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A viscometric study of the biodegradation of photographic gelatin by fungi isolated from cinematographic films

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Abstract

The biodegradation of photographic gelatin grade (Bloom 225) material was studied by viscometry in aqueous solution (at 37 °C, 6.67% w/w) using filamentous fungi isolated and identified from cinematographic film stored in different Spanish archives. From viscosity data, different variables such as molecular weight and chain scission were calculated. To ensure initial spore suspension concentration was standardized for all the biodegradation experiments, a correlation between transmittance at 530 nm of fungal spore suspensions and the corresponding cytometric determination of populations was established for all the fungal strains studied in this work. The bioassay experiments were carried out at 25 and 4 °C using an initial concentration of fungi of 4.5×10^5 conidia/mL except in the case of the genus *Alternaria,* where the concentration was 10 times lower. The fungal strains were three species of *Aspergillus,* i.e., *A .ustus*, *A. nidulans* var. *nidulans*, *A. versicolor*, seven *Penicillium chrysogenum* strains, and *Cladosporium cladosporioides*, *Alternaria alternata*, *Mucor racemosus*, *Phoma glomerata,* and *Trichoderma longibrachiatum*. All were gelatinase positive. Through the viscosity decay profiles with bioassay-time and the corresponding calculated chain scission, the relative quantitative gelatinase efficiency of these fungi has been evaluated.

Keywords: Biodegradation; Type-B gelatin; Viscometry; Filamentous fungi; Flow cytometry; *Aspergillus*; *Penicillium*; Cinematographic films conservation; Cultural heritage

1. Introduction

Gelatin is a mixture of high-molecular-mass polypeptides produced from collagenous animal tissues that is used in a variety of applications—mainly food, pharmaceutical, and photographic applications. Gelatin is an important raw material for all silver halide-based photographic materials (Szücs, 1996). Here, mostly alkaline-processed bone type-B gelatins are used, cross-linked by chemical products (Kobayashi, 1996) to increase mechanical strength and stability. Gelatin enables the coating of several emulsion layers at the same time on the film base (Wards and Courts, 1977), traditionally cellulose ester supports (acetates and nitrates), and now, substituted by poly(ethylene terephtalate), (PET). Gelatin has been present in all photographic materials throughout cinematographic history. Many attempts have been made to replace it with synthetic polymers, but none are fully satisfactory substitutes.

Among the strict specifications of properties required of gelatin for cinematographic applications, a high gel strength or bloom value is one of the most important properties. The bloom value is a measure of the gelatin quality and is measured by a bloom gelometer during its manufacture. Commercial gelatines vary from 50 to 300 g Bloom (force in grams required to depress an standard plunger 4 mm into the gel prepared from a 6.67% aqueous solution of gelatin and cooled at 10 °C).

Many methods have been developed to assess microbiological degradation and deterioration of polymeric materials (Gu, 2003; Gu and Gu, 2005). Current knowledge

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about the cellulosic supports of cinematographic films indicates that increasing the degree of substitution values (estherification of the hydroxyl groups of the cellulose structure) makes the polymers less biodegradable (Buchanam et al., 1993). In contrast, gelatin, as a component of the photographic emulsion, remains material sensitive to attack by microorganisms under convenient ambient conditions (Pickett et al., 1991).

Gelatin is rapidly degraded when the contamination and the environmental conditions are suitable. During production, biodegradation by thermotrophic, aerobic, proteolytic endospore-forming bacteria has been described; all might be present in the production factory (De Clerck and De Vos, 2002). *Bacillus* and related endospore-forming genera together with others members of the genera *Salmonella*, *Kluyvera*, *Staphylococcus*, *Burkholderia*, *Enterococcus*, *Pseudomonas*, *Yersinia*, *Streptococcus,* and *Brevundimonas* have been detected in gelatin manufacture and show capability to liquefy gelatin.

