

Monitoring Mold growth and VOC Emissions from Wood Wool Insulations under Unfavorable Hygrothermal Conditions

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ABSTRACT

Mold growth on indoor surfaces can lead to severe human health effects. The presence of bio-based surfaces under high humidity conditions is expected to favor its development. This study used a custom-built wall at a 1:1 scale mimicking the building structure of real walls of a French public building containing wood wool as a bio-sourced insulation material. The wall was placed at ambient temperature and relative humidity of 70±5% in order not only to characterize mold development, but also Volatile Organic Compounds (VOCs) emissions. Moreover, a 60 x 60 cm piece of the wood wool was inoculated with about 700 CFU/cm² of the fungus *Aspergillus niger* to evaluate the proliferation of micro-organisms under these conditions and eventually the emission of microbial Volatile Organic Compounds (mVOCs). The surface emission rates (ER) of Total Volatile Organic Compounds (TVOCs) and mVOCs were thus measured from the inoculated and non-inoculated surfaces.

No mold development was observed by the naked eye on the inoculated or on the non-inoculated surface of the wood wool after one month and this was confirmed by counting the CFU number. Only four mVOCs were detected from both surfaces with a low ER of less than 1 µg m⁻² h⁻¹. The ER of TVOCs was 90.3 µg m⁻² h⁻¹ from the non-inoculated surface and 71.2 µg m⁻² h⁻¹ from the inoculated surface. The obtained results show that the selected hygrothermal conditions are not sufficient for mold development on wood wool and that the detection of mVOCs is not a reliable indicator for microbial growth.

KEYWORDS

Mold growth, VOC emission, wood wool, humidity, fungal inoculation

INTRODUCTION

People in France spend more than 90% of their times indoors (ANSES, 2014). During this time, they are exposed to a variety of indoor air pollutants. Microbial growth is considered a key element of indoor air pollution (WHO, 2009). Visible molds are found in 14-

20% in French housings, appearing in the form of dark spots on food or surfaces in damp environments (ANSES, 2016). Indoor exposure to molds was shown to have potential human health effects on the respiratory, nervous, hematological, dermatological, and immune systems (Curtis et al., 2004; WHO, 2009).

Recently, bio-based building materials are replacing conventional materials due to their lower production energy and ecological properties (Silva et al., 2018). However, because of their chemical composition, these materials can be considered a nutritional source for the development of bacteria and fungi (Ginestet et al., 2020). Therefore, future new buildings are potentially more sensitive to mold development.

Mold growth in indoor environments is greatly affected by changes in temperature and relative humidity (RH) (Abe, 1993). The optimal temperature for mold development is between 20 and 40 °C with a critical RH value of 80-90% (Viitanen, 1994). However, spores might still germinate at 70% RH under favorable temperatures (Sedlbauer, 2001). The existence of leaking roofs or pipes and water condensation can cause moisture problems which in turn might lead to mold development at ambient temperature (EPA, 2017). Therefore, the presence of a nutritive bio-based source at favorable hygrothermal conditions can lead to spore germination or mold development in indoor environments.

mVOCs are produced in the metabolism of bacteria and fungi. Health risks caused by these compounds were raised in the 1990s and analyzed in indoor air environments for the first time during the same period (Korpi et al., 2009). VOCs can be considered as a fingerprint of fungal development. Moularat et al. (2008) have identified 19 VOCs, including mVOCs, whose emission originates either from the metabolism of fungi or from the degradation of the growth substrate (Moularat et al., 2008, 2011). Since these compounds are volatile, they are thus emitted into indoor air and can deteriorate Indoor Air Quality (IAQ).

The aim of this study is therefore to examine mold development on the indoor surface of bio-based insulation material found in a laboratory-reproduced

wall and placed under relatively favorable hygrothermal conditions. This is done through characterizing and comparing VOC emissions from the inoculated and non-inoculated surfaces and by counting the number of Colony Forming Units (CFU) on the inoculated surface.

METHODS

Construction of the wall

In order to characterize mold development and VOC emissions, a wall at a 1:1 scale was constructed at the Laboratoire des Technologies Innovantes (LTI) at the University of Picardie Jules Verne in Amiens, France (figure 1a). The structure of this wall and the used materials were similar to the real walls at the town hall of Moncheaux in the North of France and are representative of the renovated building structures in this region.

