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Fungal biodetector: a real-time indoor air quality monitoring

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ABSTRACT
Fungi are common biocontaminants of indoor environments, and numerous studies have demonstrated how they can degrade the materials they colonise (e.g. wood, textiles, paper, pigments, varnishes, etc.), which can even result in total destruction of these substrates.
Today, fungal contamination is an increasing problem in houses, working places, schools, hospitals, elderly care centers and cultural heritage.

Current techniques are unable to detect mould at an early stage in their development or hidden contaminants. Moularat et al. (2008) has established chemical fingerprints of mouldy development from Volatile Organic Compounds (VOCs) arising specifically from fungal metabolism (Moularat *et al.* 2008). This approach has the advantage of detecting fungal development both reliably and rapidly before any visible signs of contamination could be detected. Since the development of this Fungal Contamination Index (FCI), other specific indexes have been developed to monitor Invasive Nosocomial Aspergillosis in hospitals or *Serpula Lacrymans* in dwellings. Their applications constitute a new approach for diagnosis (Moularat *et al.* 2008; Moularat and Robine 2011; Moularat and Robine 2011)

However, even if the FCI has been widely tested (Joblin *et al.* 2010; Moularat *et al.* 2010; Hulin *et al.* 2013), VOCs'analysis by GC/MS, which is required for index calculation, is incompatible for indoor environment real-time monitoring strategy. So having such a device, which could be set up in buildings and able to provide almost instantaneous information on prospective fungal development, constitutes a breakthrough.
In this context, researches around FCI exploitation have been followed up in order to provide a device widely deployable which enables mould development real-time monitoring. This innovative microsystem is the result of the miniaturization of an analytical chain for portable, reliable and low-cost applications (Anton *et al.* 2016).
This biodetector was the subject of patent applications by the CSTB (Moularat *et al.* 2010; Moularat *et al.* 2011).

KEYWORDS
Mold detection; Fungal Contamination index; Indoor air quality; Monitoring device; Sensor array

INTRODUCTION
Among the pollutants from indoor environments figure micromycetes or mold. This microbial contamination is not without consequence. Indeed, fungi are growing to the detriment of the colonized support, ultimately with possible degradation of its mechanical properties in particular.
In addition to the biodegradation of the products which they colonize, mold can induce occupants various diseases, in particular respiratory pathologies such as allergies, infections or

toxi-infections. Thus, several epidemiological studies have shown an association between the increasing prevalence of asthma or respiratory symptoms on one hand, and the presence of molds (or excessive humidity) in the internal spaces on the other hand (Moularat, Flamant-Hulin et al. 2010; Hulin, Moularat et al. 2013).
Studies in Europe and North America on the fungal contamination of dwellings, showed that 14-35 % of the investigated environments were concerned (Escamilla-Garcia 1997). This finding was corroborated by the Observatory of Indoor Air Quality in 2008. Thus, 15% of French real estate present fungal infections, among these 15 %, there are 2% of cases (610,000 units) with contaminated surfaces over 1m² (Moularat, Derbez et al. 2008).
Thus, provide tools for monitoring and / or quick diagnosis of a fungal development constitutes a major issue which makes it even more urgent that requirements to reduce energy consumption in buildings expressed by the government during the Grenelle 2 will lead to buildings whose permeability has been reduced and therefore 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; promote conditions convenient to fungal growth.
In an optics of early detection, the work of CSTB rely on the emission, in the early hours of fungal development, 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. Thus a method, based on these MVOCs emitted by 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 towards the species / substrate (Moularat 2007). The FCI enables finally to rule on an active fungal growth, including the cases of early or "hidden" contaminations.

In order to detect these MVOCs, preliminary tests have been performed using different strains covered by the study of Joblin, Moularat et al. 2010 (Joblin, Moularat et al. 2010). These tests made it possible to obtain specific responses using different polymer sensors exposed to mouldy 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 a comparison with a reference (control) which 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 mould, they are not still enough selectively to realize the identification of every VOC, 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 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 samples pre-treatment (a module of concentration also enabling air collection and a module of separation) and a VOCs' analysis module. Laboratory experiments have been performed to validate that each of these modules

has the desired features such as collection, separation and detection of VOC from the Fungal Contamination Index so that the microsystem could calculate it.

