International Biodeterioration & Biodegradation 101 (2015) 1–7

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Contribution to the characterization of foxing stains on printed books using infrared spectroscopy and scanning electron microscopy energy dispersive spectrometry

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ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form
29 January 2015
Accepted 27 February 2015
Available online 24 March 2015

Keywords:
Molds
Microfungi
Foxing
Paper discoloration
ATR-FTIR
SEM-EDS

ABSTRACT

This study is part of research on the biological foxing formation affecting some old documents and explores the potential of ATR-FTIR spectroscopy to characterize foxing spots on paper from nine printed books, dating from early 19th century to mid-20th century. Preliminary assays allowed to highlight clear differences in FTIR spectral profiles between molds and the paper substrate on which they grew. However, spectra of foxed paper spots showed solely the absorption bands of cellulose due to very low amount of fungal elements inside the spots. Foxing spots were successfully differentiated from unstained areas of the paper by a slight intensity increase in the 1500–1700 cm^{-1} region and the appearance of small representative band near 1520 cm^{-1} which could be used as a marker for biological foxing. Comparative elemental analysis by SEM-EDS was also carried out with the intention of revealing other significant differences between the inside and the outside of spots. At this stage of the study, the findings are not totally conclusive. However, the overall results achieved by these two techniques encourage further research to gain a better understanding of biological foxing.

1. Introduction

Biodeterioration by filamentous fungi poses a serious threat to the conservation of artworks, especially graphic documents and their eradication represents often a challenge (Sequeira et al., 2014). Molds are always present inside buildings such as storage areas of collections. Unable to assimilate atmospheric CO_2, these heterotrophic microorganisms feed on organic matter found on the colonized substrates. The materials of organic origin composing the structure of the documents are then all vulnerable to fungal attack. Their susceptibility to fungal biocontamination depends on their chemical composition, their physicochemical properties and their state of conservation. But in addition to these factors related to the nature of the substrate, some environmental conditions such as a suitable temperature, oxygen and moisture are required to promote the growth of mold. Moisture is the most important of these factors, because water is an essential element of the exchanges of substances with the environment. The quantity of water available in a matrix which allows the development of the microorganisms is defined by its water activity (Aw), which varies between 0.65 and 0.98 for each type of mold. This quantity of available water is related to the relative humidity in the environment. There is a permanent exchange of moisture between the two until they reach a balance.

At the time of a biocontamination, settle mold spores on materials will germinate within a few hours and will give rise to germ tubes which will become hyphae. This filament will first grow on the surface and form soft spots of circular form easily recognizable. The degradation is both physical and chemical. Physical by the penetration and the propagation of the hyphae in the substrate that disorganizes cellulose fibers of paper. At the same time, chemical degradation is due to the secretion of enzymes, toxins, pigments and other substances often acidic, which causes the degradation characteristic of acidity. The hyphae will quickly form a group called mycelium to colonize larger volumes of the substrate and will produce spores by specialized structures, which will be disseminated and will contaminate other surfaces. Contamination

Abbreviation: CRCC, Centre de Recherche sur la Conservation des Collections.

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http://dx.doi.org/10.1016/j.ibiod.2015.02.031
0964-8305/

then appears like dust. Although any organic material may be a substrate for the growth of molds, optimum growth conditions may vary from one species to another, each having a different degree of adaptation to the environment. Some molds require high humidity to grow while others prefer much less. What we have just described, is normally what happens in the case of a conventional biocontamination.

Among the biological alterations encountered in the documents, foxing is a special case. Foxing is the name of random circular and irregular yellowish to brownish-red stains on the surface of old paper. Old books, documents, artworks, maps, etc. are prone to developing fox spots. The stains grow following a three dimensional network migrating through adjacent paper sheets of a book, diminishing with the number of sheets distanced from the coated paper insertion (Ligterink et al., 1991; Peters, 2000). Stains frequently fluoresce when examined under ultraviolet radiation. Fluorescence areas are larger than colored stains and some fluorescence occur also in clear zones, without any visible discoloration. UV fluorescence is detected in the early stage of foxing and is considered as a precursor of colored stains (Pedersoli et al., 2001).

Microscopic observations reveal evidence of fungal growth in some foxed areas and around them (Florian, 1996; Florian and Manning, 2000; Arai, 2000; Montemartini Corte et al., 2003; Choi, 2007). Other authors have detected the presence of bacteria in the foxing spots (De Paolis and Lippi, 2008). Indeed, even if molds are the most visible, bacteria, especially *Bacillus*, are common biocontaminants in paper industry and also in documents repositories (Borrego et al., 2012; Lavin et al., 2014).

