Journal of Cultural Heritage xxx (2017) xxx–xxx

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Original article

Disinfection of ancient paper contaminated with fungi using supercritical carbon dioxide

Fernanda S. Teixeira[a,*], Tatiana Alves dos Reis[b], Leonardo Sgubin[a], Lucia Elena Thomé[c], Isabel Wilmers Bei[c], Rejane Elias Clemencio[d], Benedito Corrêa[b], Maria Cecília Salvadori[a]

[a] Institute of Physics, University of Sao Paulo, Sao Paulo, Brazil
[b] Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
[c] Institute of Brazilian Studies, University of Sao Paulo, Sao Paulo, Brazil
[d] Museum of Contemporary Art, University of Sao Paulo, Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 9 May 2017
Accepted 12 September 2017
Available online xxx

Keywords:
Supercritical CO_2
Fungal disinfection
Ancient paper biodeterioration

ABSTRACT

Fungi continue to be the main cause of biodeterioration in libraries. In addition to degrading paper, fungi are an important health issue for librarians and even library users. The aim of this study was to investigate the application of supercritical carbon dioxide ($SCCO_2$) to ancient paper contaminated with fungi. For this purpose, $SCCO_2$ was applied in two processes to treat samples of paper pieces: addition of 4% and 8% ethanol (w/w) at a pressure of 150 bar and temperature of 40 °C for 1 hour. Control samples (no processing) and processed samples were directly plated onto culture media to evaluate the frequency of fungal growth. Morphological and molecular analysis of the 294 samples showing mold growth on paper revealed that *Aspergillus niger*, *Aspergillus flavus*, and *Eurotium amstelodami* were the most frequently isolated fungi. In the control group, 47.6% of the samples were contaminated with fungi. This percentage was only 1.9% after treatment with both processes. The difference between unprocessed control samples and samples treated under the two conditions was statistically significant ($P < 0.1$) for a confidence interval of 90%.

1. Research aims

The objective of this work is to show the successful results of the use of supercritical carbon dioxide ($SCCO_2$), with ethanol additive (2% and 4% w/w), to disinfect fungi on ancient paper.

$SCCO_2$ is an inexpensive green solvent that has been used in many industrial applications such as extraction and dry cleaning. As it will be duly referenced in the following, other authors reported the deacidification of ancient paper using ($SCCO_2$), evidencing the improvement of mechanical strength of the paper, even without the use of additives. One of the procedures most used for disinfection of the paper is the gamma irradiation, but this method can cause paper acidification and consequent yellowing, besides the reduction of mechanical strength due to the structural damage. In this way, with the use of $SCCO_2$ it is possible to disinfect the ancient paper preserving the paper structure.

2. Introduction

Ancient paper is the basis of numerous sources of information on history and culture in the form of books, art pieces, documents, and maps. The state of deterioration that those items can reach is very familiar and ranges from the paper becoming yellow to pages falling apart. Among the causes of deterioration such as acidification caused by oxygen, humidity, heat and other physical and chemical conditions, biodeterioration plays an important role. Biodeterioration can be caused by insect infestation and microbial growth. Despite environmental control and the efforts commonly made by libraries to conserve their collections, fungi continue to be a major problem. In addition to degrading the paper, fungi are an important health issue for librarians and library users.

Fungal contamination of documentary heritage is still an underestimated concern [1]. This matter becomes important because of its risks to public health since fungi are associated with a number of allergic diseases in humans [2]. Inhalation or ingestion is the main route of exposure to fungal propagation. The prevalence of respiratory allergy to fungi ranges from 20 to 30% in atopic individuals and is 6% in the general population [3].

* Corresponding author.
E-mail address: nandast@if.usp.br (F.S. Teixeira).

http://dx.doi.org/10.1016/j.culher.2017.09.009
1296-2074/

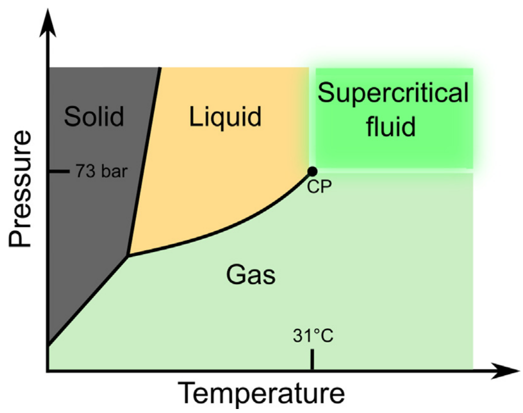

Fig. 1. Phase diagram of supercritical carbon dioxide ($SCCO_2$). CP: critical point.

When a fungus attacks a substrate, its water activity changes sufficiently to support the growth of other species (fungi and bacteria), as observed in natural successions [4,5]. Secondary colonizers are species with high resistance to water stress. Moreover, poor ventilation and surface temperature dynamics can produce water condensation and local microclimates with localized peaks of water activity in the substrate greater than the indoor surrounding environment [6]. These conditions favor the proliferation of fungal species in seemingly hostile environments [7].

