actinomycetes, bacteria, and

foliage). The three scenarios fall into the two categories of primary ((i) and (ii), differentiated by the colonisation time) and secondary (iii) bioreceptivity.

In all cases except the control, which was kept in a completely separate Petri dish for comparison purposes of the environmental alteration of surfaces, reference samples are colonised, and there is mycelium growth over the glass samples in the less nutritive medium (Tween solution). Moreover, it could be verified that the PDA (secondary bioreceptivity) does not increase the mycelium density for *Aspergillus niger*, contrary to *Penicillium chrysogenum*. It is possible to assume that the PDA does not play a determinant role for the development on glass surfaces of the AS1, but it has some effect on the growth of the mycelium in case of the PS1. In terms of damage to the surfaces, it is possible to observe that, after the cleaning procedure, AS1 in PDA contributed to the formation of a larger amount of crystals than the inoculation with spores of the same species in the Tween solution.

The reference glass are a substrate with no culture media or Tween, having been originally sterilised and thus representing the best example of primary and intrinsic bioreceptivity. However, the small amount of dust particles visible in the control (Figure A1) throughout the tooling marks (glass blowing marks) may provide some hints of some additional nutritional source that could be collecting on the surface at the same time as the spores. Consequently, this demonstrates that fungi can colonise and develop over newly formed glass surfaces, clean and with solely dust particles. The ability of fungi to grow on nutrient-free media solely with silica compounds was also elsewhere reported [6,34], and apparently some fungi have the ability to grow by extracting the necessary elements from deteriorating glass [35]. Curiously, spots and stains are visible after 6 months, but there is no clear PS1 mycelium growth (Figure 2a). On the opposite, it can be observed that the PS1 genera is able to grow its mycelium into a dense level without a nutritional source after the 6 months incubation period (Figure 2a). This suggests that the two species have different colonisation patterns, progression, or needs. These differences may be a consequence of the physiology of the two fungal genera, as discussed below.

The semi-quantitative evaluation of the chromatic scale in Figure 6, consistently differentiated through the ANOVA results, indicate that the primary bioreceptivity of the glass samples inoculated with *Aspergillus niger* results in a lower damage degree than the secondary bioreceptivity.

4.2. Measuring Damage: Two Species, One Glass

When comparing the behaviours of the two fungi over the same glass substrate, it is possible to observe that: (i) the mycelia over the samples inoculated with *Aspergillus niger* is less dense than the ones with spores of *Penicillium* at t_6 in PDA, as spores and even in the contaminated reference samples; (ii) the biopitting and formation of crystals over the surfaces is more pronounced with AS1, in particular when inoculated with the nutritive medium (PDA). The damage patterns differ according to the fungi specimen (*c.f.* Figure 2a), i.e., the *Penicillium chrysogenum* hyphae fingerprints and craters, stains and iridescence are more visible than the *Aspergillus niger* ones, and the biopitting and crystals are less dominant in PS1. The inoculated fungi were also able to biodeteriorate SPB-87 glass to different degrees. This behaviour is rather different from what can be observed in the control samples (i.e., early stages of glass alteration), as the authors know well from similar soda-lime silicate glass environmental alteration, that at this point (a maximum of 6 months) has almost negligible damage compared to the one observed in the inoculated samples (e.g., Rodrigues et al. [21], Palomar and Rodrigues [36]).

The μ-Raman analysis revealed minerals that remained in the glass after the cleaning procedure, something also previously reported [4,6,7]. Most crystalline structures found in our experiment ($CaCO_3$ and $CaC_2O_4 \cdot H_2O$) are rich in Ca. Nonetheless, the leaching of the bivalent ion Ca^{2+} is not usually preferential compared to Na^+ in soda-rich silicate glasses such as SPB-87 [21,37]. Drewello and Weissmann [5] reported that the enrichment of some elements in specific areas can be induced by biomineralisation or produced by the mobilisation of elements by microorganisms. Calcium carbonate ($CaCO_3$) is know to be a common water corrosion product

widely reported in accelerated or long term ageing, mostly in silicate glass immersion experiments (e.g., Greiner-Wronowa and Stoch [38], Palomar et al. [39]). Short-term or environmental glass alteration experiments in soda-lime-silicate glasses (e.g., Rodrigues et al. [21], Palomar and Rodrigues [36]) seldom report the presence of this corrosion product in early stages (up to one year exposure), except if pollutants or other external factors are contributing to accelerate the glass surfaces deterioration [40]. The fact that $CaCO_3$ is present in PS1 and AS1 inoculated samples but is not observed in reference samples, and even less in the control, point to the role of fungi the formation of the observed $CaCO_3$. It has been well-established, for instance, that the rate of silicate mineral and basaltic glass weathering promoted by environmental microorganisms is usually higher than that of pure chemical weathering [41,42]. Additionally, other authors already reported the presence of similar biominerals (calcite, kalicinite, and whewellite) in different glass types due to the presence of *Penicillium aurantiogriseum* [43].

