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MICROSCOPIC TECHNIQUES IN THE DETERMINATION OF THE BIODETERIORATION IN CINEMATOGRAPHIC FILMS

I. Vivar[a]*, S. F. Borrego[a], A.M. García[b], D.A. Moreno[b]

[a] Laboratorio de Conservación Preventiva, Archivo Nacional de la República de Cuba, Havana, Cuba.
[b] Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (UPM), Madrid, Spain.

*Corresponding author, e-mail: belvi270281@gmail.com, tlf: +53 7 861 2513.

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ABSTRACT
For years, the researchers have been interested in studying the deterioration caused by microorganisms in the cinematographic heritage. To study the biodeterioration caused by microorganisms not only techniques of identification of microorganisms are used but also the microscopic techniques to observe of their structures and the damages in the materials. In Cuba, electron and epifluorescence microscopy are applied in multiple branches of scientific research but so far it is unknown to use it to study the affectation that may have the biodeteriorated documentary supports in our heritage institutions. Therefore the aim of the present work is to apply different microscopic techniques to study the biodeterioration caused by microorganisms in cinematographic films of patrimonial value. Optical microscopy, environmental scanning electron microscopy (ESEM), vacuum scanning electron microscopy (SEM) and epifluorescence microscopy (EPI) were used. An important microbial colonization, fundamentally fungal on both sides of the films was evidenced with the ESEM and SEM. In addition, a dense biofilm and a bio-fouling formed mainly by mites and pollens were observed. The presence of small holes in the fungal structures as a result of the lytic activity of the bacteria was observed. For the other hand, fluorescence microscopy showed that the majority of the microorganisms on the films were still viable and active. For the first time in Cuba, the microscopic techniques were used to evidence the presence of viable and active microorganisms. Those microorganisms are responsible of the biodeterioration in cinematographic films of heritage value.

Keywords: cinematographic-films; deterioration; microorganisms.

LAS TÉCNICAS MICROSCÓPICAS EN LA DETERMINACIÓN DEL BIODETERIORO EN PELICULAS CINEMATOGRÁFICAS

RESUMEN
Desde hace años es interés de los investigadores el estudio del deterioro provocado por microorganismos en el patrimonio cinematográfico. El biodeterioro microbiológico no solo se estudia empleando técnicas de identificación de los microorganismos si no también mediante la observación microscópica de sus estructuras y el daño de los materiales. En Cuba, las microscopías electrónicas y de epifluorescencia nunca antes se habían utilizado para estudiar el grado de afectación que pudieran tener los soportes documentales biodeteriorados que se encuentran en instituciones patrimoniales. Por ello el objetivo del presente trabajo fue aplicar diferentes técnicas microscópicas en el estudio del biodeterioro provocado por microorganismos en películas cinematográficas de valor patrimonial. Se emplearon la microscopía óptica, la microscopía electrónica de barrido ambiental (MEBA), la microscopía electrónica de barrido al vacío (MEB) y la microscopía de epifluorescencia (EPI). Con las microscopías MEBA y MEB se evidenció una importante colonización microbiana, fundamentalmente fúngica por ambas caras de las películas. Se observó además, una biopelícula densa y un bioensuciamiento formado fundamentalmente por ácaros y pólenes. También fue posible ver pequeños agujeros en las estructuras fúngicas producto de la actividad lítica de las bacterias. Se comprobó que la mayoría de los microorganismos colonizadores de las películas cinematográficas aún estaban viables y activos. Se emplearon por primera vez en Cuba diferentes técnicas microscópicas para evidenciar la presencia de microorganismos viables y activos que están biodegradando y dañando los soportes cinematográficos de valor patrimonial estudiados.

Palabras clavesbiodeterioro, microorganismos, películas-cinematográficas.

