Are microbial volatile organic compounds (MVOC) useful predictors for a hidden mould damage?

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ABSTRACT

Laboratory trials were performed in order to search for the variety of the production of microbial volatile organic compounds (MVOC), which could be used as indicators for hidden mould damage. Concerning the MVOC production the experiments showed a dependency on the building materials used as substrate and on the genus or species. It could be proved that the production of certain MVOC is not consistent at all times. On the whole low emission rates in terms of $\mu g\ h^{-1}$ of the MVOC were found. Extrapolating the emission rates from the laboratory trails to an indoor air situation results in concentrations below the analytical detection limit in most cases. According to these results only heavy fungal contaminations might be detected by this method in indoor air. But the method could yet be very useful for the search for microbial damages in small cavities with normally very low air exchange rates.

INDEX TERMS

Microbial volatile organic compounds (MVOC); Indicator of fungal damage; Emission spectrum; Emission rate; Substrate dependency; Quality of indicators

INTRODUCTION

Damages caused by moulds, bacteria and other microorganisms are frequently found in different parts of buildings. The reasons are water damages, surface temperatures below the dew point due to building insuffiencies, over-utilization of indoor spaces and reduced air exchange rates. Almost all usual materials used for construction or furnishings like wallpaper, flooring, textiles and wood can serve as a substrate for microorganisms. The growth of microorganisms in indoor spaces must always be suppressed or eliminated, since microorganisms produce and spread a number of products causing adverse health effects. Spores of moulds may cause allergies, asthma and sometimes contain mycotoxins. Microorganisms also produce many volatile organic compounds (MVOC), which sometimes provoke an unpleasant smell. The toxicological relevance of the MVOC is probably negligible as they occur at very low concentrations. But these compounds are used more and more frequently for the search for hidden mould growth. There was much hope in the analysis of MVOC, as various studies have shown that 'traditional' microbiological examinations failed to predict hidden mould damage. This is especially true for the measurements of airborne fungal spores, as there is frequently no significant difference in the spore concentrations between microbiologically infested and non-infested buildings. This occurs especially when the fungal damage is concealed, since fungal spores are usually too large to penetrate, e.g. carpeting or insulating material. The possibility to diffuse through carpets, wallpapers and other tissues was regarded as main advantage of the MVOC analysis.

Knowledge about emitted MVOC spectra is usually gained from laboratory experiments. First, such trials have to prove that the systems are free from blank values, so that laboratory

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contaminants are not misinterpreted as MVOC. Second, in many laboratory trials microbial nutrients like Petri dishes were used, as fungal growth and MVOC production are satisfying under these conditions. But one has to bear in mind that the growth on 'real' substrates like building material may be less abundant and that the emission rate of MVOC therefore might be possibly reduced. Furthermore, one has to take into consideration that the formation of the so-called secondary metabolites is strongly dependent on the substrate.

AIMS OF THE STUDY

This study aimed at three points:

1. Which emission rates of MVOC can be expected by moulds growing on typical building materials in terms of µg/week or µg/hour?
2. How is the emission spectrum of the MVOC influenced by different moulds causing typical indoor damages?
3. Will those emission rates lead to measurable concentrations in the indoor air depending on the size of the infected site and the air exchange rate?

METHODS

Experimental Set

In carefully cleaned and sterilized glass incubation chambers five typical indoor building materials (see Table 1) were inserted under sterile conditions. The building materials were inoculated with spores of *Aspergillus versicolor* # 1943, a strain delivered by the German collection of microorganisms and cell cultures (DSMZ). To investigate the MVOC spectrum produced by different genera the following four moulds were inoculated on ingrain wallpaper: *Penicillium brevicompactum*, *Aspergillus versicolor*, *Eurotium amstelodami* and *Chaetomium globosum* (see Table 2). Each species tested comprised two strains from certified collections and three wild strains isolated from recent mould damages. All trials are based on at least four sets of measurements, in which both parallel and independent replicate trials were included.