The main mechanisms underlying microorganisms–silicates interaction have been reported as including: (i) the destruction of the lattice via redox; (ii) the exchange of charge-balancing cations (K^+, Na^+, Ca^{2+}) for protons at the surface; and (iii) the production of metabolic by-products (e.g., extracellular enzymes, chelating agents, organic acids), which form network-destabilising surface complexes, or metal complexes [41,44]. Elements such as Al, Ca, Fe, K, Mg, and Na (released from the material) are assimilated by the biomass [35,45]. Nonetheless, the crystalline structures and chemical alterations observed here for each fungi present some differences that deserve attention.

On the one hand, in the case of AS1, the apparent dissolution and redeposition of elements such as Mg, Zn, and Al increased significantly over time with the growth of the biomass (see Table 3). Species of the same genera have been reported to possess the ability to promote dissolution and complexation processes with aluminium, as well as zinc [29,41,46]. The ability of this genera to acidify its micro-environment and exude high quantities of low molecular weight organic acids, which results in the extraction of relevant concentrations of aluminium from mineral phases, has been reported [29]. Other actors in this process are the well-known siderophores—specific ligands for ferric and other metal ions [42,47,48]—which are reported as being able to bind and remove Al^{3+} from the silicate network in the absence of Fe [41,42], thus being responsible for remarkable etching rates [47]. Due to the low content and lack of Fe and P in the SPB-87 glass itself, as well as in the inoculation with spores or the contaminated reference samples, the possible response in the form of a high production and accumulation of exometabolites may be occurring [49] and be related to the damage caused by AS1. The reported phenomena seem to be in accordance with the dominant biopitting and crystal formation in the SPB-87 glass studied here.

On the other hand, in the case of PS1, there is an apparently lower degree of dissolution of elements such as Zn and Al, despite species of this genera also being reported to be able to leach these metal ions [46,50]. Besides Ca^{2+}, Mg^{2+} are reputably a preferential leachate of this genus. In fact, it has been reported in the literature that Ca, Mg, and Zn are able to be solubilised from silicates by *Penicillium* (by Henderson and Duff [51] and by Ehrlich and Rossi (1990), cited by Castro et al. [52], Wei et al. [53]). Additionally, a good adaptation of this genera to the substrate in all cases can be perceived, causing an overall higher damage degree, considering all parameters: mycelium growth, chromatic alteration, biopitting, deposition of exometabolites, crystal formation, and surface corrosion/chemical alteration (Table 3).

In summary, the bioreceptivity and the biodeterioration consequences promoted by the two fungal species in each of the three different nutrient levels were verified to be different, being the influence of the nutritive medium dependent on the fungal genus. The observed variations could be associated with some of the genus-specific physiological characteristics. Some hypothesis can be placed for *Penicillium*'s similar growth and damage in both PDA and in Tween solution, such as (a) the ability of most species of *Penicillium* to mobilise other elements besides carbon and nitrogen from inorganic sources, with little or no requirements for their complex growth, (b) their good response in terms of development

in liquid media, or (c) the stimulus of the calcium ions in the glass, which are reported to be associated with increasing sporulation [32]. In addition, some hypothesis for the *Aspergillus* species studied in terms of a lower degree of damage and the differences between the high and low nutrient situations can also be conjectured, such as (a) the fact that extrinsic factors tend to have a more profound influence in the growth of *Aspergilli*, (b) the possibility of low water activity and its effect on germination and sporulation, and even on the subsequent mycelial growth of the species of this genus, (c) the fact that the mycotoxin production can be determined by the availability of nutrients, and (d) the general behaviour of species of this genus is that they can tolerate adverse conditions in one variable (e.g., water, nutrients), provided that the others are optimal [33].

4.3. Damage Degree: What about Time?

As aforementioned, both fungal genera *Aspergillus* and *Penicillium* are air-borne fungi and they are able to colonise distinct types of glass. According to Kerner-Gang [20], *Aspergillus* has been reported to cause glass biodeterioration in only 5 days, while *Penicillium* is said to biodeteriorate glass after 40 days of incubation [43]. The present study—where two species of these two genera are inoculated in a soda-lime silicate glass—shows evidence of damage in similarly short periods of time in the present case, the minimum observation time was 4 months. The ability of *Aspergillus niger* to cause biodeterioration in very different types of glass (optical glass [8,19,20,54] and potassium lime silicate [3,43]) is known from the literature, and once more it is demonstrated that some species (such as the one used here) can promote damage in a soda-lime glass as well. Additionally, damages were also reported for different *Penicillium* species (potassium-lime- or potassium-silicate [4,43], soda-lime silicate [14], mixed alkali silicate [4], borosilicate [14], and optical glass [8]). The presently studied *Penicillium* species also produced damage in the surface of the contemporary soda-lime silicate formulation, in a relatively short period of time, as was observed for instance by some of the authors for different glass types [4].

The passage of time also leaves a mark on the damage caused over the glass surfaces. The observations made on the current work are evidence that the damage increases with increasing time (see Figure 6). After a short period of inoculation (4 months) the damages caused by both *Penicillium chrysogenum* and *Aspergillus niger* include depletion and deposition of elements, hyphae fingerprints and crystal formation. When 6 months have passed, an increase in the amount of damage is also observed (e.g., mycelium growth and surface corrosion in *Penicillium chrysogenum* in PDA, or biopitting and amount of crystalline structures in *Aspergillus niger* in PDA—see Figure 6).