INTRODUCTION

Cinematographic films, as part of documentary heritage, are vulnerable to chemical deterioration as well as biodeterioration [1-7]. For some years researchers have studied the deterioration caused by microorganisms in the cinematographic heritage for the conservation of this valuable legacy. The microbiological biodeterioration (microbiodeterioration) is understood as an undesirable change in the properties of a material being caused by the vital activity of the microorganisms [8]. In relation to cultural heritage, microbiodeterioration is the physical, chemical and aesthetic damage caused biologically by fungi, bacteria, microalgae and lichens. This microbiodeterioration is not only studied using identification techniques of microorganisms but also by microscopic observation of their structures. Microscopic optics is the conventional technique used in the field of biology for the study of microscopic structures of living matter. Currently, electron microscopy is one of the most used techniques to obtain information on the morphology of microorganisms as well as the configuration of the supports [9]. Another technique employed at the present is epifluorescence microscopy, since it is a quick tool for determining the viability of microorganisms [10]. In Cuba, microscopy is applied in multiple branches of scientific research but so far it is unknown to use it to study the degree of affectation that may have the biodeteriorated documentary supports that are in our heritage institutions. Therefore the aim of the present work is to apply different microscopic techniques to study the biodeterioration caused by microorganisms in cinematographic films of patrimonial value.

MATERIALS AND METHODS

Samples were taken at random from six films of cellulose acetate in color that belong the Cuban Institute of Art and Film Industry of Cuba (ICAIC) [10]. For selection, the visible affectations by microorganisms at the beginning of the rolls of film and along the edges thereof were taken into account. Fragments of each film selected for the study were cut.

The repository, at the time of sampling (2009), did not have the appropriate conditions for the conservation of this type of special material. The average temperature was 31^0C and the average relative humidity was 65%.

The microscopic observations were made in the Biodeterioration Laboratory at the Technical University of Madrid (UPM), Spain.

Preliminary observation

To obtain a first impression of the biodeterioration state of the film fragments and the selection of the study areas, the stereo microscope (SZX12, Olympus) was used. Areas of interest were selected and small fragments were cut with sterile scissors for other microscopic observations.

Electron microscopy

The samples of the unprocessed films were observed under the environmental scanning electron microscope (ESEM) (INSPECT, QUANTA 200, 20-25 kV).

The samples of the films were also observed in the scanning electron microscope (SEM) (DSM 960, Zeiss, 15 kV) and previously the fragments were fixed and were metallized with gold [10].

Both microscopies were used to see the degree of deterioration of the support and the colonizing microorganisms.

Study of microbial viability by microscopy

Epifluorescence microscopy was the technique used and the procedure followed was previously described by Vivar et al [10].

RESULTS AND DISCUSSION

Cinematographic films can be biodeteriorated by fungi and bacteria [10,11], especially in hot and humid

environments of tropical countries. In Cuba these organisms grow and develop easily in the archives [8,12-14] if the climatic conditions of the environments in the repositories are not controlled. That was precisely what happened with the films studied, where the damage is visible to the naked eye.

The fungal growth in all the films studied was confirmed by observations in the stereomicroscope (Fig. 1). Film 1 was completely covered by a dense biofilm and the rest only showed dark spots. The development of microorganisms, preferably by the edges of the perforations in the films, was also observed (Fig. 2). Abrusci [15] obtained similar results in films from the film archives of Madrid, Las Palmas de Gran Canaria and Barcelona in Spain as well as Bučková *et al.* [16] observed also the development of fungi on the edge of photographic negatives of cellulose acetate, materials very similar in composition to the cinematographic films studied. These areas are more susceptible to colonization, possibly, because they can retain moisture and there is greater availability of oxygen. In any case, microbial development can continue from a small contamination under appropriate environmental conditions and therefore the film can be lost.

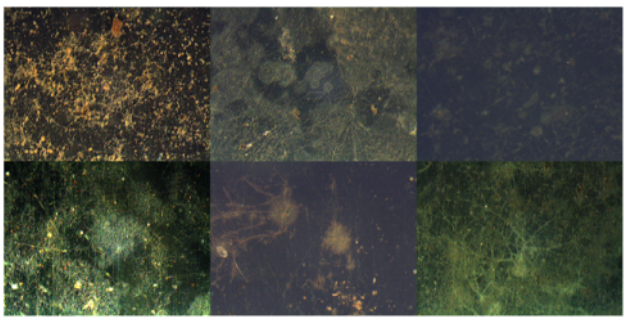

Fig. 1. Images obtained by optical microscopy (20X) that show the microbial colonization of the analyzed films.