The tested wall (181 x 188 cm) was made up (from the interior to the exterior) of consecutive layers of two plasterboards of 18 mm thickness each, a 60 mm wood wool insulation material, followed by a water vapor barrier for damp proofing, another wood wool insulation of 120 mm thickness, a 40 mm air gap, and finally a brick wall. The temperature and RH were set at 22 °C and 70 ± 5%, respectively from the interior side of the wall and at 12 °C and 65% from the exterior side in the aim of creating a hygrothermal flux that promotes mold development on the insulation surfaces of the wall. 9 hygrothermal sensors (Vaisala HMP60) were placed at each surface of the constructed wall upon construction to continuously monitor the T and RH at different positions as presented in figure 1b.

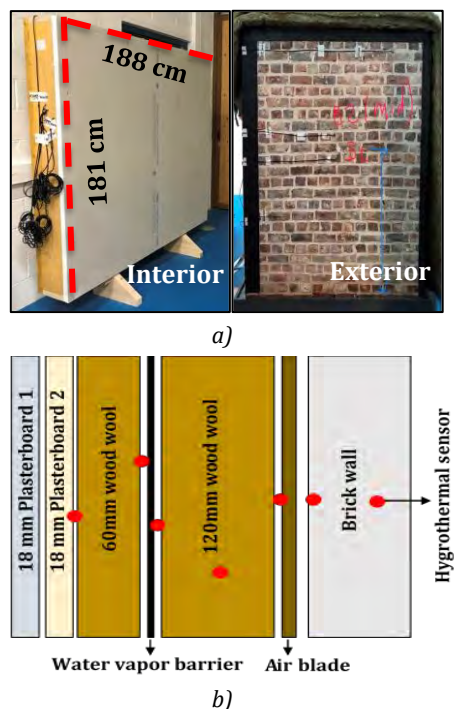


Figure 1. a) The constructed wall from the interior and exterior sides; b) A schematic representation of the tested wall and hygrothermal sensors (red dots) positions

Experimental procedure

After 4 weeks of hygrothermal conditioning of the wall, two FLECs (Field and Laboratory Emission Cell) were placed on the interior surface of the wall in order to characterize the emission of VOCs. One FLEC was installed on the top half of the wall while the other was placed on the lower half, as shown in figure 2, in order to evaluate the homogeneity of VOC emissions within the wall. A 500 mL/min flow of clean and humidified (70 ± 5%) air entered each FLEC. Sampling occurred after one day of their installation as recommended by the European standard ISO 16000-10 (International Organization of Standardization, 2006).

VOC samples were actively collected on DNPH cartridges (2,4-Dinitrophenylhydrazine) to collect the emitted carbonyls while Tenax TA tubes were used to trap all the other VOCs. Two DNPH cartridges were placed in series to avoid the breakthrough of carbonyls as they are highly volatile. The sampled volume was equal to about 25 L (2-hour sampling with an air flow rate of about 200 mL/min). The FLECs were removed after sampling. Then a 60 x 60 cm piece of the top part of the plasterboard, on which the FLEC was previously installed, was taken off to spray the wood wool behind it with a solution containing 3.85×10^5 CFU/mL of the fungus *Aspergillus niger*. An average of 700 CFU/cm² was thus initially deposited on the surface. The plasterboard was then put back and left for another month under the same hygrothermal conditions in order to give time for the spores to germinate.

After this period, the FLECs were reinstalled again in the same positions to not only to monitor VOC emissions due to potential mold development on the inoculated surface, but also to assess the evolution of VOC emissions from the non-inoculated surface after one month of exposure to humidity.

The collected DNPH cartridges were then eluted by 3 mL acetonitrile and analyzed using an HPLC/UV (High Performance Liquid Chromatography coupled to Ultra Violet Detector, Dionex Ultimate 3000, Thermo Scientific U.S.A.) equipped with an Acclaim RSLC Carbonyl column (2.2 µm, 2.1 x 150 mm, Thermo Scientific, U.S.A.). The Tenax TA tubes were analyzed using TD-GC-MS/FID system (Thermal Desorption Gas Chromatography, Clarus 680 - Mass Spectrometry and Flame Ionization Detection, Clarus SQ 8T, Perkin Elmer, U.S.A.) with a CP-Sil 5CB column (60 m x 0.25 mm x 1 µm, Agilent U.S.A.). The HPLC/UV and TD-GC-MS/FID methods used by Tobon-Monroy were used in this study (Tobon-Monroy, 2020). Calibrations were run before each HPLC and GC analysis. Carbonyls were quantified using their individual calibration coefficients in HPLC while a calibration of the TD-GC-MS/FID using 10 VOCs occurred. All other VOCs were quantified as toluene equivalent. The limit of detection (LOD) of the GC method for toluene was equal to 0.004 µg m⁻³ while that of the HPLC ranged from 0.2 to 0.6 µg

m^{-3} , depending on the compound, for an air sampled volume equal to 25 L.