4.4. Contemporary Glass Art: From Biodeterioration to Creativity

Both this study and in others [5,55], the deposits formed in the glass surfaces that are associated with the reaction between glass components and fungi metabolites, and the biofilm itself and its interaction with glass, can cause serious damage on '*historical stained glass windows not only from a chemical point of view but also from an aesthetic and iconographic one, since glass loses its transparency*' [3]. The same applies to SPB-87 glass, since this material is the medium of many contemporary glass artists, where the loss of transparency could create great damage from an aesthetic and iconographic point of view. Such as in heritage glass and even modern materials [7,56], the biofilm growth on contemporary glass surfaces may alter their transparency and smoothness, and could even compromise the artistic value. Moreover, it is also noticed that the cleaning procedure (a 1:1 solution of water:ethanol) does not completely remove the fungi, which has been reported previously [4]. Therefore, further research is necessary on glass cleaning procedures, in order to be able to preserve both the past and the contemporary glass legacies.

On the other hand, the artistic process could also build upon this knowledge and include the methods and results of the current study for new creations. Often the scientific and artistic investigations run in parallel [11], where the artistic processes are many times inspired and grounded on the material and natural sciences. Hence, concerning

this dichotomy between biodeterioration and creativity, this study offers several insights, resulting that the outputs can have the potential to serve both the scientific and the artistic communities. In addition, when looking at the conservation of SPB-87 glass, the knowledge on the bioreceptivity of this glass and on the potential of fungal species to cause damage is of primary importance. The need for further developments in finding effective cleaning procedures becomes evident. Moreover, artists are now better informed on how to explore the interaction and surface alteration patterns resulting from the action of these microorganisms on SPB-87 glass. The effect of these microorganisms on the glass surfaces can be further explored in their creative processes. The inspiration in nature and the interaction of the environment and organisms with the matter is, and always will be, an intrinsic part of art created by man.

5. Conclusions

The results of the present work regard the role of each fungal species (*Penicillium chrysogenum* and *Aspergillus niger*) on the degree and pattern of damage of the soda-lime silicate contemporary glass SPB-87 used by artists worldwide. They showed that both genera inoculated were able to grow and produce damages on this glass to different degrees. In terms of bioreceptivity, it was verified that the glass samples with PDA (more nutritive medium) produced more damages than the ones without this nutritional particularly for *Aspergillus niger*. Regarding the nutritive medium relevance for each species, it was verified that the secondary bioreceptivity for *Aspergillus niger* does not significantly influence the mycelium growth, but it does influence the chemical damage of the glass surfaces, contrary to *Penicillium chrysogenum*, where the mycelium growth is more or less similar in the two different nutritive conditions, as is the damage degree.

The new and in-depth understanding of the bioreceptivity and of this glass present in contemporary art and our cultural heritage is very relevant for the scientific, conservation, and artistic communities–either for the protection of cultural materials, or for the artists from the point of view of innovative and inspirational ways of creating glass art in the future.

Author Contributions: Conceptualisation, A.R., M.A., and M.F.M.; methodology, M.A. and M.F.M.; validation, A.R. and M.F.M.; investigation, M.A.; resources, M.F.M. and A.Z.M.; data curation, A.R., M.A., and S.G.-P.; original draft preparation, A.R.; writing—review and editing, M.A., M.F.M., S.G.-P., and A.Z.M.; supervision, M.F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by The Portuguese Science Foundation (project Pest-OE/EAT /UI0729/2014, UIDB/00729/2020). A.Z.M. was supported by the Ramon y Cajal contract RYC2019-026885 from the Spanish Ministry of Science and Innovation (MCIN). S.G.-P. was supported by the intramural project PIE_20214AT021 from the Spanish National Research Council (CSIC).

Data Availability Statement: The data presented in this study are available within this article and Appendix part. Raw data not included can be made available on request.

Acknowledgments: The authors acknowledge Daniela Gomes for the collaboration in the SEM observations and late Professor Solange Muralha for the μ-Raman insights.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Supplementary Figures

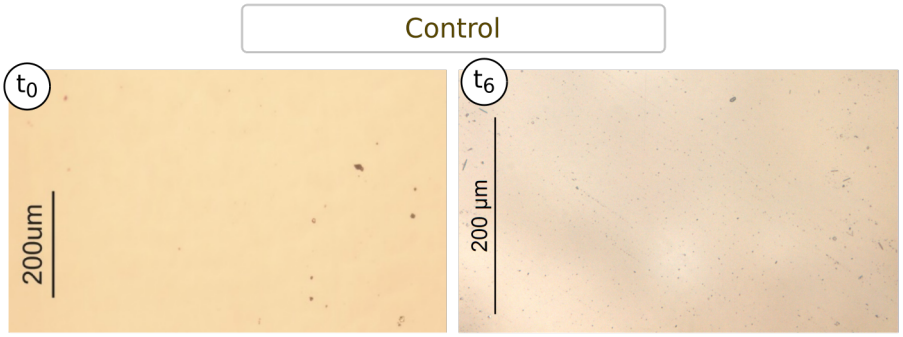

Figure A1. Optical microscopy results form the control samples.