Air samples were taken in intervals during the second incubation week. The supply air was purified by activated charcoal (250 mg) to avoid blank values. In order to prevent laboratory air from being sucked into the chambers, the purified air was pumped in the incubation chambers and through the sampling tubes. All connections were made of glass and metal to prevent sorption processes. To anticipate microbial contaminations particle filters with a pore size of 0.2 µm were used.

The air samples were analysed for the following 36 compounds by GC/MS in the SIM (selected ion monitoring) mode:

dimethylsulphide, 2-methylfuran, 3-methylfuran, 3-methyl-2-butanone, 3-methyl-2-butanol, 2-pentanone, 2-pentanol, 3-methyl-1-butanol, pyrazine, 2-methyl-1-butanol, 2-methyl-1-butanol, dimethyldisulphide, 1-pentanol, 2-butanonoxime, 2-hexanone, 3-methoxy-1-butanol, furfural, dimethylsulphoxide, 1-hexanol, 2-heptanon, 1-heptanol, 1-octen-3-ol, 3-octanone, 3-octen-2-ol, 3-octanol, 2-n-pentylfuran, 2-octanol, 2-ethyl-1-hexanol, cis-3-octen-1-ol, trans-2-octen-1-ol, 1-octanol, 1-nonanol, 4-hydroxyanisole, 1-decanol, 2,4,6-trimethylbenzaldehyde, diphenylsulphide.

RESULTS

MVOC Emissions Rates and Spectra in Dependency to the Building Material

The strain *Aspergillus versicolor* (DSMZ # 1943) produced a variety of MVOC, which are indicated in Table 1. Only 2-ethyl-1-hexanol was produced by *Aspergillus versicolor* on all

five substrates. 3-Methylfuran, pyrazine and 2-methyl-1-butanol were emitted by *Aspergillus versicolor* on four substrates, 2-pentanol was produced on three substrates.

Table 1 Emission of MVOC by *Aspergillus versicolor* (DSMZ[a] # 1943) on different substrates typically used as building materials

MVOC	Material (substrate)				
	Gypsum board	Spruce wood	Pine wood	Ingrain wallpaper w/o glue	Ingrain wallpaper with special glue*
3-Methylfuran	+	++			+
2-Methylfuran				+	
2-Pentanol		++	++	+	
3-Methyl-2-butanol	+			+	+
3-Methyl-1-butanol	+			+	
Pyrazine	++	++	+	+	
2-Methyl-1-butanol	+	++	+	+	
2-Butanonoxime		+			
1-Octen-3-ol	++			++	
3-Octanone	+++			+	
3-Octen-2-ol		++			
3-Octanol	++				
2-Octanol		++			
2-*n*-Pentylfuran				++	
2-Ethyl-1-hexanol	+++	++	++	++++	++
cis-3-Octen-1-ol		++			

[a]DSMZ: German collection of microorganisms and cell cultures.
[b]A special heavy-duty glue consisting of starch ether and polyvinyl acetate.
+, 0.001–0.01; ++: 0.01–0.1; +++: 0.1–1; ++++: 1–10 μg/week.

MVOC Spectrum Subject to Different Mould Strains

The results concerning the MVOC production in particular on ingrain wallpaper are given in Table 2. The compounds 2-methylfuran, 3-methyl-2-butanol, 2-pentanol, 3-methyl-1-butanol, pyrazin, 2-methyl-1-butanol, 1-octen-3-ol, 3-octanone, 3-octanol, 2-n-pentylfuran, 2-ethyl-1-hexanol and 2,4,6-trimethylbenzaldehyde were produced by all four strains. The homogeneity between wild strains from fungal damages and laboratory strains from collections was good. But it has to be mentioned that, in some cases, certain MVOC could not be detected in every trial round.