After the second VOC sampling, the plasterboards were removed and five samples for microbial analysis were cut from the inoculated wood wool piece. Extraction of microorganisms was performed using an MgSO_4 solution ($5 \times 10^{-3} \text{ mol L}^{-1}$, 0.25% Tween-20) followed by 1.5 hr of agitation. A 1 mL aliquot was sprayed on DRBC agar to characterize the proliferation of the deposited fungus by counting the CFU. The water content of the five samples was also measured by Karl Fischer titration method to verify if the humidity conditions were adequate for microbial development or not.



Figure 2. Installation of the FLECs on the constructed wall for VOC measurements

RESULTS AND DISCUSSION

4 types of samples were collected: non-inoculated samples from the top (NIT) and the bottom (NIB) surfaces of the wall after four weeks of hygrothermal conditioning, non-inoculated samples from the same bottom surface after another month of hygrothermal conditioning (NIB'), and inoculated samples from the top surface (IT) after 1 month of inoculation. A replicate of each sample was made for more precise results.

90 compounds were quantified in total from both inoculated and non-inoculated surfaces. However, only compounds having an emission rate (ER) $> 0.5 \mu\text{g m}^{-2} \text{ h}^{-1}$ in GC or higher than the limit of quantification (LOQ) in HPLC will be presented throughout this paper. The emission rate of TVOCs has been determined from the different surfaces as the sum of individual VOCs sampled on Tenax TA tubes and eluted between hexane (C_6) and hexadecane (C_{16}) as recommended by the European Standard ISO 16000-6 (International Organization of Standardization, 2011). Since the

difference in ER between the two replicates ranged from 10 to 30% for most of the characterized compounds, indicating the robustness of our experiments, results will be presented as their average throughout this paper. The difference in emission between surfaces was considered significant when higher than that between replicates ($> 30\%$). Non-parametric statistical tests on our samples seemed incoherent, probably due to errors induced by the small-sized populations (Columb & Atkinson, 2016),

Characterizing the homogeneity of VOC emissions within the constructed wall

Several numerical models are being developed nowadays to characterize the emission of indoor pollutants, such as VOCs, from building and consumer materials into indoor environments (Cox et al., 2002; Huang & Haghghat, 2002; Yang et al., 2001). Most of these models consider the emission sources, and thus VOC emissions, homogeneous which enables simpler model development and requires fewer input parameters.

To characterize the homogeneity of VOC emissions from different parts of the wall, emissions of some VOCs from the NIT and NIB surfaces are compared (table 1). The emission of TVOCs in addition to other VOCs such as 1,2-ethanediol, propylene glycol, the coelution of benzoic and octanoic acids, and furfural from the same surface at two different positions was compatible with a relative difference of less than 20% while for the other compounds such as propanone, acetaldehyde, and formaldehyde a big difference was observed between the two parts of the same surface.

Therefore, considering a surface as homogeneous upon developing numerical models to estimate the emission of individual VOCs should be reconsidered since it may lead to under- or overestimation of VOC emissions from building materials into indoor environments.

Table 1. Comparison of VOC emission rates ($\mu\text{g m}^{-2} \text{ h}^{-1}$) from the non-inoculated top (NIT) and left (NIB) surfaces

VOC	NIT	NIB
1-Pentanol	0.7	0.7
1,2-Ethanediol	3.1	2.8
Propanone	16.3	122.5
Propylene glycol	47.4	54.6
Acetaldehyde	7.2	46.4
Coelution of Octanoic and Benzoic acids	1.1	1.0
Coelution of 1-Butanol and Benzene	1.3	7.7
Methyl Vinyl Ketone (MVK)	3.0	11.9
Methyl Ethyl Ketone (MEK)	2.5	5.4
o-Tolualdehyde	< LOQ	3.3
Nonanal	0.4	0.6
Formaldehyde	9.0	1.8
Hexanal	4.4	2.3
Furfural	6.3	5.2
TVOCs	90.3	112.4

Characterizing VOC emissions as function of relative humidity (RH)

Relative humidity has a considerable effect on VOC emissions. Placing the constructed wall at a relatively high humidity level (RH = 70 ± 5%) for about 1 month can have an effect on the ER of VOCs. In order to characterize the change in VOC emission due to RH, VOC ERs from the non-inoculated surface (NIB) were compared to those from the same surface after one month of hygrothermal conditioning (NIB') (table 2).