METHODS

Description of modules constituting the microsystem

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.

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.

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 non-contaminated environments (Joblin, Moularat et al. 2010). Presently, 8 different polymers are used in this study.

Description of modules' validation setups

Biological material

The selected mould specie for these experiments is a strain from the Institute of Hygiene and Epidemiology in Brussels (IHEM 05077): *Aspergillus brasiliensis*. Indeed, during Index development, more than 15 mould species have been tested and the *A. brasiliensis* strain was the one which was able to emit all the index VOCs. That is why this strain is used as a

reference in this study. Moreover, it is the most emissive strain in terms of VOCs' concentration.

Chemical materials

Twenty-one standard compounds (SIGMA-ALDRICH -> 95% purity) representing different chemical VOC families and that are characteristic or non-characteristic of fungal metabolism, are identified. The characteristic ones have been selected during Moularat et al. 2008 study. The non-characteristic ones have been chosen because they belong to the same chemical families but with different carbon chains. The followings VOCs are studied: a linear alkane (VOC 1), 5 alkenes (VOC 2, VOC 3, VOC 4, VOC 5 and VOC 6), 5 linear primary alcohol (VOC 7, VOC 8, VOC 9, VOC 10 and VOC 11), 2 branched primary alcohols (VOC 12 and VOC 13), 2 secondary alcohols (VOC 14 and VOC 15), 3 ketones (VOC 16, VOC 17 and VOC 18) and rings (VOC 19, VOC 20 and VOC 21). Each of these 21 molecules has been tested separately.

Emission chambers

Modules' validation tests are performed on 300 ml emission chambers. These chambers are used to generate experimental atmospheres and are made of materials (glass and PTFE) that remain inert in the presence of VOC.
Chambers contain either contaminated substrates (glass fabric) by mould for pre-concentration and separation modules' validation or chemical compound for polymer sensors' validation.
For the mould contaminated chamber, glass fabric is used as growth substrate. This material is cut and autoclaved (121 °C, 45 min) before adding distilled water. A pool of 5 ml of water helps to keep the chamber water saturation during the test (RH> 99%). The moisture level is maintained at saturation throughout tests. The obtained load rate is 7.10^{-2} cm^2 / cm^3. The spore suspension preparation used for surface contamination by *Aspergillus brasiliensis* is carried out by pouring 50 ml of ultrapure water on the strain sub-cultured on oats. Air filtered through activated charcoal is passed on for 30 minutes into each chamber. Then each contaminated chamber is placed in a stove for 7 days at 25 °C in total darkness.

For polymer sensor tests, two types of atmospheres are created, one is regarded as reference in order to generate filtered air and the second one contains with a VOC. To constitute a reference chamber, Tenax TA tubes are introduced at each end of valves, then closed. Activated charcoal is introduced to filter the incoming air. To constitute a VOC emission chamber, Tenax TA tubes are introduced at each end of valves, then closed. 300 µl of each VOC are introduced into this chamber to create a saturated atmosphere. Then the chamber is placed in a stove at 25 ° C for at least 30 minutes prior to a test.

RESULTS

Concentration module validation

In order to validate the pre-concentrator, the air from the chamber contaminated by *Apergillus brasiliensis* is sampled through the module. It is then heated at 140 °C to desorb the sampled VOCs in a chamber with a vial. The sample from the pre-concentrator contained in the vial is then analyzed on GC-MS. Eight compounds identified as coming from the fungal metabolism

(Moularat, Robine et al. 2008) are then searched. After chromatogram analysis, all these VOC have been found (Figure 1).

