Chemosphere xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

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

A new approach to detect early or hidden fungal development in indoor environments

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HIGHLIGHTS

- Moularat et al., in 2008 has developed the Fungal Contamination Index (FCI).
- This index has the advantage of detecting fungal development reliably and rapidly.
- This device is based on VOCs' selection, concentration and polymer sensors' array.

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 21 June 2015
Accepted 22 June 2015
Available online xxxx

Keywords:
Mold detection
Fungal Contamination index
Indoor air quality
Monitoring device
VOC analysis
Sensor array

ABSTRACT

In addition to the biodegradation problems encountered in buildings, exposure of their occupants to mold is responsible for numerous diseases such as respiratory infections, immediate or delayed allergies and different types of irritations. However, current techniques are unable to detect mold at an early stage of development or hidden contaminants.

Moularat et al., in 2008 has established chemical fingerprints of moldy growth from Volatile Organic Compounds (VOCs) arising specifically from fungal metabolism and developed the Fungal Contamination Index (FCI) (Moularat et al., 2008a,b). This index has the advantage of detecting fungal development both reliably and rapidly before any visible signs of contamination could be detected.

However, even though the FCI has been widely tested, VOCs' analysis by GC/MS, which is required for index calculation, is incompatible with real-time monitoring strategy for indoor environments.

In this context, researches around FCI exploitation have been followed up in order to provide a monitoring device widely deployable which is the result of the miniaturization of an analytical chain for portable, reliable and low-cost applications. This device is based on one hand the selection and concentration of chemical compounds from the sample of interest and on the other hand the development of an array of different conducting polymer based sensors in order to obtain a specific footprint.

This fungal contamination detection device was the subject of patent applications by the CSTB.

The modularity of the system (ability to vary both the elements of detection polymers and retention time of interest) allows for expansion of its use to other pollutants.

1. Introduction

Micromycetes or mold feature among the pollutants from indoor environments. This microbial contamination is not without consequence. Indeed, fungi are growing on substrates with possible degradations of their mechanical properties in particular.

In addition to the biodegradation of the products which they colonize, mold can induce various diseases to occupants, in particular respiratory pathologies such as allergies, infections or toxi-infections (Kuhn et al., 1995). Thus, several epidemiological studies have shown an association between the increasing prevalence of asthma or respiratory illness symptoms on one hand, and the presence of molds (or excessive humidity) in the internal spaces on the other hand (Garrett et al., 1998; Kilpelainen et al., 2001; Moularat et al., 2011a,b; Hulin et al., 2013).

Studies in Europe and North America on the fungal contamination of dwellings, showed that 14–35% of the investigated environments were of concern (Tsongas, 1994; Pirhonen et al., 1996; Escamilla-Garcia, 1997). This finding was corroborated by the Observatory of Indoor Air Quality in 2008. Thus, out of 15% of French real estate present fungal infections, among these 15%, there are 2% of cases (610,000 buildings) with contaminated surfaces over 1 m^2 (Moularat et al., 2008a–c).

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http://dx.doi.org/10.1016/j.chemosphere.2015.06.072
0045-6535/

Therefore, providing tools for monitoring and/or quick diagnosis of a fungal development constitutes a major issue which becomes it even more urgent due to the requirements to reduce energy consumption in buildings expressed by the French government during the Grenelle 2. This has led to reduction in permeability of these buildings therefore making them more susceptible to dysfunction of aeration devices. However, if improving the tightness of the building envelope is effective, it is feared that excess moisture whether it is of accidental origin, related to the activities of the occupants, or a dysfunction of the ventilation will promote conditions conducive to fungal growth.

With the aim of early detection, the work of CSTB relies on the emission, in the early hours of fungal growth, of Microbial Volatile Organic Compounds (MVOCs). These diffuse into the environment and constitute a specific biochemical fingerprint whose measure is exploitable for diagnosis or monitoring of confined spaces.

Techniques conventionally used to diagnose a fungal contamination in an environment are based on visual examination and culture of fungal spores in the air. While in many situations of infestation, this approach is efficient, it does not allow for much the detection of contamination either "hidden" (behind a partition on ventilation filters for example) or early (in the early stages of mold growth).

However, early in their development, fungi emit volatile compounds (MVOCs) from either their metabolism or degradation of the material by enzymes or acids they produce (Wessen et al., 1995; Korpi et al., 1997, 1999; Wilkins, 2002; Moularat et al., 2008a–c).

Moreover, unlike spores, these gaseous compounds diffuse into the environment without being restrained by the media.