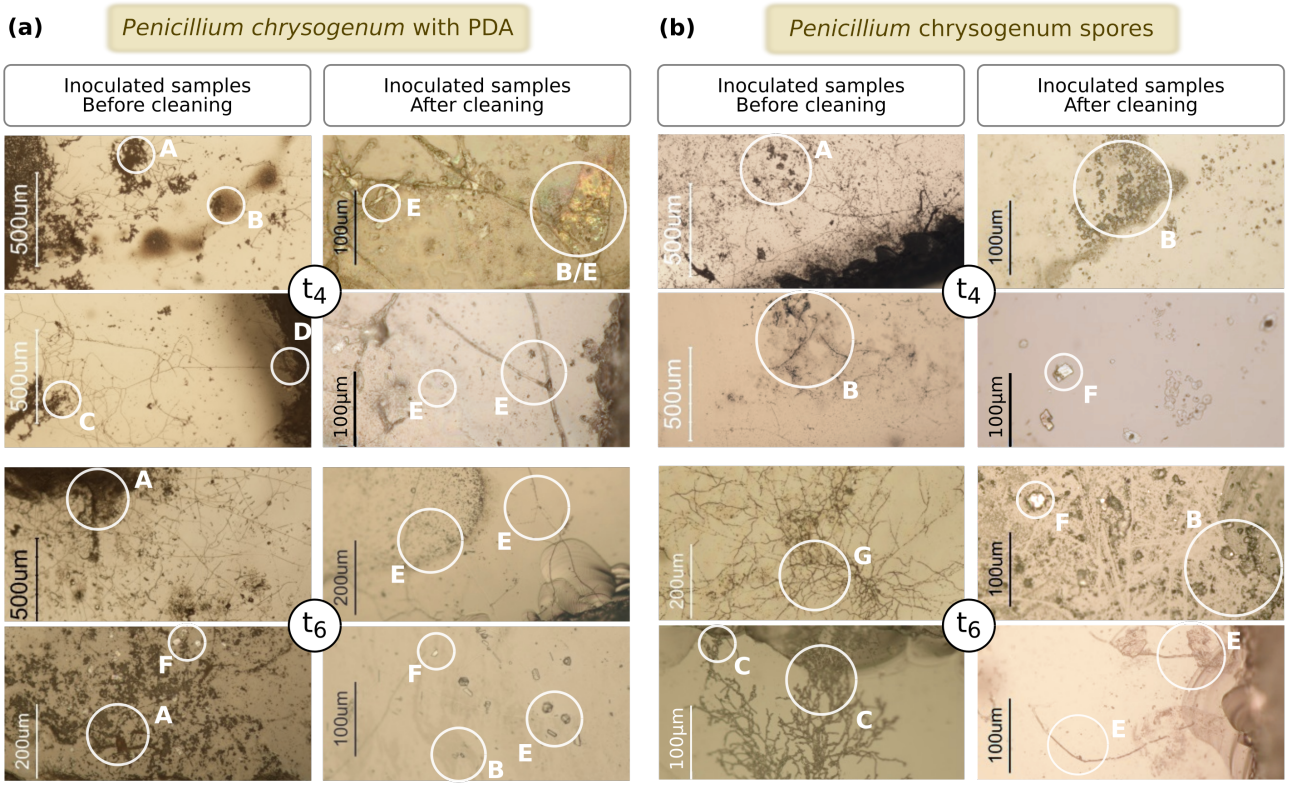

Figure A2. Optical microscopy results from the inoculation of SPB-87 with (**a**) *Penicillium chrysogenum* in PDA and (**b**) *Penicillium chrysogenum* spores in Tween solution. Legend as in Figure 2.