In general, the surface emission of VOCs decreased upon prolonged exposure to relatively high humidity which is well reflected in the 60% decrease in the emission rate of TVOCs between the surfaces NIB and NIB'. However, two major emission behaviors of individual VOCs were observed: decreased and increased emissions.

As shown in table 2, the ERs of 12 compounds out of 17 decreased significantly with time. This can be partly explained by the prolonged exposure of the wall to relatively high humidity. VOCs are known for their high vapor pressure or volatility and they are thus susceptible to leach out of the material into indoor environments, especially at high temperatures and variable RH. Under relatively humid conditions, water molecules compete with VOCs for occupying the adsorbent sites of the building materials and can replace these compounds leading to higher VOC concentrations at first (Lin et al., 2009). However, materials are considered finite VOC sources and upon prolonged exposure to humidity, VOC emissions become diffusion-controlled (Fang et al., 1999). In this case, the VOC load in the bulk decreases resulting in lower emission rates with time and progressive depletion of VOCs from the material (Harb et al., 2018).

On the other hand, the ERs of most of the aldehydes (formaldehyde, hexanal, 2,5-dimethylbenzaldehyde, and furfural) from the NIB surface increased significantly (up to 10 µg m⁻² h⁻¹) where they were approximately multiplied by 2 after one month of hygrothermal exposure. According to Pohleven et al., VOC emissions from wood are dominated by aldehydes (Pohleven et al., 2019); therefore, it seems very likely that the emission of these compounds originates from the wood wool insulation materials. Since aldehydes are readily soluble in water (Stephenson, 1993), they may be dissolved after emission in the water molecules occupying the active sites of the material leading to an increase in the initial emittable concentration (C₀) of VOCs with time and thus an increase in their surface emissions (Liang et al., 2016). As a result, even if building materials are considered finite sources of VOCs, there is no need for the emission to decrease or become zero as long as the source is emitting (Harb et al., 2018).

Table 2. Comparison of VOC emission rates (µg m⁻² h⁻¹) from the same non-inoculated surface after one month

VOC	NIB	NIB'	
1-Pentanol	0.7	0.3	↓
1,2-Ethandiol	2.8	3.0 x 10 ⁻²	↓
Propanone	122.5	9.9	↓
Propylene glycol	54.6	0.5	↓
Acetaldehyde	46.4	3.3	↓
Coelution of Octanoic and Benzoic acids	1.0	0.5	↓
Coelution of 1-Butanol and Benzene	7.7	2.2	↓
Methyl Vinyl Ketone (MVK)	11.9	4.8	↓
Methyl Ethyl Ketone (MEK)	5.4	2.8	↓
o-Tolualdehyde	3.3	< LOQ	↓
Nonanal	0.6	0.7	↑
Formaldehyde	1.8	3.6	↑
Hexanal	2.3	5.5	↑
2,5-Dimethylbenzaldehyde	< LOQ	4.8	↑
Furfural	5.2	9.8	↑
TVOCs	112.4	48.1	↓

Characterizing microbial development

Through microbial extraction

After the extraction of micro-organisms from the five samples of the inoculated surface, the number of viable fungal cells (CFU) was counted and reported.

No CFUs were detected. This indicates that the initially deposited CFUs of *A. niger* were dead due to unfavorable hygrothermal conditions even if the nutritive support (wood wool) was present. Therefore, the selected hygrothermal conditions in this study seemed unsuitable for mold development on wood wool. The obtained results are coherent with those obtained by Block where mold growth was observed at humidity levels higher than 85% (Block, 1953; Tobon et al., 2020).

To further validate these results, the water content in the five samples was also determined by Karl Fischer titration. The obtained results are shown in table 3.

When characterizing microbial development on insulation materials, Tobon-Monroy found that a minimum water content of 15.3 ± 1.0% is needed for microbial development to occur on wood wool (Tobon-Monroy, 2020). The obtained average water content of wood wool was equal to 9.5 ± 1.5% in this study. These results validate that humidity levels higher than 70 ± 5% are required for microbial development.