The role of fungi is still not clearly understood, whether they accompany the formation of stains or they are opportunists taking advantage of a favorable ground to develop. For microbiologists, containment of fungal growth inside spots, has not yet found a satisfactory explanation. Species identification is then an essential step if we want to advance assumptions to explain the presence of mold. In previous studies, as cultivation-dependent methods were often unsuccessful, molecular biology techniques were preferably used to identify species (Rakotonirainy et al., 2007). Thirteen types of fungi were identified, distributed into 24 species from various pages of a 19th century book. No bacterial species was detected. No DNA was found in unstained areas. Furthermore, significant viable elements were highlighted inside the foxing stains by ATP bioluminescence assay (Rakotonirainy and Dubar, 2013). Then, the identified species may be alive. Moreover, mycelia observed under microscope are turgid and not lysed, which means that they are probably still active.

As mentioned earlier, the limiting factors for mold growth are availability of nutrients and moisture content. In the case of foxing, cellulose-based nutrients are sufficiently available in paper support. The limited growth of strains may be the result of inhibiting elements present in the paper support. Consequently, it is therefore important to characterize the papers items and to assess their conditions. As mentioned by Zotti et al. (2008) “the source of cellulose (linen rags, cotton and wood pulp), sizing compounds (starch, gelatin and rosin), coatings (starch, natural/synthetic adhesives), inorganic compounds (carbonates, sulphates, clay) and contaminants (metals) play an important role in the fungal attack”. This limited growth of strains inside foxed spots may be due to a low level of humidity. But how to explain the lack of growth when cultivating pieces of foxed papers on humid conditions (RH>90%) ? So, in the case of foxing, the moisture content and availability of nutrients cannot be considered as the only limiting factors of the mold growth.

Fourier transform infrared spectroscopy (FTIR) is a non-destructive and powerful tool largely used to identify the main components of papers (Trafela et al., 2007; Cséfalvayová et al., 2010) and its degradation state (Calvini and Gorassini, 2002; Lojewska et al., 2005). FTIR spectroscopy is also used in microbiology for the identification of filamentous fungi (Fischer et al., 2006; Linker and Tsror, 2008; Santos et al., 2010; Lecellier et al., 2015). It was demonstrated that each fungal species exhibits a typical infrared spectrum. Zotti et al. (2011) showed that it is possible to detect the presence of mold on biodeteriorated paper, but also, in the same time, to identify paper components.

The aim of this paper is to apply the attenuated total reflection Fourier transform infrared spectrometry (ATR-FTIR) technique on nine printed books deteriorated by foxing, dated from early 19th century to mid-20th century, in order to better differentiate the foxing spots and the clear zones and to detect the presence of mold on stains. The advantage of this technique is its capacity to investigate directly samples without preparation. Some authors have already used FTIR analyses to study the foxing phenomenon and highlighted the presence of oxide groups (Bicchieri et al., 2002; Buzio et al., 2004; Manso et al., 2009). The scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) analysis was used for comparative elemental composition assessment of papers inside and outside the foxing stains. The mechanism of stains formation is outside the scope of this paper.

2. Material and methods

2.1. Foxed book samples

For this study, samples consist of 9 printed books published between 1823 and 1963 and damaged by foxing. They will be named L1 to L9. These books were kept in CRCC (Centre de Recherche sur la Conservation des Collections) facilities in the dark for at least 10 years in non-controlled climatic conditions (20–40 °C, 45–50% RH). Unfortunately, storage conditions prior to their arrival are unknown.

Foxing spots are scattered on the inside pages without any privileged location; while most of the 9 book covers are totally foxing-free, and are not taken into consideration in this study. The stains, yellowish to pale brownish in color, may migrate through 2 or 3 successive pages, suggesting that the foxing stains were developed after the books were manufactured. The appearance of spots on all the books fit the description of “snowflake” foxing of Cain and Miller (1984). As expected, foxed spots fluoresce under UV lamp (312 and 365 nm), but also some fluorescent spots that were not visible to the naked eye. Examination under microscope showed that barely visible brownish stains begin to appear in these fluorescent spots.

2.2. Composition of the paper materials

Signatures pages were held together with stitches and bound into a softcover (paper or cardboard). The overall composition of the paper materials is determined by identifying fibers with a microscope, and detecting lignin and/or sizing agents (gelatin, starch, rosin) with a series of specific spot tests. In the field of cultural heritage conservation, the use of spot tests is recommended for basic evaluation of paper composition, because they are simple and quick procedures (Odegaard et al., 2005). Spot tests are based on chemical reactions and consist of putting a drop of a solution on a surface of the paper. Lignin is detected with phloroglucinol in hydrochlorid acid. A purple coloration indicates the presence of lignin. Aluminum is detected with a pink color solution of aluminon (ammonium salt of aurintricarboxylic acid), becoming darker pink or red when aluminum salts are present. Rosin is detected with the Raspail test. A pink to raspberry color indicates rosin is present. Starch is detected using the iodine test. A blue to black color