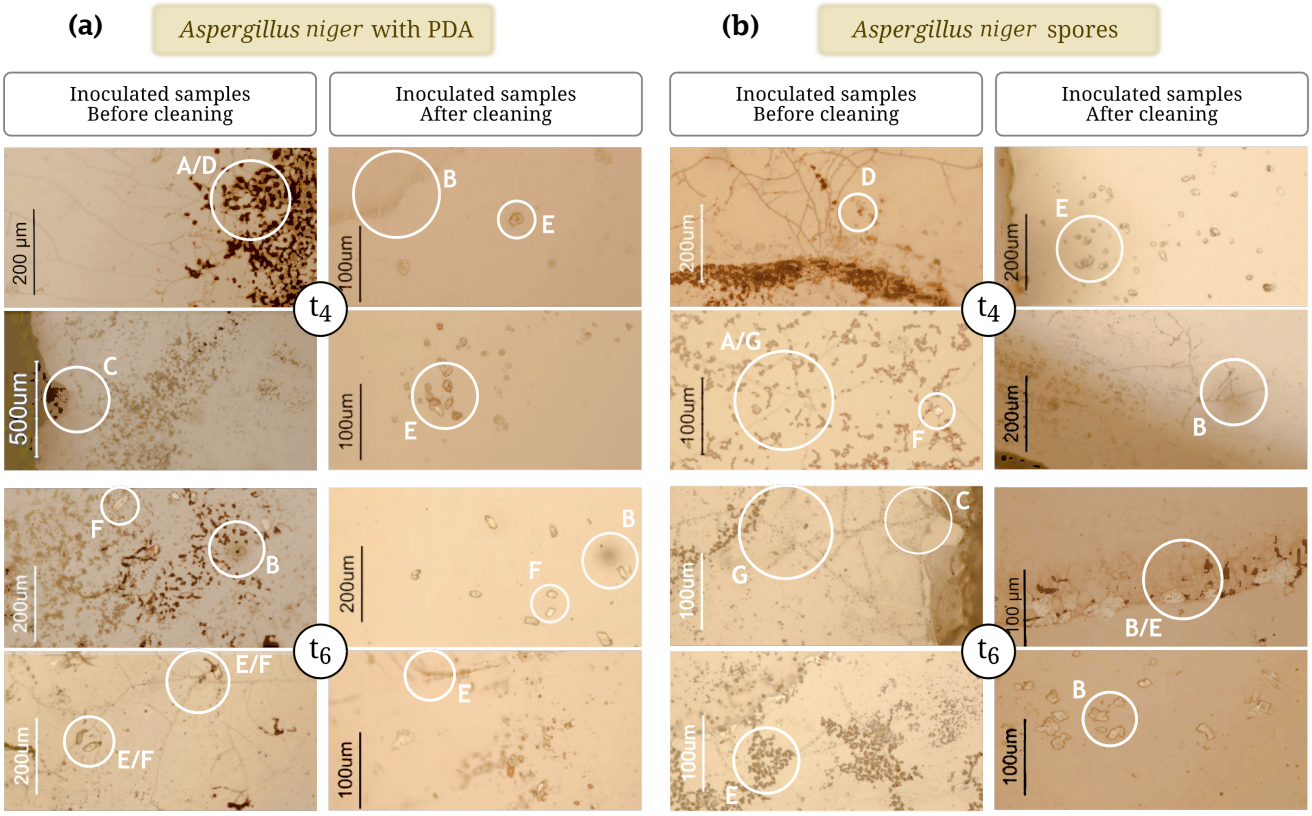

Figure A3. Optical microscopy results from the inoculation of SPB-87 with (**a**) *Aspergillus niger* in PDA and (**b**) *Aspergillus niger* spores in Tween solution. Legend as in Figure 2.

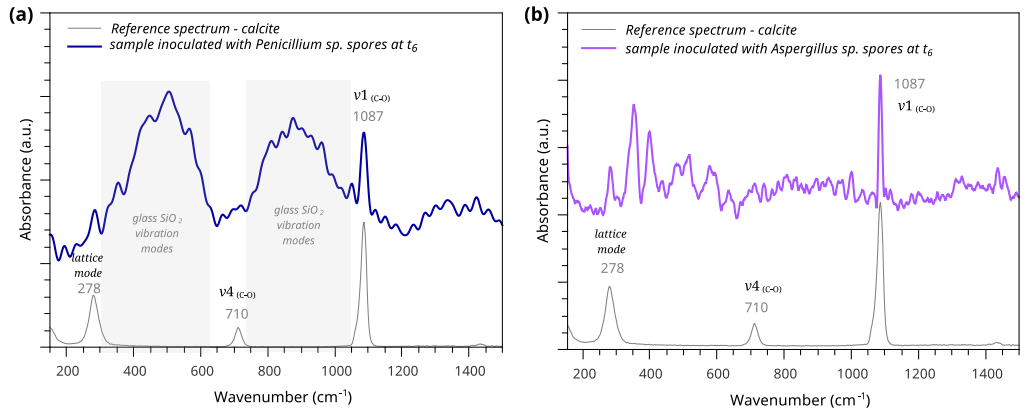

Figure A4. Raman spectra of the identified crystalline products on the glass surfaces inoculated with (**a**) *Penicillium chrysogenum* spores in Tween, according to literature data [22,23], and (**b**) *Aspergillus niger* spores in Tween, identified according to literature data [23].

Appendix B. Supplementary ANOVA Tables

The ANOVA analysis is used to test the null hypothesis that the means of several populations are all equal. If $F > F_{crit}$, we reject the null hypothesis. First we applied the ANOVA single factor from Excel software to all the damage data, using a p value of 0.05.

Table A1. ANOVA Single Factor Results obtained from the values of the damages produce on glass by *Penicillium* and *Aspergillus* with PDA and in Tween.

Summary of All the Groups						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Penicillium PDA	12	38	3.167	0.333		
Penicillium Spores	12	31	2.583	0.992		
Aspergillus PDA	12	34	2.833	0.515		
Aspergillus spores	12	27	2.25	0.386		
ANOVA Results of all the groups						
Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	5.417	3	1.806	3.243	0.031	2.816
Within Groups	24.5	44	0.557			
Total	29.917	47				

This analysis results showed $F > F_{crit}$ and $p < 0.05$, which indicates that the mean values of the damages that occurred on glass were significantly different, or that at least one mean is different. Since the group of *Aspergillus* spores presented the lower average value, a new ANOVA test was made without this group.

Table A2. ANOVA Single Factor Results comparing *Penicillium* and *Aspergillus* with PDA, and *Penicillium* in Tween.

Summary of 3 First Groups						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Penicillium PDA	12	38	3.167	0.333		
Penicillium Spores	12	31	2.583	0.992		
Aspergillus PDA	12	34	2.833	0.515		
ANOVA results of 3 groups						
Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	2.056	2	1.028	1.675	0.203	3.285
Within Groups	20.25	33	0.614			
Total	22.306	35				

The ANOVA results for the three groups presents a $F > F_{crit}$ and a p value > 0.05, indicating that the mean value of these groups, their damage degree, are not significantly different. The *Aspergillus* spores produced a lower damage on glass than the other fungi.