The images obtained by SEM and ESEM confirmed that both sides of the films had microbial growth fundamentally by fungi, and the gelatine emulsion side was the most affected (Fig. 3). This result was to be expected because it is known that a large variety of microorganisms can degrade gelatin [11,17,18]. The degrees of colonization were different between the films and the film 1 was the one that showed a denser biofilm (Fig. 4).

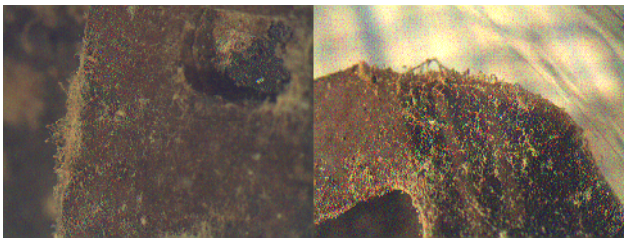

Fig. 2. Images obtained by optical microscopy (20X) that show the microbial growth in the perforations of the films.

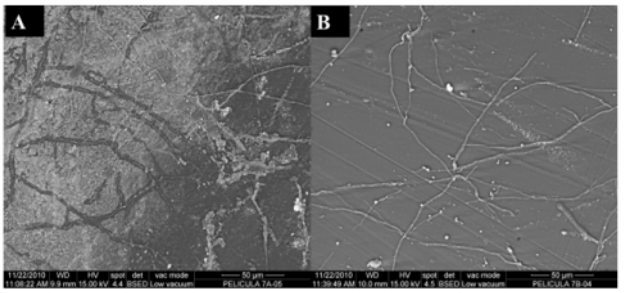

Fig. 3. ESEM micrographs that show the different microbial colonization on both sides of one of the films. a) in the gelatin emulsion. b) in the cellulose acetate based.

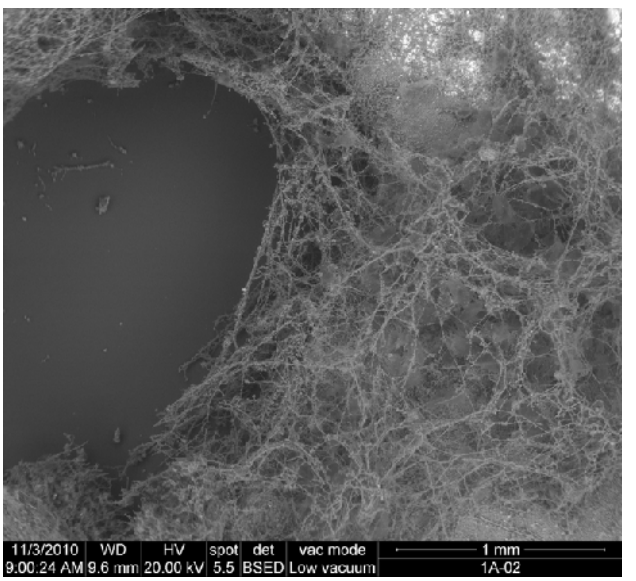

Fig. 4. Micrograph obtained by ESEM in one film that shows the dense biofilm.

The growth and metabolic activity of the fungi contribute to the fragility and breakage of the films support, as was proved by SEM, since in some films the fungal hyphae penetrated the support, causing mechanical damage as well as chemical deterioration (Fig. 5a). Likewise, it was observed that after preparation for this analysis, the fungi remained strongly adhered to the surface and the presence of extracellular polymeric substances was detected as part of the biofilm produced by the fungi (Fig. 5a).