In the photographic industry, contamination by bacteria from the genera *Bacillus* and *Pseudomonas,* as well as the yeast-like *Aureobasidium* sp. has been described (Stickley, 1986) along with their effectiveness in decreasing gelatin quality (viscosity and bloom value). Also, in a recent paper we discussed the biodegradation of type-B gelatin by bacteria (Abrusci et al., 2004) isolated from cinematographic films supplied by the Spanish archives. From the 14 studied bacteria only six were gelatinase active. They were primarily members of the genus *Bacillus* (*B. amyloliquefaciens*, *B. subtilis*, *B. megaterium*, *B. pichinotyi*, *B. pumilus*), and *Staphylococcus hominis*.

The biodegradation of cinematographic materials by microorganisms could be an important problem if in the archive (or during film use) the films are contaminated and the necessary moisture for their growth is present. Moisture levels above a relative humidity value of 60% can result in colonization of the materials. Fungi can grow under different moisture regimes (Klamer et al., 2004). The fungal contamination of a photogram is shown in Fig. 1. Damage to the image is not usually immediate, but further growth may liberate substances that affect the dyes and the images. The presence of microorganisms on material surfaces can have a profound effect on materials performance (Beech et al., 2005). Uncontrolled growth of microorganisms is an important problem in cinematographic archives in countries with tropical climates or warm summers. Cinematographic film manufacturers recommend prevention (Kodak Publication AE-22, "Prevention and Removal of Fungus on Prints and Films") and, in the case of early detection, removal of the contamination as soon as possible, and isolation of the material. Also, when storage conditions are not totally controlled, the materials should be inspected regularly for microbial problems.

We carried out research to isolate and identify microorganisms from selected samples of cinematographic films collected from archives around Spain. Together with the above-mentioned bacteria, 17 fungal strains (Abrusci et al., 2005), corresponding to nine different fungal species, were identified. Here we present the results of studies of the ability of fungi to biodegrade photographic gelatin in solution. The quantitative evaluation of their biodegradation activity was determined by viscosity measurements. Bioassays were carried out at two temperatures, 25 and 4 °C. Flow cytometry was used to determine cell number in the conidia suspensions and by correlation with corresponding optical density values, the population of the fungal inoculum was maintained constant in all the biodegradation experiments except for the case of the fungi *Alternaria,* where concentration was 10 times lower.

2. Materials and methods

2.1. Materials

Gelatin (type B, Aldrich Chemicals) was used as received. It had a Bloom o gel strength value of 225 g.

2.2. Fungal cultures and flow cytometry data acquisition and analysis

Nine fungal species were isolated and identified from samples supplied by Filmoteca Española and collected from different archives around the

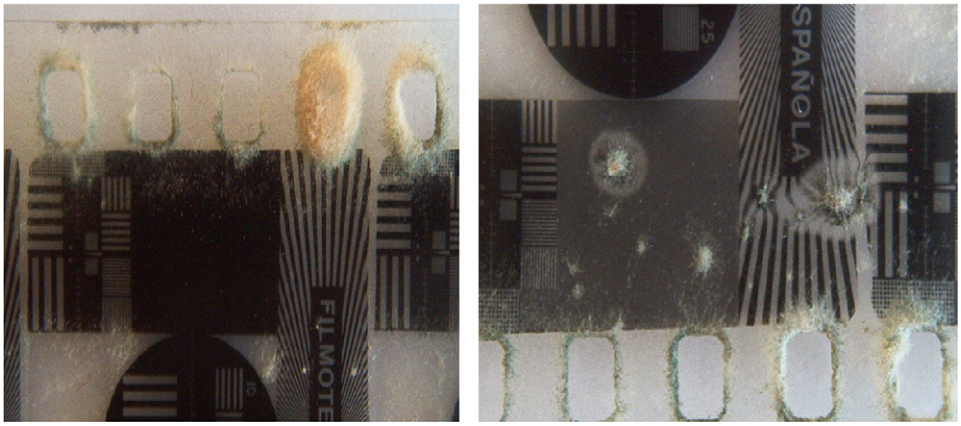

Fig. 1. Contaminated photograms colonized by *P. chrysogenum*. Film fragments were cultured at 30 °C for a week.