Table A3. ANOVA Single Factor Results comparing *Aspergillus* with PDA and in Tween.

Summary of the 2 *Aspergillus* Groups						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Aspergillus PDA	12	34	2.833	0.515		
Aspergillus spores	12	27	2.25	0.386		
ANOVA results of the 2 *Aspergillus* groups						
Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	2.042	1	2.042	4.529	0.045	4.301
Within Groups	9.917	22	0.451			
Total	11.958	23				

$F > F_{crit}$ and $p < 0.05$ indicate that the two groups are significantly different.

Table A4. ANOVA Single Factor Results comparing *Penicillium* with PDA and in Tween.

Summary of the 2 *Penicillium* Groups						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Penicillium PDA	12	38	3.167	0.333		
Penicillium Spores	12	31	2.583	0.992		
ANOVA results of the 2 *Penicillium* groups						
Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	2.0417	1	2.042	3.08	0.093	4.301
Within Groups	14.583	22	0.663			
Total	16.625	23				

$F < F_{crit}$ and $p > 0.05$ indicate that the two groups are not significantly different.

References

1. Macedo, M.F.; Vilarigues, M.G.; Coutinho, M.L. Biodeterioration of Stained Glass and Ceramic Tiles: Recent Developments. In *Recent Developments in Heritage Microbiology*; Clifford, J., Mitchell, R., Vasanthakumar, A., Eds.; Archetype Publications: London, UK, 2021.
2. Guillitte, O. Bioreceptivity: A new concept for building ecology studies. *Sci. Total Environ.* **1995**, *167*, 215–220. [CrossRef]
3. Carmona, N.; Laiz, L.; Gonzalez, J.M.; Garcia-Heras, M.; Villegas, M.A.; Saiz-Jimenez, C. Biodeterioration of historic stained glasses from the Cartuja de Miraflores (Spain). *Int. Biodeterior. Biodegrad.* **2006**, *58*, 155–161. [CrossRef]
4. Rodrigues, A.; Gutierrez-Patricio, S.; Miller, A.Z.; Saiz-Jimenez, C.; Wiley, R.; Nunes, D.; Vilarigues, M.; Macedo, M.F. Fungal biodeterioration of stained-glass windows. *Int. Biodeterior. Biodegrad.* **2014**, *90*, 152–160. [CrossRef]
5. Drewello, R.; Weissmann, R. Microbially influenced corrosion of glass. *Appl. Miciobiology Biotechnol.* **1997**, *47*, 337–346. [CrossRef]
6. Gorbushina, A.A.; Palinska, K.A. Biodeteriorative processes on glass: Experimental proof of the role of fungi and cyanobacteria. *Aerobiologia* **1999**, *15*, 183–191. [CrossRef]
7. Corrêa Pinto, A.M.; Palomar, T.; Alves, L.C.; da Silva, S.H.; Monteiro, R.C.; Macedo, M.F.; Vilarigues, M.G. Fungal biodeterioration of stained-glass windows in monuments from Belém do Pará (Brazil). *Int. Biodeterior. Biodegrad.* **2019**, *138*, 106–113. [CrossRef]
8. Kerner-Gang, W.; Theden, G. Durch Pilze verursachte Schäden an optischen Gläsern-Ein Bildbericht über Laboratoriumsversuche (Damage to optical glass caused by fungus). *Glastech Ber.* **1964**, *37*, 256–259.
9. Macedo, M.F.; Vilarigues, M.G.; Coutinho, M.L. Biodeterioration of Glass-Based Historical Building Materials: An Overview of the Heritage Literature from the 21st Century. *Appl. Sci.* **2021**, *11*, 9552. [CrossRef]
10. Piñar, G.; Piombino-Mascali, D.; Maixner, F.; Zink, A.; Sterflinger, K. Microbial survey of the mummies from the capuchin catacombs of palermo, italy: Biodeterioration risk and contamination of the indoor air. *FEMS Microbiol. Ecol.* **2013**, *86*, 341–356. [CrossRef] [PubMed]
11. Alves, M. Entre o Homem e a Matéria. Master's Thesis, Faculdade de Belas Artes, Universidade de Lisboa, Faculdade de Ciências e Tecnologia & Universidade Nova de Lisboa, Lisbon, Portugal, 2015.
12. Sterbenk, Y.; Kritzeck, M. Corning Museum of Glass Exhibitions Focus on Harvey K. Littleton and Dominick Labino, Founders of American Studio Glass Movement, Corning Museum of Glass. 2011. Available online: http://www.cmog.org/article/harvey-k-littleton-and-american-studio-glass-movement (accessed on 28 February 2022).
13. Byrd, J.F. Harvey K. Littleton and the American Studio Glass Movement, Corning Museum of Glass. 2011. Available online: http://www.cmog.org/press-release/corning-museum-glass-exhibitions-focus-harvey-k-littleton-and-dominick-labino-founder (accessed on 28 February 2022)
14. Callot, G.; Maurette, M.; Pottier, L.; Dubois, A. Biogenic etching of microfractures in amorphous and crystalline silicates. *Nature* **1987**, *328*, 147–149. [CrossRef]
15. Gallien, J.P.; Gouget, B.; Carrot, F.; Orial, G.; Brunet, A. Alteration of glasses by micro-organisms. *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. At.* **2001**, *181*, 610–615. [CrossRef]
16. Booth, C. *Fungal Culture Media*; Academic Press Inc.: Cambridge, MA, USA, 1971; Volume 4, pp. 49–94. [CrossRef]
17. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. Guide Methods Appl.* **1990**, *18*, 315–322.
18. Zhang, Z.; Schwartz, S.; Wagner, L.; Miller, W. A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **2000**, *7*, 203–214. [CrossRef] [PubMed]
19. Bartosik, M.; Zakowska, Z.; Cedzińska, K.; Rozniakowski, K. Biodeterioration of optical glass induced by lubricants used in optical instruments technology. *Pol. J. Microbiol.* **2010**, *59*, 295–300. [CrossRef] [PubMed]
20. Kerner-Gang, W. Zur frage der entstehung von schimmelpilzspuren auf optischen gläsern. *Mater. Org.* **1968**, *3*, 1–17.
21. Rodrigues, A.; Fearn, S.; Palomar, T.; Vilarigues, M. Early stages of surface alteration of soda-rich-silicate glasses in the museum environment. *Corros. Sci.* **2018**, *143*, 362–375. [CrossRef]
22. Frost, R.L. Raman spectroscopy of natural oxalates. *Anal. Chim. Acta* **2004**, *517*, 207–214. [CrossRef]