Table 3. The determined water content in the five extracted samples from the inoculated surface after one month of inoculation

Sample	Water content (%)
1	8.2
2	10.2
3	9.2
4	11.0
5	8.9

Through VOC emissions

Certain VOCs can be emitted upon microbial development either from the fungal metabolism or due to the biodegradation of the substrate (Moularat et al., 2008). In order to evaluate the potential presence or absence of mold development, a comparison of VOC emissions from the same surface before (NIT) and after inoculation (IT) took place (table 4).

No “newly emitted” compounds were observed, i.e. the emitted VOCs after inoculation were also detected before inoculation, with observed variations in their emission rates. When comparing VOC emissions from the NIT and IT surfaces, we observe here again two major behaviors, decreased and increased emissions, which can be explained similarly based on the previously built hypotheses discussed in part b.

Since mVOCs can be considered as an index for mold development, it is interesting to consider their emissions in this part even though their emission rates are low ($< 0.5 \mu\text{g m}^{-2} \text{h}^{-1}$). Based on the mVOCs classification by (Korpi et al., 2009), only four mVOCs: 1-butanol, benzene, 2-heptanone, and 2-ethyl-1-hexanol were detected in this study and their ERs are shown in table 4. The emission rates of 2-ethyl-1-hexanol and 2-heptanone were very low while that of 1-butanol and benzene decreased with time. This is corroborated by the results of the microbial analysis showing the absence of microbial growth, as discussed previously. It is likely thus that the detected mVOCs are mainly emitted from the building materials used for the wall construction, which is further shown by the decrease over time of the ER of 1-butanol and benzene due to hydrothermal conditioning. Since no spores germination or mold development was detected neither by microbial extraction nor by VOC emissions, it seems that the detection of mVOCs might not be a specific and reliable indicator for mold growth in indoor environments, as previously observed in other studies (Schuchardt & Strube, 2013).

Table 4. Comparison of VOC emission rates ($\mu\text{g m}^{-2} \text{h}^{-1}$) from the same surface before (NIT) and after inoculation (IT)

VOC	NIT	IT	
1-Pentanol	0.7	0.4	↓
1,2-Ethanediol	3.1	1.8	↓
Propanone	16.3	4.8	↓
Propylene glycol	47.4	27.7	↓
Acetaldehyde	7.2	3.0	↓
Coelution of Octanoic and Benzoic acids	1.1	0.7	↓
Coelution of 1-Butanol + Benzene	1.3	0.7	↓
Hexanal	4.4	3.5	↓
2-Ethyl-1-hexanol	0.1	6.0×10^{-2}	↓
2-Heptanone	6.0×10^{-2}	8.0×10^{-2}	↑
Methyl Vinyl Ketone (MVK)	3.0	4.5	↑
Methyl Ethyl Ketone (MEK)	2.5	2.7	↑
Nonanal	0.4	0.8	↑
Formaldehyde	9.0	9.6	↑
2,5-Dimethylbenzaldehyde	< LOQ	2.3	↑
Furfural	6.3	6.2	=
TVOCs	90.3	71.2	↓

CONCLUSION

A 1:1 scale wall was constructed in this study and placed under specific hygrothermal conditions ($T = 22 \text{ }^\circ\text{C}$ and $\text{RH} = 70 \pm 5\%$) to characterize the possibility of mold development on bio-based insulation materials (wood wool). This was done through the measurement of surface VOC emissions before and after the wall inoculation with the fungi *A. niger*.

Our results show that VOC emissions can show significant differences within a same wall under the same hydrothermal conditions. Therefore, the homogeneity in emissions of materials should be considered with caution.

Although some mVOCs were detected, the VOC emissions showed no presence of mold development and that the emitted VOCs originate solely from the building materials. The decrease in emission rate of certain VOCs with time might be due to the prolonged exposure to relatively high humidity level which led to their depletion from the materials while the increase in the emission rates of aldehydes can be due to their high water affinity.

The absence of biologically emitted mVOCs was further validated by the microbial results. No CFUs were detected indicating that 70% relative humidity is inadequate for spore proliferation or mold development.

For this reason, a new emission test will be conducted following the same procedure, but this time under higher humidity levels and using a combination of two fungi (*Aspergillus niger* and *Penicillium*) to further characterize the conditions needed for mold development or spores germination.

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