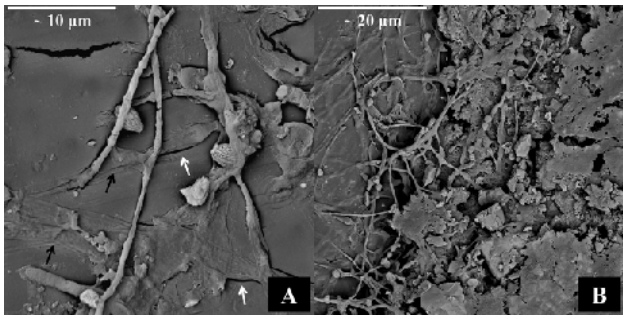

Fig. 5. SEM Micrographs. a) Fungal hyphae that penetrate the support (black arrows) causing mechanical damage (white arrows). b) Extracellular polymeric substances produced by fungi as part of the biofilm.

The presence of small holes in the fungal structures as a result of the lytic activity of the bacteria was detected (Fig. 6) [19], which shows their presence as part of the biofilm.

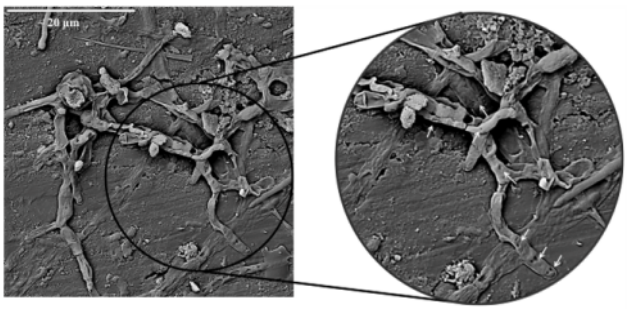

Fig. 6. SEM micrographs of one of the films, the small holes in fungal structures (white arrows) resulting from the lytic activity of the bacteria can be observed.

In addition to fungi and bacteria, exoskeletons of mites and pollens were observed by SEM (Fig. 7). It is known that arthropods play an important role in the deterioration of documents since they can act as contaminating vectors between the environment and the collections because fungal propagules can be transported in your body [20]. The dust mites, being arthropods, could contribute to the fungal development and therefore to the biodeterioration of the films studied. On the other hand, their exoskeletons along with the pollens particles were part of biofouling that favored biodeterioration, since they are organic matter that could be used as an additional nutritional source by some fungi. Similar results were previously obtained [22].

For the other hand, fluorescence microscopy showed that the majority of the microorganisms on the films were still viable and active (Fig. 8), consequently, their harmful effect on the analyzed films could continue.

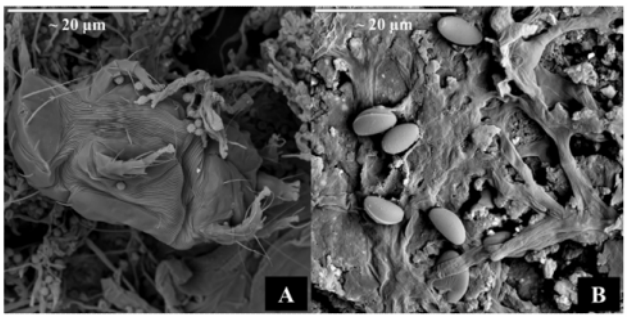

Fig. 7. Micrographs that show the biofouling of the films studied. a) Exoskeletons of dust mites (ESEM). b) Pollens (SEM)

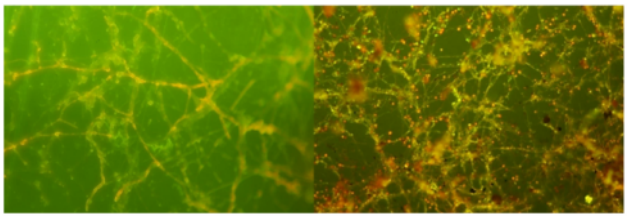

Fig. 8. Epifluorescence micrograph (40X) of film. Fungal mycelia can be observed. (live cells in green and dead cell in red).

CONCLUSIONS

For the first time in Cuba, the microscopic techniques were used to evidence the presence of viable and active microorganisms on films. Those microorganisms are

responsible of the biodeterioration in cinematographic heritage.

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