23. DeCarlo, T.M. Characterizing coral skeleton mineralogy with Raman spectroscopy. *Nat. Commun.* **2018**, *9*, 9–11. [CrossRef] [PubMed]
24. Jehlicka, J.; Edwards, H.G.; Culka, A. Using portable Raman spectrometers for the identification of organic compounds at low temperatures and high altitudes: Exobiological applications. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2010**, *68*, 3109–3125. [CrossRef] [PubMed]
25. Colomban, P.; Paulsen, O. Non-destructive determination of the structure and composition of glazes by Raman spectroscopy. *J. Am. Ceram. Soc.* **2005**, *88*, 390–395. [CrossRef]
26. Furukawa, T.; Fox, K.E.; White, W.B. Raman spectroscopic investigation of the structure of silicate glasses. III. Raman intensities and structural units in sodium silicate glasses. *J. Chem. Phys.* **1981**, *75*, 3226–3237. [CrossRef]
27. Fox, T.R.; Comerford, N.B. Influence of Oxalate Loading on Phosphorus and Aluminum Solubility in Spodosols. *Soil Sci. Soc. Am. J.* **1992**, *56*, 290–294. [CrossRef]
28. Sayer, J.A.; Kierans, M.; Gadd, G.M. Solubilisation of some naturally occurring metal-bearing minerals, limescale and lead phosphate by Aspergillus niger. *FEMS Microbiol. Lett.* **1997**, *154*, 29–35. [CrossRef] [PubMed]
29. Polák, F.; Urík, M.; Bujdoš, M.; Uhlík, P.; Matúš, P. Evaluation of aluminium mobilization from its soil mineral pools by simultaneous effect of Aspergillus strains' acidic and chelating exometabolites. *J. Inorg. Biochem.* **2018**, *181*, 162–168. [CrossRef] [PubMed]
30. Polák, F.; Urík, M.; Bujdoš, M.; Matúš, P. Aspergillus niger enhances oxalate production as a response to phosphate deficiency induced by aluminium(III). *J. Inorg. Biochem.* **2020**, *204*, 110961. [CrossRef] [PubMed]
31. Sand, W. Microbial mechanisms of deterioration of inorganic substrates—A general mechanistic overview. *Int. Biodeterior. Biodegrad.* **1997**, *40*, 183–190. [CrossRef]
32. Moss, M.O. Morphology and physiology of Penicillium and Acremonium. In *Penicillium and Acremonium*; Chapter 2; Peberdy, J.F., Ed.; Springer Science+Business Media: New York, NY, USA, 1987; pp. 37–71.
33. Kozakiewicz, Z.; Smith, D. Physiology of Aspergillus. *Aspergillus* **1994**, 23–40. [CrossRef]
34. Wainwright, M.; Al-Wajeeh, K.; Grayston, J. Effect of silicic acid and other silicon compounds on fungal growth in oligotrophic and nutrient-rich media. *Mycol. Res.* **1997**, *101*, 933–938. [CrossRef]
35. Krumbein, W.E.; Urzì, C.E.; Gehrmann, C. Biocorrosion and biodeterioration of antique and medieval glass. *Geomicrobiol. J.* **1991**, *9*, 139–160. [CrossRef]
36. Palomar, T.; Rodrigues, A. Environmental degradation of Modern non-balanced glasses. *Ge-conservacion* **2020**, *17*, 226–232. [CrossRef]
37. Rodrigues, A.; Fearn, S.; Vilarigues, M. Mixed reactions: Glass durability and the mixed-alkali effect. *J. Am. Ceram. Soc.* **2019**, *102*, 7278–7287. [CrossRef]
38. Greiner-Wronowa, E.; Stoch, L. Influence of environment on surface of the ancient glasses. *J. Non-Cryst. Solids* **1996**, *196*, 118–127. [CrossRef]
39. Palomar, T.; Redol, P.; Almeida, I.C.; da Silva, E.P.; Vilarigues, M. The Influence of Environment in the Alteration of the Stained-Glass Windows in Portuguese Monuments. *Heritage* **2018**, *1*, 365–376. [CrossRef]
40. Robinet, L.; Eremin, K.; Del Arco, B.C.; Gibson, L.T. A Raman spectroscopic study of pollution-induced glass deterioration. *J. Raman Spectrosc.* **2004**, *35*, 662–670. [CrossRef]
41. Hu, R.; Li, F.; Yu, H.; Yang, J. Weathering of Basalts by Aspergillus sp. FS-4 Strain: Glass Compositions are Prone to Weathering. *Geomicrobiol. J.* **2020**, *37*, 101–109. [CrossRef]
42. Perez, A.; Rossano, S.; Trcera, N.; Huguenot, D.; Fourdrin, C.; Verney-Carron, A.; van Hullebusch, E.D.; Guyot, F. Bioalteration of synthetic Fe(III)-, Fe(II)-bearing basaltic glasses and Fe-free glass in the presence of the heterotrophic bacteria strain Pseudomonas aeruginosa: Impact of siderophores. *Geochim. Cosmochim. Acta* **2016**, *188*, 147–162. [CrossRef]
43. Drewello, U.; Weissmann, R.; Rölleke, S.; Müller, E.; Wuertz, S.; Fekrsanati, F.; Trolle, C.; Drewello, R. Biogenic surface layer on historical window glass and the effect of excimer laser cleaning. *J. Cult. Herit.* **2000**, *1*, S161–S171. [CrossRef]
44. Bennett, P.C.; Rogers, J.R.; Choi, W.J.; Hiebert, F.K. Silicates, silicate weathering, and microbial ecology. *Geomicrobiol. J.* **2001**, *18*, 3–19. [CrossRef]
45. Kaiser, J.P.; Trumpler, S.; Raschle, P. Wachstum von taeniolina deightonii auf mittelaterlichen glasern. *Werkst. Korros.* **1994**, *45*, 125–127. [CrossRef]
46. Bakri, M.M. Tri-Calcium and Zinc Phosphates Solubilization by Aspergillus niger and Its Relation to Organic Acids Production. *BioNanoScience* **2019**, *9*, 238–244. [CrossRef]
47. Ehrlich, H.; Demadis, K.D.; Pokrovsky, O.S.; Koutsoukos, P.G. Modern views on desilicification: Biosilica and abiotic silica dissolution in natural and artificial environments. *Chem. Rev.* **2010**, *110*, 4656–4689. [CrossRef] [PubMed]
48. Haas, H. Fungal siderophore metabolism with a focus on Aspergillus fumigatus. *Nat. Prod. Rep.* **2014**, *31*, 1266–1276. [CrossRef] [PubMed]
49. Ahammad, S.Z.; Gomes, J.; Sreekrishnan, T.R. Wastewater treatment forproductionofH2S-free biogas. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 1163–1169. [CrossRef]
50. Urík, M.; Bujdoš, M.; Milová-Žiaková, B.; Mikušová, P.; Slovák, M.; Matúš, P. Aluminium leaching from red mud by filamentous fungi. *J. Inorg. Biochem.* **2015**, *152*, 154–159. [CrossRef]

