Journal of Chromatography A, 1409 (2015) 30–45

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Optimization of headspace solid phase microextraction for the analysis of microbial volatile organic compounds emitted by fungi: Application to historical objects

Tomasz Sawoszczuk[a,*], Justyna Syguła-Cholewińska[a], Julio M. del Hoyo-Meléndez[b]

[a] Cracow University of Economics, Faculty of Commodity Science, Department of Microbiology, Rakowicka 27 St., 31-510 Cracow, Poland
[b] Laboratory of Analysis and Non-Destructive Investigation of Heritage Objects, National Museum in Krakow, Piłsudskiego 14 St., 31-106 Cracow, Poland

ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 11 July 2015
Accepted 13 July 2015
Available online 17 July 2015

Keywords:
SPME
GC–MS
Biodeterioration
Fungi
Historical objects

ABSTRACT

The main goal of this work was to optimize the SPME sampling method for measuring microbial volatile organic compounds (MVOCs) emitted by active molds that may deteriorate historical objects. A series of artificially aged model materials that resemble those found in historical objects was prepared and evaluated after exposure to four different types of fungi. The investigated pairs consisted of: *Alternaria alternata* on silk, *Aspergillus niger* on parchment, *Chaetomium globosum* on paper and wool, and *Cladosporium herbarum* on paper. First of all, a selection of the most efficient SPME fibers was carried out as there are six different types of fibers commercially available. It was important to find a fiber that absorbs the biggest number and the highest amount of MVOCs. The results allowed establishing and selecting the DVB/CAR/PDMS fiber as the most effective SPME fiber for this kind of an analysis. Another task was to optimize the time of MVOCs extraction on the fiber. It was recognized that a time between 12 and 24 h is adequate for absorbing a high enough amount of MVOCs. In the last step the temperature of MVOCs desorption in the GC injection port was optimized. It was found that desorption at a temperature of 250 °C allowed obtaining chromatograms with the highest abundances of compounds. To the best of our knowledge this work constitutes the first attempt of the SPME method optimization for sampling MVOCs emitted by molds growing on historical objects.

1. Introduction

Biodeterioration constitutes a risk for cultural heritage objects, especially the one caused by molds. Spores of various molds species are a typical contamination of indoor air in museums, libraries and archives [1]. Moreover, it has been proved that molds are able to grow even when small amounts of organic compounds are available. For example, molds have been observed on dust layers deposited on the surface of historical objects [2]. The growth of molds on objects causes: color changes, occurrence of stains, mechanical failure of the object's structure, and the chemical biodeterioration, which is a consequence of the molds metabolic activity [3]. Hence, molds activity constitutes a risk for historical objects, but it can also be hazardous for museum visitors and museum staff if they become in contact with molds' hyphae, spores, and mycotoxins present in indoor air, as well as with volatile metabolites emitted by molds to the ambient air [2,4–15]. The latter are known as microbial volatile organic compounds – MVOCs.

MVOCs are assumed to be an indicator of molds activity [16,17]. The majority of articles that deal with the MVOCs measurements are studies carried out on building and construction materials infested by molds or on contaminated interiors. These studies are concentrated on the health risk assessments of workers or people residing in places with possible active molds growth, especially in the context of the sick building syndrome [2,4–15]. Only a few publications deal with the analysis of MVOCs emitted by molds growing on historical objects, like library paper [18] or cinematographic film [19]. An interesting work about detection of molds by volatile organic compounds in application to heritage conservation was presented by Joblin et al. [20].

Microbial volatile organic compounds are emitted by fungi at every stage of their growth. What is more important, MVOCs can be used to point out the presence of active forms of molds even if they are growing inside the structure of a historical object, because they

* Corresponding author. Tel.: +48 12 293 5807; fax: +48 12 293 58 74.
E-mail addresses: tomasz.sawoszczuk@uek.krakow.pl (T. Sawoszczuk), sygulaj@uek.krakow.pl (J. Syguła-Cholewińska), jdelhoyo@muzeum.krakow.pl (J.M. del Hoyo-Meléndez).

http://dx.doi.org/10.1016/j.chroma.2015.07.059
0021-9673/

might easily diffuse through porous barriers [21]. In this case, the classical microbial test will not confirm the infestation. MVOCs can be sampled with various techniques [22]. One of the most frequent techniques applied for MVOCs sampling is absorption in sorption tubes [4–11,20,21,23–25]. The analyzed volatiles are caught in the sorbent bed, which is inside a stainless steel or glass tube. The most popular sorbents are Tenax TA and Carbopack B. The sampling of volatiles can be done in active or in passive way. The chromatographic analysis of volatiles collected on sorbents is carried out by thermal desorption coupled with gas chromatography–mass spectrometry.