Table 2: **Emission of MVOC on ingrain wallpaper by 4 mold strains: Percentage of detection in independent trails**

Mold species / **MVOC**	*Penicillium brevicompactum* [%]	*Aspergillus versicolor* [%]	*Eurotium amstelodami* [%]	*Chaetomium globosum* [%]
3-methylfuran	0	0	11	0
2-methylfuran	70	100	94	93
3-methyl-2-butanone	100	0	64	96
3-methyl-2-butanol	70	91	82	62
2-pentanone	20	0	47	69
2-pentanol	50	25	24	27
3-methyl-1-butanol	40	8	41	62
pyrazin	45	50	65	12
2-methyl-1-butanol	70	67	53	81
dimethylsulfide	10	0	29	0
1-pentanol	0	0	0	4
2-hexanone	0	0	18	19
1-hexanol	0	0	0	8
2-heptanone	0	0	0	4
1-octen-3-ol	70	91	100	4
3-octanone	55	96	76	81
3-octanol	5	17	12	54
2-n-pentylfuran	25	17	41	23
2-octanol	0	0	0	19
2-ethyl-1-hexanol	100	100	100	96
2-ethylhexylacrylate	0	33	12	0
1-decanol	0	4	0	0
2,4,6-trimethyl-benzaldehyde	5	4	6	4
diphenylsulfide	0	8	12	8

DISCUSSION

One aim of the study was to decide whether MVOC emissions lead to measurable concentrations in indoor air. Therefore theoretical air concentrations were extrapolated from the laboratory experiments. The calculations are based on a relevant size of a mould-infected site (0.25 m^2), a given room volume (50 m^3) and an interval from the last window ventilation prior to the potential measurement (8 h). Hereby different air exchange rates ranging from 0.1 to 1 exchanges per hour were taken into account.

The following equation was used; the results are given in Table 3:

$$C(t) = E \cdot R_v^{-1} \cdot n^{-1} \cdot (1 - \exp - n \cdot t)$$

Table 3 Calculated indoor air concentrations from results of laboratory experiments

Emission rate in laboratory incubation chambers	Calculated emission rate based on a defined area	Calculated indoor air concentrations by an mould infected site of 0.25 m^2			
Area = 20 cm^2 (µg/week)	Area = 0.25 m^2 (µg/h)	AER = 0.1 (µg/m^3)	AER = 0.2 (µg/m^3)	AER = 0.5 (µg/m^3)	AER = 1.0 (µg/m^3)
0.001	0.00074	0.000082	0.000059	0.000029	0.000015
0.005	0.0037	0.00041	0.00030	0.00015	0.000074
0.01	0.0074	0.00082	0.00059	0.00029	0.00015
0.05	0.037	0.0041	0.0030	0.0015	0.00074
0.1	0.074	0.0082	0.0059	0.0029	0.0015
0.5	0.370	0.041	0.030	0.015	0.0074
1	0.74	0.082	0.059	0.029	0.015
5	3.7	0.41	0.30	0.15	0.074

Infected site: 0.25 m^2. Air exchange rate (AER) = 0.1, 0.2, 0.5 and 1.0 h^{-1}.
Room volume = 50 m^3. Time elapsed after ventilation and closure of the room = 8 h.

As shown in Table 3 only emission rates above 1 µg/h will lead to measurable indoor air concentrations above a detection limit of 0.1 µg/m^3. If the VOC load is elevated in realistic indoor air situations, the detection limit for MVOC is often significantly worse. In this case even higher source strength is needed to find mould growth by MVOC analysis. Due to these results the 'MVOC' concentrations found indoors are mainly due to other, mostly chemical sources.

A further shortcoming of the MVOC concept is that there is a clear dependence of the mould genus and the respective strain (see Table 2). Furthermore, the reproducibility of the MVOC production is sometimes poor. For example, the typical MVOC 1-octen-3-ol was produced only in 70% of all trials by *Penicillium brevicompactum*.

CONCLUSION

From the results given here we conclude that in the majority of cases there are poor chances to detect hidden mould damage in most indoor air situations by the MVOC method. Exceptions might be mould damages of large dimensions or of a high density. But the MVOC technique might still be useful for detecting hidden mould growth in closed spaces and cavities with a relatively low volume and a negligible air exchange rate.