Thus a method, based on the detection of several VOCs from fungal metabolism, has been proposed as a Fungal Contamination Index (FCI). The presence and/or absence of different tracers are taken into account, the index is incremented according to their specificity toward species/substrate (Moularat, 2007). The FCI can finally decide on an active fungal growth, including cases of early or "hidden" contaminations.

This tool has already been used in many studies:

- Applied to gaseous samples taken at the National Housing Campaign Observatory Air Quality (OQAI) it has a description of the state of fungal contamination of French housing (Moularat et al., 2008a–c).
- Used in an epidemiological study, the association between fungal growth and asthma or symptoms similar to chronic bronchitis (Hulin et al., 2013) has been demonstrated.
- Applied to 94 homes in the area of Clermont-Ferrand, in France, in the context of another epidemiological study (ISAAC), this tool has also demonstrated the relationship between fungal development and childhood asthma (Hulin et al., 2010).

Studies in collaboration with agencies responsible for safeguarding the heritage such as the Research Laboratory of Historical Monuments, National Archives or the National Library of France have also shown interest in biochemical fingerprints as a means of detecting fungal growth (Joblin et al., 2010; Nguyen et al., 2012; Hulin et al., 2013).

Preliminary tests performed using different strains covered by the study of Joblin et al., 2010 made it possible to obtain specific responses using different polymer sensors exposed to moldy environments. Thus in the laboratory, the sensors studied were thus able to distinguish contaminated from non-contaminated environments.

However, the use of a profile requires it to be compared it with a reference (control) that is not applicable in indoor environments. Besides, if these Electronic Conductive Polymers (ECP) layers showed themselves effective for the detection by the identification of a global VOC fingerprint emitted by mold, they are not still enough selectively to realize the identification of every COV, essential for the index calculation. So, these sensors remain inadequately specific to permit their use *in situ*.

Regarding these stakes, having a tool which can be installed in buildings and is capable of supplying an almost immediate information about a possible fungal development could be a major step forward.

Now, if the FCI was widely used, the analysis of VOCs' by Gas Chromatography coupled with Mass Spectrometry (GC–MS) necessary for its calculation, is incompatible with a strategy of real-time control of the indoor environments.

In this context, we propose a monitoring beacon. Our efforts of research concerned the miniaturization of the analytical chain for portable and reliable applications at moderate costs. The final system consisted of two modules of pre-treatment of samples (a module of concentration also enabling air collection and a module of separation) and of a VOCs' analysis module. Laboratory experiments have been performed to validate that each of these modules has the desired features to collect, separate and detect VOC from the Fungal Contamination Index so that the microsystem could calculate it.

2. Materials and methods

2.1. Description of modules constituting the microsystem

2.1.1. Concentration module

The concentration microstructure or pre-concentrator developed in this study consists of a silicon substrate in which 60 mm length microchannels with a 500 μm width were etched. The sealing of the silicon substrate with glass enables the realization of closed cavities. The microstructure is then packed with a granular adsorbent, Tenax TA, with an average diameter of 120 μm. Fluidic ports adapted to this type of microstructure enables equipping the micro-module openings with capillaries for pump connection and air circulation through the structure.

Tenax TA tube that is classically used for FCI calculation contains a 1 cm^3 volume with average bead diameter of 300 μm. The beads' size used for the microstructure (average diameter of 120 μm) enables to increase the specific surface. Consequently, in spite of the volume decrease (0.20 cm^3), the equivalent active surface is preserved. So, the concentration efficiency (retention and drilling volume) of this microstructure remains equivalent to a classic tube.

2.1.2. Separation module

The micro-column developed also consists of a silicon substrate in which a micro-channel is etched. The sealing of the silicon substrate with glass enables the realization of this micro-channel. The fabricated micro-chip with a 5 m-long, 150 μm-wide, 200 μm-deep separation column is coated with polydimethylsiloxane (Sylgard® 184, Dow corning) as the stationary phase using static coating method. Indeed, because compounds to be detected in the FCI belong essentially to alkenes, ketones and esters and are polar, an apolar stationary phase is chosen. As for the concentration microstructure, adapted fluidic connectors enable equipping the micro-module's openings with capillaries.

2.1.3. Detection module

Interdigitated Electrodes (IDE) with different geometries are implemented in the array to increase the sensor dynamic range variability. Conducting polymers are chosen as sensing materials as they enable differentiation between contaminated and

Please cite this article in press as: Anton, R., et al. A new approach to detect early or hidden fungal development in indoor environments. Chemosphere (2015), http://dx.doi.org/10.1016/j.chemosphere.2015.06.072