A second popular method of MVOCs sampling is solid phase microextraction (SPME) [16,17,19,26–36]. In this case, volatiles are absorbed on a small amount of sorbent or mixture of sorbents attached to the top of a needle [26]. Sorbents are hidden before and after measurements inside a steel housing in order to be isolated from ambient air. This sampling device is called an SPME fiber. With this technique, VOCs sampling is carried out on a passive way after the fiber is moved out and exposed to the investigated atmosphere. Once sampling is finished volatiles are thermally desorbed in the injection port of a gas chromatograph. The great advantage of the SPME technique is the possibility of sampling just from above the tested surface or just from the surface itself when working in contact mode (placing the sorbent directly on the surface of the object). This can be very useful for investigating MVOCs emitted by molds growing on historical objects. Classical microbial tests require sampling of the investigated surface. Sometimes it is impossible to take a sample from the surface of a historical object because of its value. In this limiting circumstance, SPME technique offers a better alternative because it is non-destructive and non-invasive. Furthermore, the size of the SPME fiber is small enough to acquire the MVOCs directly from the mycelium present on the object by the contact method (to check if mold is active or inactive). This sampling method excludes the influence of other volatiles present in the surroundings of the infested place and allows avoiding MVOCs dilution that can occur during sampling in an open system as a result of diffusion phenomena. Another advantage of sampling with an SPME fiber in a contact mode is the possibility of obtaining the reference sample (background VOCs emission) in a simple way by placing the fiber on a non-infested surface aside the place with mycelium (maintaining an adequate distance).

Among the many available techniques for qualitative and quantitative analysis of MVOCs (sampled with various methods), gas chromatography (GC) is the most frequently used [20,27]. The choice of this technique for analysis is obvious since the analyzed compounds are volatile and are sampled with methods related to gas chromatography. When MVOCs are collected in the sorption tube, the volatiles are analyzed in a thermal desorber (TD)–gas chromatograph (GC)–mass spectrometer (MS) system [5–11,20,21,23–25], whereas MVOCs gathered on the SPME fiber are analyzed in a GC–MS system [16,17,19,26–31,33–38]. Liquid chromatography is a less popular technique for this type of analysis [39]. Sometimes MVOCs are analyzed with very sophisticated methods like e-nose [18,40], ion mobility spectrometry [41] or GC–TOFMS [42].

Before applying the SPME method for sampling MVOCs from moldy historical objects the technique itself has to be optimized, as in any other cases afore SPME is introduced [32]. The main goal of this work was to refine the SPME sampling method for measuring MVOCs emitted by active molds that may deteriorate historical objects. The procedure was carried out for selected mold species inoculated on artificially aged model materials that resemble those found in historical objects. First of all, a selection of the most efficient SPME fibers was carried out because there are six different types of fibers commercially available, each one with various combinations of sorbents exhibiting different sorption properties. It was important to find a fiber that absorbs the biggest number and the highest amount of MVOCs. Another task was to optimize the temperature of MVOCs desorption in the GC injection port as well as the time of MVOCs extraction on the fiber to obtain chromatograms with the highest abundances of compounds, high enough to carry out their quantitative and qualitative analysis. To the best of our knowledge this work constitutes the first attempt of SPME method optimization for sampling MVOCs emitted by molds growing on historical objects.

2. Experimental

2.1. Chemicals

The chemicals used as reference compounds and for preparing the microbial broth were of analytical grade (Avantor Performance Materials, Poland). While some of the broths were purchased as a ready formula (BTL, Poland). Various types of microbial broths were used for cultivation of the selected molds species. They were chosen based on the major metabolic activity that mold represent, e.g. cellulolytic, keratinolytic, collagenolytic or fibroinolytic. Based on the list of broths ingredients which were produced by the BTL Company on special order, it was assumed that they do not contain a source of organic carbon (or if than very low concentration, as it was ordered) that could be available for fungi. However, it was not confirmed whether the broths contain some organic impurities. The idea of this experiment was to ensure that the main nutrient for molds had to be an organic model material that was placed on the surface of the broth. Firstly this was a way to confirm that the chosen mold really has protolithic or cellulolytic properties. Furthermore, this procedure gives a very high probability that the volatiles emitted by molds originated mostly from enzymatic decomposition of the model material and not from the metabolism of sugar in the broths. The medium that was used for cultivation of keratinolytic and fibroinolytic molds was a modified version of Weary and Canby's medium (W&C) [43,44]. It did not contain sources of neither carbon nor nitrogen because they were supplied in the samples of protein materials placed on the broth (wool and silk). The second type of medium employed was a modified version of Czapek–Dox broth (CzD – BTL, Poland). Similar to the previous one, this medium did not contain carbon and nitrogen and it was used for cultivation of collagenolytic molds (a sample of parchment was a source of nutrients).

An exemption was SNA (Synthetic Nutrient Deficient Agar – BTL, Poland), which was used for cultivation of a cellulolytic mold – *Cladosporium* sp. This medium contains only 0.2% (w/w) of glucose and 0.2% (w/w) of saccharose as a source of carbon. In this case, the small quantity of organic compounds in broth can somehow imitate the presence of organic debris that can cover the surface of historical objects made of cellulose. The presence of a small amount of organic compounds enables some molds to start growing on cellulosic materials, since this polymer is not easily metabolized by some of them, like for example *Cladosporium* sp. This mold is common in indoor air [45,46] and it was isolated from cellulose-containing objects [46,47]. However, it has been shown that it grows much slower on cellulosic items, than for example *Chaetomium globosum*, and emits less volatiles [23].

2.2. Materials

The model organic materials were used for optimization of the SPME sampling method provided for measurements of MVOCs emitted by active molds that can deteriorate historical objects. The following materials were chosen: