VOLATILE METABOLITES FROM MICROORGANISMS GROWN ON BUILDING MATERIALS

A-S Claeson[1], J-O Levin, G Blomquist and A-L Sunesson

National Institute for Working Life, Program for Chemical Exposure Assessment, Umeå, Sweden

ABSTRACT
Microorganisms are able to produce a variety of volatile organic compounds. Microbially produced substances are one possible explanation of odour problems and negative health effects in buildings affected by microbial growth. In this study emissions of volatile compounds from mixed cultures of five fungi grown on three different humid building materials were investigated. Six different sampling methods were used, to be able to collect both non-reactive volatile organic compounds and reactive compounds such as volatile amines, aldehydes and carboxylic acids. Analysis was performed using gas chromatography, high-pressure liquid chromatography and ion chromatography. Mass spectrometry was used for identification of compounds.

The main microbially produced metabolites found on pinewood were ketones and alcohols. Compounds such as nitromethane and dimethoxymethane were found on particleboard. On all materials the emission of aldehydes decreased during microbial growth. No amines or carboxylic acids were identified.

INDEX TERMS
Building material, Microorganisms, Fungi, MVOC, Reactive compounds

INTRODUCTION
Over 300 volatile organic compounds (VOCs) have been identified in indoor air and the main sources are human activities or emissions from building products (Ezeonu *et al*. 1994). In damp buildings some of these compounds can be of microbial origin. Microbial contamination and the volatiles produced during growth are one possible explanation of different building related health problems. Microorganisms are known to produce a variety of compounds such as hydrocarbons, alcohols, ketones, sulphur compounds, terpenes and terpene derivatives and amines.

In recent years microbial volatile organic compounds (MVOC) have been used as an indicator of fungal growth when evaluating microbial contamination in buildings. A group of certain MVOCs, such as 1-octen-3-ol, 2-heptanone, 3-methyl-1-butanol etc have been used to detect mould growth (Ström *et al*. 1994). Since both the species and the substrate influence the MVOC pattern emitted by the microorganisms (Wilkins *et al*. 2000), the lack of knowledge of the MVOC composition during different growth conditions still limits the use of marker compounds in buildings with health complaints. Also, the compounds found so far cannot alone explain indoor eye- and airway irritation, because most of the identified metabolites are non-reactive and found in low concentrations in the indoor air. Other sampling techniques must be used order to find more reactive compounds (Pasanen *et al*. 1998).

[1] Contact author e-mail: anna-sara.claeson@niwl.se

In this study a mixture of five fungal species were cultivated on three different sterile humid building materials. The aim of the study was to investigate the emissions of volatile compounds from mixed cultures of fungi grown on building materials with a focus on reactive compounds.

METHODS

Mixtures of five fungi were cultivated on gypsum board, pinewood and particleboard. The following strains were used *Aspergillus versicolor* (UPSC 2027), *Fusarium culmorum* (UPSC 1981), *Penicillum chrysogenum* (UPSC 2020), *Ulocladium botrytis* (UPSC 3539) and *Wallemia sebi* (UPSC 2502). The isolates were obtained from the Uppsala University culture collection of fungi (UPSC). The gypsum board (13 mm) and the particleboard (12 mm) were bought from retail dealers and pieces of freshly cut pinewood deal were bought at a sawmill. The building material were cut into small pieces (approximately 8*1.5 cm) and sterilised by exposure to UV radiation.

Spore suspensions from the fungi were mixed and poured into cultivation flasks. A total amount of 100 ml autoclaved demineralized water was poured into each flask together with the spore suspensions. For each media, two cultivation flasks and one blank were prepared. The blank was treated as above except the spore suspensions. The cultivation was performed in 2-liters culture flasks made of glass as previously described by Sunesson (1995). The cultivation was performed at room temperature for 63 days and samples were taken continuously.

The volatile metabolites released by the cultures were sampled with six different adsorbents and chemosorbents by connecting sampling tubes at the outlet of the culture flask. Stainless steel tubes (Perkin Elmer) packed with Tenax TA (mesh 60-80, Chrompack) and Carbopack B (mesh 60-80, Supelco) were used as described by Sunesson (1995). The sampling time was 60 min and the sampling flow was 30 ± 2ml/min. The tubes were thermally desorbed at 225ºC (Tenax TA) and 270ºC (Carbopack B) for 10 minutes in an ATD 400 (Perkin Elmer) which was coupled to an Agilent GC/MS system (5973/6890). A reference masspectra library, NIST was used for identification of the volatiles. When necessary (library search qualifier below 80% or an unknown compound found), reference compounds were used to ensure the identity of the detected compounds. The analysed metabolites were quantified as equivalents of toluene (Sunesson 1995).

Sampling of aldehydes was performed using a DNPH (2,4-dinitrophenylhydrazine) impregnated adsorbent and the samples were analysed using a Waters HPLC with an UV-detector (365nm) (Levin *et al*. 1994). The sampling volume varied between 13 and 150 litres. The metabolites were identified and quantified using reference compounds.

Tertiary amines were collected using XAD-2 and charcoal tubes (SKC) and volumes of 25 – 120 litres were sampled (Andersson *et al*. 1989). The chromatographic separations were performed using an Agilent GC/MS system (5973/6890). The gas chromatograph was operated in splitless mode starting at 60ºC and raising 20ºC/min until reaching 150ºC. Primary and secondary amines were sampled (sampling volume 15-160 litres) with NIT (naphtylisothiocyanate) impregnated XAD-2 tubes (SKC) (Lindahl *et al*. 1993). Analysis was performed using HPLC-MS (Claeson *et al*.).

Carboxylic acids were sampled with silica tubes (ORBO-53, Supelco) according to the NIOSH method for sampling of formic acid (NIOSH-method 2011, 1994) and the sampling

volume varied between 12-290 litres. Ion chromatography (a Coricon IC 21 series II column oven and JD 21 series conductivity detector) was used for analysis and chromatographic separation.

RESULTS

A large number of microbially produced volatiles were detected on the three building materials. Dimethyl disulphide was the only compound produced on all media. The growth medium strongly affected the pattern of metabolites. On pinewood the metabolites consisted almost entirely of ketones and alcohols. A greater variety of compounds were found on particleboard and gypsum board. On particleboard the most abundant group was hydrocarbons such as 2-methyl-1,3-butadiene, but metabolites like 1,3,5-trioxepane and dimethoxymethane, that to our knowledge not have been found before, were also identified. The emissions from microbial growth on gypsum board consisted mainly of terpenes and alcohols.

On all building materials aldehyde emissions decreased faster in the cultivation flask than in the sterile flasks. The fungal mixture produced some nitrogen containing compounds, such as pyridine, 2-methyl-pyridine, nitromethane, hexanenitrile and ammonia, during growth on particleboard. No lightweight organic acids could be detected. The identified compounds are summarised in Table 1.

Table 1. Microbially produced compounds from cultures on building materials

	Pinewood	*Particle board*	*Gypsum board*	*Q*
COMPOUNDS				
Hydrocarbons				
1-Hexene	X	X		90
1-Methyl-4-(1-methylethyl)-benzene			X	90
1-Octene		X		93
1,2,4,4-Tetramethylcyclopentene		X		91
2-Methyl-1,3-butadiene		X		90
Hexane		X		90
Heptane		X		83
Nonane		X		96
Octane		X		94
Styrene	X			97
Alcohols				
1-Heptanol	X			86
1-Hexanol	X	X		91
1-Octen-3-ol	X		X	90
1-Pentanol			X	90
2-Heptanol	X			83
2-Pentanol	X			72
2-Methyl-1-butanol	X		X	83
2-Methyl-1-propanol	X		X	90
3-Methyl-1-butanol	X		X	86
3-Methyl-2-buten-1-ol	X			78
2-Methyl-3-buten-2-ol		X		91
4-Methyl-1-hexanol	X			72
4-Methyl-1-3-cyclohexen-1-ol			X	96

Methylbutenol	X			80
Ketones				
2-Butanone	X	X		80
2-Heptanone	X	X		91
2-Hexanone	X	X		91
2-Methyl-5-1-cyclohexanone	X			86
2-Nonanone	X			97
2-Octanone	X		X	90
2-Pentanone	X	X		90
3-Methyl-2-pentanone	X			80
3-Octanone	X			87
4-Methyl-2-hexanone	X			83
4,4-Dimethyl-2-2-cyclopentene-1-one		X		78
Acetone			X	86
2,6,6-Trimethyl-bicyclo-heptan-3-one		X		90
Cyclopentanone	X			83
Pulgone			X	86
Ethers				
2-Methylfuran	X	X		94
2,5-Dimethylfuran	X			90
Esters				
n-Propyl acetate	X			83
Pentyl acetate	X			86
Methyl acetate			X	R
Propanoic acid ester (Cas:74367-33-2)			X	64
Propanoic acid ester (Cas:74367-34-3)			X	90
Nitrogen compounds				
2-Methylpyridine		X		93
Ammonia		X		R
Hexanenitrile		X		87
Nitromethane		X		78
Pyridine		X		90
Terpenes				
3-Carene			X	95
3-Thujen-2-ol			X	72
Camphene			X	97
D-Limonene			X	93
Alpha-Pinene			X	96
1,3,3-Trimethyl-tricycloheptane			X	81
D-Verbenone			X	80
Acids				
Hexanoic acid			X	83
Others				
1,3,5-Trioxepane		X		83
2-Butyl-2-octenal			X	80
Dimethoxymethane		X		86
Dimethyl disulphide	X	X	X	97

Q = Qualifier (MS library fit) in %, R = Reference compound

DISCUSSION

Both 2-alkanones and straight chain alcohols are commonly found as fungal metabolites and they are formed by the breakdown of fatty acids (Kinderlerer 1989). The problem with using these as fungal tracers is that many of these compounds, as well as aldehydes and terpenes, are emitted from building materials. 2-methyl-1-propanol, 3-methyl-1-butanol, 3-octanol, 1-octen-3-ol, 3-octanone, 3-methylfuran and geosmin are often identified as fungal metabolites from different substrates and are therefore regarded as suitable tracers of microbial contamination. The result of this study reveals that these compounds are not completely reliable for the detection of mould growth. All of them except geosmin were identified but none of them were produced on all materials.

On pinewood and particleboard, aldehyde emissions decreased faster during microbial growth than in the sterile building material flasks. Some aldehydes have previously been reported as fungal metabolites, but in recent studies it has been found, as in this study, that they are rather consumed than produced, a phenomenon yet to be explained (Korpi *et al*. 1998).

Compounds containing nitrogen are interesting because they are reactive and potential airway irritants. The fungal mixture produced some nitrogen containing compounds during growth on particleboard. Some of them have been identified earlier as metabolites produced by microorganisms grown on different artificial media (Kaminski *et al*. 1974; Wilkins *et al*. 1995). This study indicates that these compounds can also be expected to be found in the indoor air. No amines, except pyridine and 2-methylpyridine, could be identified, with a detection limit for tertiary amines analysed with GC/MS of 0.08 ng/µl (triethylamine standard). The detection limit for primary and secondary amines analysed with LC/MS in full-scan mode was 0.3ng/µl and significantly lower with selected ion monitoring (SIM). The fungi needs to produce 0.8 µg tertiary amine/m^3 pumped air (5 µg/m^3 for primary and secondary amines) to be detected by the analytical system. This means that if the fungal mixture produce amines, the levels must be low. Few studies have been able to identify amines as microbial metabolites. Two studies, one performed by Rivers *et al*. (1992) and the other by Miller *et al*. (1973) reported trimethylamine as a metabolite from bacteria. Also, in an ongoing study of metabolites produced by *Streptomyces albidoflavus*, both primary and secondary amines have been identified (unpublished results).

CONCLUSIONS

A large number of fungal metabolites were identified. The metabolite production is very dependent on the substrate. The fungal mixture produced only one compound (dimethyl disulphide) on all materials. To be able to properly evaluate indoor microbial contamination, more metabolites produced by different microorganisms on various building materials need to be identified.

Few reactive compounds could be identified in this study, although they are very interesting from a health point of view. New methods more specific, with better sensitivity for sampling of reactive compounds need to be developed to be able to sample the low levels found in indoor environments.

REFERENCES

Andersson B and Andersson K. 1989. Determination of Tertiary Amines in Air. *Appl. Ind. Hyg.* Vol.4 (7) pp 175-179.

Claeson A S, Östin A, Levin J O and Sunesson A L. Development of an LC/MS method for the analysis of volatile primary and secondary amines sampled with NIT

(naphtylisothiocyanate). *Manuscript in Preparation*.
Ezeonu I M, Price D L, Simmons R B, Crow S A and Ahearn D G. 1994. Fungal production of volatiles during growth on fiberglass. *Applied and Environmental Microbiology*. Vol.60 (11) pp 4172-4173.
Kaminski E, Stawicki S and Wasowicz E. 1974. Volatile Flavor Compounds Produced by Molds of Aspergillus, Penicillum an Fungi imperfecti. *Appl. Microbiol.* Vol.27 (6) pp 1001-1004.
Kinderlerer J L. 1989. Volatile metabolites of filamentous fungi and their role in food flavour. *J. Appl. Bacteriol. Symp. Suppl.* Vol.67 pp 133S-144S.
Korpi A, Pasanen A L and Pasanen P. 1998. Volatile Compounds Originating from Mixed Microbial Cultures on Building Materials under Various Humidity Conditions. *Appl. Environ. Microbiol.* Vol.64 (8) pp 2914-2919.
Levin J O and Lindahl R. 1994. Diffusive air sampling of reactive compounds-A review. *Analyst*. Vol.119 pp 79-83.
Lindahl R, Levin J O and Andersson K. 1993. Determination of volatile amines in air by diffusive sampling, thiourea formation and high-performance liquid chromatography. *J. Chromatogr.* Vol.643 pp 35-41.
Miller A, Scanlan R A, Lee J S and Libbey L M. 1973. Volatile Compounds Produced in Sterile Fish Muscle (Sebastes melaops) by Pseudomonas putrefaciens, Pseudomonas fluorescens, ans an Achromobacter Species. *Appl. Microbiol.* Vol.26 (1) pp 18-21.
NIOSH-method 2011. 1994. Formic acid. *Manual of Analytical Methods (NMAM)*. Vol. (1).
Pasanen A L, Korpi A, Kasanen J P and Pasanen P. 1998. Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. *Environ. Int.* Vol.24 (7) pp 703-712.
Rivers J C, Pleil J D and Wiener J W. 1992. Detection and charachterization of volatile organic compounds produced by indoor air bacteria. *J. Exp. Anal. Environ. Epidemol.* Vol.suppl.1 pp 177-188.
Ström G, West J, Wesse'n B and Palmgren U. 1994. Quantitative analysis of microbial volatiles in damp Swedish houses . *In Health Implications of Fungi in Indoor Environments. Eds R.A Samson, B Flannigan, M.E. Flannigan, A.p. Verhoeff, O.C.G. Adan, E.S. Hoekstra. Elsevier Amsterdam.* pp 291-305.
Sunesson A L. 1995. *Volatile Metabolites From Microorganisms in Indoor Environments - Sampling, Analysis and Identification*. Ph. D Thesis. University of Umeå, Sweden.
Wilkins K and Larsen K. 1995. Variation of volatile organic compound patterns of mold species from damp buildings. *Chemosphere*. Vol.31 (5) pp 3225-3236.
Wilkins K, Larsen K and Simkus M. 2000. Volatile metabolites from mold growth on building materials and synthetic media. *Chemosphere*. Vol.41 pp 437-446.