Sequeira et al. [8] provided an interesting review about the use of antifungals for paper conservation. The authors reported several chemical and physical methods and their main advantages and drawbacks. A routinely used physical method is gamma irradiation. However, according to these authors, gamma irradiation diminishes the mechanical resistance of the paper and causes its acidification and consequent yellowing. Chemical agents also usually damage the paper. For instance, the use of an aqueous solution of 70% ethanol (v/v) can cause the loss of gloss, and damage to the book parts.

Supercritical carbon dioxide ($SCCO_2$) is the physicochemical state that CO_2 assumes when confined in a chamber with temperature and pressure conditions above its critical point [9]. In the case of $SCCO_2$, the critical point corresponds to a temperature of 31.1 °C and a pressure of 73.8 bar (Fig. 1 shows the phase diagram of CO_2). Under supercritical conditions, a fluid is dense as a liquid, but possesses viscosity and diffusivity similar to those of a gas. Thus, the supercritical state exhibits several interesting properties and possibilities, such as fine-tuning of substance density, solvent properties and high diffusivity. Moreover, $SCCO_2$ has great appeal as an inexpensive green, abundant and non-flammable solvent. In this paper, we will refer to the treatment or the use of $SCCO_2$ inside a chamber for a certain period of time as "$SCCO_2$ process".

The $SCCO_2$ process was not listed by Sequeira et al. [8] as a fungicidal or fungistatic treatment for paper conservation. Some studies reported its use only for the deacidification of paper [9–11]. Although some of these studies briefly mention that $SCCO_2$ cleans the material by removing dust and fungi, no systematic data are provided. However, several advantages of $SCCO_2$ for paper treatment are reported. The $SCCO_2$ process does not damage parts of the book such as ink, bindings or leather [9] and, even without the use of additives, provides a certain degree of deacidification and creates an alkaline reserve [9–11]. In addition, the $SCCO_2$ process does not affect the mechanical resistance of cellulose fibers. Actually, Selli et al. [11] reported improved mechanical resistance of the paper after the use of $SCCO_2$ without additives. Thus, a clear advantage of the $SCCO_2$ process is the possibility to treat wholes books in one process cycle. From a practical point of view, there are many industrial applications [9] of supercritical fluids in the food, microelectronics, textile and pharmaceutical industry, including extraction, product formulation, and dry cleaning. In this respect, the process could readily become available for conservator.

Kamihira et al. [12] applied the $SCCO_2$ process to sterilize microorganisms, but did not use any kind of paper as a substrate. Fungi (among other microorganisms) in dry and wet states were sterilized successfully with $SCCO_2$ using alcohol as an additive (2% to 10%, w/w). However, there are no studies on the application of $SCCO_2$ with or without ethanol to treat paper contaminated with fungi. In an interesting study, Sequeira et al. [14] used ethanol as an antifungal agent for paper conservation. Thus, ethanol was chosen as the additive for the $SCCO_2$ process.

In this study, we report original results on the use of $SCCO_2$ with alcohol as an additive for fungal disinfection of ancient paper.

3. Material and methods

3.1. Ancient paper and sampling

A specialist in conservation and restoration selected a representative book in a second-hand bookstore for this study. The title of the book in Portuguese is "A emancipada – La garçonne", published by "Empreza de Publicações Modernas" (Rio de Janeiro, 1922). Fig. 2 shows a photo of the book. It is possible to note several stained areas caused by fungal activity (foxing) [15].

The fiber morphology of the book was analyzed using the method established by the ISO 9184-1 standard (Paper, board and pulps – Fibre Furnish Analysis – Part 1: General Method, Part 4: Graff "C" staining test). A Leica DM 4000 B light microscope equipped with a DFC 310 FX camera and LAS 3.8.0 software was used for analysis at an original magnification of 200×. Fig. 3 shows the microscopic images revealing the morphology of the fibers and their characteristic size. As can be seen, the paper exhibits the halo patterns typical of the coniferous fibers. Thus, the paper was classified as being produced by mechanical pulping.

The selected book had 312 pages and 5 pages (measuring 10.8 × 15.6 cm) were randomly chosen. Each page (referred to as page A, page B, page C, page D, and page E) was cut into 117 sample pieces measuring 12 × 12 mm. For each page, 60 pieces or three groups of 20 pieces each were randomly chosen. Each group was transferred to a small envelope of Kraft paper for processing in the $SCCO_2$ chamber. The following groups were established: control consisting of samples not submitted to processing; process 4% consisting of samples processed in a solution of $SCCO_2$ at 4% [4% ethanol (w/w)]; process 8% consisting of samples processed in a solution of

Fig. 2. Photo of the book selected for the experiments.