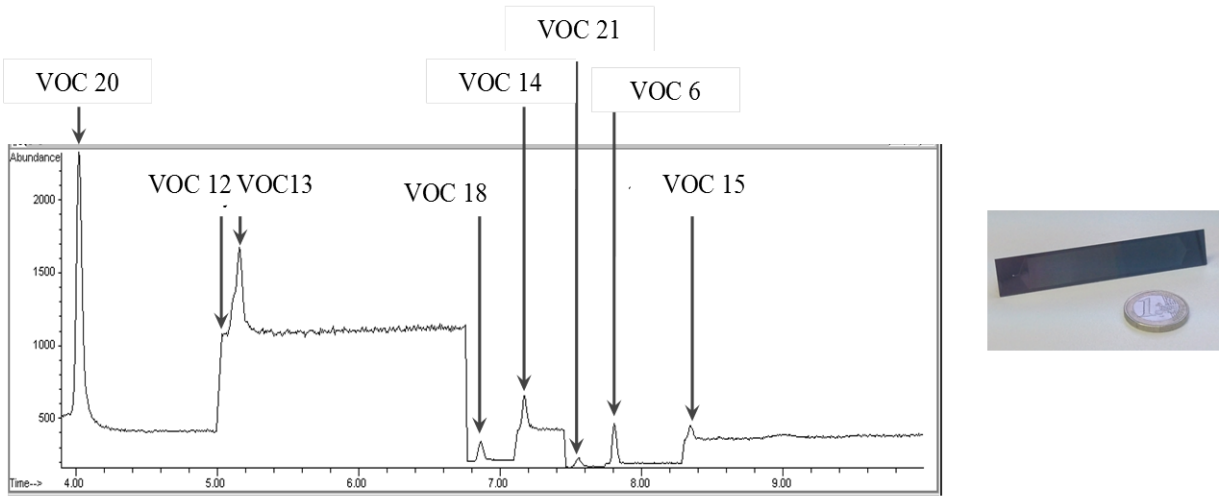


Figure 1: Chromatogram from emissions of a chamber containing 8 fungal VOC's (separation on a standard column) and photograph of the concentration module

The results repeatability is checked with three successive measurements. Thus, the results enable to validate both the trapping and desorption of the compounds at 140 °C.

Separation module validation

Once the chromatographic micro-column is realized and filled with PDMS, tests are carried out to verify the retention and separation effectiveness of various targeted compounds of the study. In order to validate this module, the air from the chamber contaminated by *Apergillus brasiliensis* is collected in the vial and analyzed by GC-MS. After chromatogram analysis, all VOCs have been found (Figure 2).

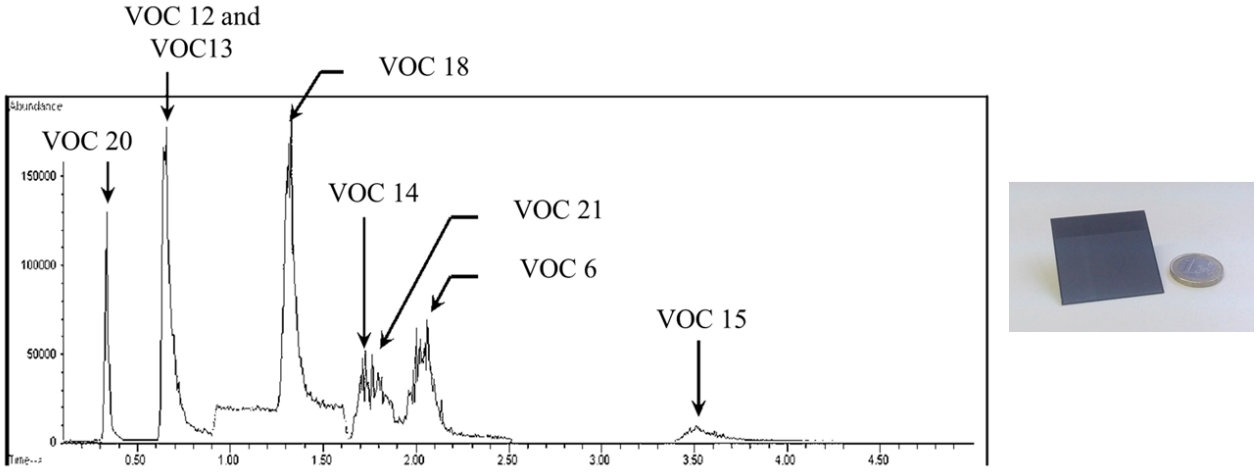


Figure 2: Chromatogram from emissions of a chamber containing 8 fungal VOC's and photograph of separation module

Thus, different retention times are found for the various families, proving the efficiency of the micro-column separation. These tests prove that the micro-column is able to separate eleven targeted VOC for fungal contamination index calculation. The separation tests in this study

confirm that this stationary phase provided sufficient separation for our problem, despite the short length of the micro-column.