51. Henderson, M.E.; Duff, R. The release of metallic and silicate ions from minerals, rocks, and soils by fungal activity. *J. Soil Sci.* **1963**, *14*, 236–246. [CrossRef]
52. Castro, I.M.; Fietto, J.L.; Vieira, R.X.; Trópia, M.J.; Campos, L.M.; Paniago, E.B.; Brandão, R.L. Bioleaching of zinc and nickel from silicates using Aspergillus niger cultures. *Hydrometallurgy* **2000**, *57*, 39–49. [CrossRef]
53. Wei, Z.; Liang, X.; Pendlowski, H.; Hillier, S.; Suntornvongsagul, K.; Sihanonth, P.; Gadd, G.M. Fungal biotransformation of zinc silicate and sulfide mineral ores. *Environ. Microbiol.* **2013**, *15*, 2173–2186. [CrossRef] [PubMed]
54. Ohtsuki, T. Uber das Verschimmeln der glaseren Gegenstande. *Pro. Imp. Acad. Tokyo* **1943**, *19*, 688–692. [CrossRef]
55. Bartosik, M.; Żakowska, Z.; Klimek, L.; Szumigaj, J. Korozja mikrobiologiczna szkla optycznego. *Ochr. Przed Koroz.* **2007**, *7*, 294–296.
56. Shirakawa, M.A.; John, V.M.; Mocelin, A.; Zilles, R.; Toma, S.H.; Araki, K.; Toma, H.E.; Thomaz, A.C.; Gaylarde, C.C. Effect of silver nanoparticle and TiO2 coatings on biofilm formation on four types of modern glass. *Int. Biodeterior. Biodegrad.* **2016**, *108*, 175–180. [CrossRef]