Sensor array responses

The study of the sensors' response is alternately performed with two emission chambers, the reference one with activated charcoal (2 min exposure) and the other one with VOC (15 sec exposure). The air used between injections is filtered over activated charcoal and a Tenax tube. This filtered air enables quick desorption of VOCs that have penetrated into the polymer in order to find a stable baseline.
Resistance responses of each triplicate sensor to each vapour exposure are recorded three times and the percentage change against the baseline are calculated using (1):

$$\%\frac{dR}{R} = \frac{\left(R_{sensor\ exposed\ to\ VOC} - R_{sensor\ non\ exposed\ to\ VOC}\right)}{R_{sensor\ non\ exposed\ to\ VOC}} \times 100 \quad (1)$$

where $R_{sensor\ exposed\ to\ VOC}$ is the resistance of the sensor exposed to VOC and $R_{sensor\ non\ exposed\ to\ VOC}$ is the resistance of the sensor which is not exposed to VOC.
A signal is considered as a response of the sensor to the stimulus when its intensity exceeds the detection limit defined by DL = mean (background) + 3 x standard deviation (background).
Once obtained the sensor response, average sensitivities ((dR / R) / dC) of each PCE were calculated for each VOC tested according to the following equation (2):

$$\frac{\mathrm{dR/R}}{dC} = \frac{\left(R_{sensor\ exposed\ to\ VOC} - R_{sensor\ non\ exposed\ to\ VOC}\right)/R_{sensor\ non\ exposed\ to\ VOC}}{C_{VOC}} \quad (2)$$

where C_{VOC} is the concentration of the tested VOC.

The fact of calculating the sensitivity with dR / R and non dR enable the comparison between all ECPs. For each VOC and each PCE, responses of four sensors were studied. A triplicate was carried out for each test. The average values for all the VOCs are shown in the radar chart where the radius corresponds to the sensitivity of an ECP for a particular VOC (Figure 3). This type of diagrams can differentiate positive and negative response.

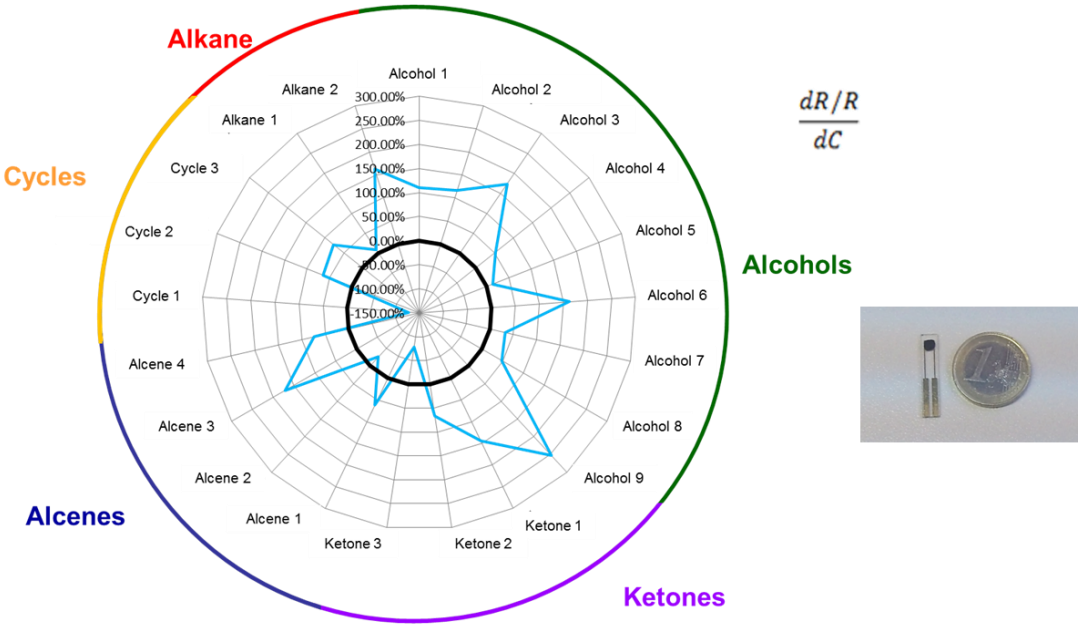


Figure 3: Radar chart for the sensitivity of a polymer for different VOC's and photograph of a polymer sensor

All the responses from these different sensors enable to create a sensor array. These differences in sensitivity and response patterns have contributed to the creation of a specific signature for VOCs. Thus, the responses matrix provides 14 specific signatures for 14 VOCs. The other 7 VOCs (VOC 9 and VOC 10; VOC 2 and VOC 4; VOC 5, VOC 19 and VOC 1) have common signatures.

DISCUSSIONS

Monitoring enclosed environments towards fungal development is already a major concern for curators but it should become urgent for human and animal health in short term. So having a tool which can be installed in buildings and can provide almost instant information about a possible fungal development constitutes therefore a major breakthrough.

This monitoring device has been developed in order to be compatible with an indoor environment real time monitoring strategy. Therefore, our research efforts have concerned the miniaturization of the analytical chain for portable, reliable and low cost applications. As the microsystem required specifications such as collection, analysis and interpretation of data, the developed microsystem integrates the steps of sampling, transfer, separation and analysis of samples.

In detail, the developed monitoring beacon has three modules: a pre concentrator, a chromatograph column dedicated to the VOCs' separation and finally the detector.

Thus, regarding the pre-concentration micro-module, the micro-structure was sized to maintain the characteristics of a conventional sampler. The concentration efficiency (retention and volume of drilling) of this microstructure is still equivalent to a conventional collection tube.

Concerning the separation module, the length of the micro-column (5 m) is shorter than that of a conventional column (60 m). Despite this decrease, better retention efficiency can be achieved with thick films of polydimethylsiloxane, enabling the analysis of light compounds such as selected tracers. Separation tests in this study confirmed that this stationary phase provides sufficient separation to our problem, despite the short length of the column.

However, it is difficult to compare these results with the literature since there are no studies on fungal development tracers.

Concerning the tested sensor array, the differences in sensitivity and selectivity of ECPs contribute to the creation of a specific signature for VOCs. This module enabled to yield specific signatures for 14 out of the 21 VOCs tested. For 7 other VOCs with identical signatures, the use of upstream the separation module allowed to differentiate them. Thus, the coupling of the retention times with the chemical signature enabled identification of the 21 VOCs tested.
So, the developed modules are suitable for an analytical micro-system that integrates air sampling, transfer, separation and analysis steps.
The modules tested in this study enabled to consider early detection of fungal growth in an indoor environment. This beacon coupled with the benefits of the fungal contamination index (detection of recent contamination and/or hidden, specificity of detection) makes it a useful tool for monitoring the microbiological quality of enclosed spaces. In addition, the modularity of the system, including the ability to vary both the elements of polymers detection and retention time of interest, and therefore the nature of VOC to analyze, allows expanding its use to other pollutants.

CONCLUSIONS

The device developed in this study enables to detect quickly fungal growth in an indoor environment. Besides this ability, this beacon coupled to the benefits of FCI (detection of recent contamination and/or hidden, specificity of detection) makes it a useful tool for monitoring the microbiological quality of confined spaces. In addition, the modularity of the system, including the ability to vary both the elements of polymers detection and retention time of interest, and therefore the nature of VOC to analyze, allows expanding its use to other pollutants. At the moment, the CSTB follows the industrialization of this device with the society named as Créative Eurecom.

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