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Use of gas chromatography-mass spectrometry/solid phase microextraction for the identification of MVOCs from moldy building materials

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Abstract

Gas chromatography-mass spectrometry/solid phase microextraction (GC-MS/SPME) was applied to identify microbial volatile organic compounds (MVOCs) in water-damaged, mold-infested building materials (gypsum board papers ($n=2$), mineral wool, and masonite) and in cultivated molds (*Aspergillus penicillioides*, *Stachybotrys chartarum*, and *Chaetomium globosum*). Three SPME fibers (65-μm PDMS-DVB, 75-μm Carboxen-PDMS, and 70-μm Carbowax-stableflex) designed for automated injection were used of which the latter showed best performance. A number of previously reported MVOCs were detected both in the building materials and the cultivated molds. In addition, methyl benzoate was identified both in the *S. chartarum* and *A. penicillioides* cultures and in the building materials. SPME combined with GC-MS may be a useful method for the determination of MVOCs emitted from mold-infested building materials.

Keywords: Building materials; GC-MS; Indoor environment; Molds; MVOCs; SPME

1. Introduction

The residential indoor environment is considered to be a significant route for human exposure to organic air contaminants. More than 300 volatile organic compounds (VOCs) have been identified in indoor air (Berglund et al., 1986) and a number of them originate from microorganisms, the so-called microbial volatile organic compounds (MVOCs). MVOCs include a broad range of compounds with boiling points from below 0 °C to about 400 °C and are predominantly produced by molds. Among the molds reported to commonly occur in damp indoor environments are *Penicillium* spp., *Aspergillus* spp., and *Alternaria* spp. (associated with asthma and atopy), *Stachybotrys chartarum* (pulmonary hemorrhage), *Cladosporium* spp., *Mucor* spp., and *Ulocladium* spp. (Samson et al., 1994; Grant et al., 1989; Fradkin et al., 1987; Etzel et al., 1998).

Solid phase microextraction (SPME) is a rapid technique for identification of VOCs that was introduced a decade ago by Arthur and Pawliszyn (1990). It has been used to analyze volatile disease markers in blood, drug metabolites in urine, volatile anaesthetic gases, and various solvents in solid and liquid materials (Arthur and Pawliszyn, 1990; Pawliszyn, 1997,

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1999; Scheppers, 1999). SPME utilizes a short fused silica fiber coated with a polymeric organic material as a stationary phase (Pawliszyn, 1997; Scheppers, 1999). The fiber is housed inside a syringe needle that allows penetration of the cap membrane above the sample in a sealed container as well as the septum in a gas chromatograph (GC). The syringe first penetrates the vial septum, then the fiber is pushed out of the needle and exposed to the sample headspace. The VOCs are thus concentrated on the fiber surface and then desorbed in the hot injector of the GC. Variables such as incubation temperature, sample agitation, and extraction and desorption time must be controlled (Pawliszyn, 1997).

SPME/GC-MS using electron impact (EI) ionization has previously been applied to detect VOCs from cultures of *Penicillium* spp. (Nilsson et al., 1996). The present study is the first that demonstrates that the technique can also be used to identify MVOCs directly from mold-infested building materials, and that EI and chemical ionization (CI) are complementary MS modes for studying MVOCs in environmental samples.

2. Materials and methods

2.1. Chemicals

2-Methylpentane, 2-pentanol, 3-methyl-1-butanol, 1-octen-3-ol, 1-pentanol, 3-methyl-2-butanol, 2-methyl-1-butanol, 2-heptanone, and 2-hexanone (all purchased from Fluka, Steinheim, Germany), 2-methyl-1-propanol (Aldrich Chem., WI, USA), and 3-octanone (purchased Sigma, Seelze, Sweden) were used. These VOCs were selected as they represent commonly reported MVOCs in indoor air. Methyl benzoate was purchased from Fluka.

2.2. Building materials and molds

Three different types of indoor building materials were used: (i) two gypsum board papers collected from the floor of an exhibition hall; (ii) one sample of mineral wool from a living room wall; (iii) one sample of masonite from an office floor. On the day of arrival, fungal cultivation was commenced from pieces taken from the most affected areas of each sample, as determined by the observed degree of material degradation and the amount of fungal growth (judged by stereomicroscopy). Parts of the same pieces were used for the direct SPME/GC-MS analysis. Fungal spores were eluted from the sample by lateral shaking in buffer containing (g/l): KH_2PO_4 (0.0425), $MgSO_4$ (0.25), NaOH (0.008), and Tween 80 (0.2 ml/l) (pH 7.0 ± 0.1). Fungal colonies were grown on DG18 (Oxoid, Basingstroke, England, CM729) and malt extract agar (MEA) without added sugar (Oxoid CM59). Both media contained (g/l) chloramfenicol (0.05) and chlortetracycline (0.05) to prevent bacterial growth. Mold fungi and yeast plates were incubated 7 days at 25 °C before subculturing.

Subcultivation of mold fungi (*Aspergillus penicillioides* from mineral wool, *S. chartarum* ($n = 2$) from the two gypsum board papers, and *Chaetomium globosum* from masonite) was done as three-point-inoculations on MEA except for *A. penicillioides*, which was grown on DG18. Subcultures were grown at 25 °C for 10 days and then stored at 4 °C before SPME/GC-MS analysis. Identification was done by microscopy.

2.3. SPME fibers

Three different types of SPME fibers that are compatible with autosampling using Merlin microseals (65-μm PDMS-DVB, 75-μm Carboxen-PDMS, and 70-μm Carbowax-stableflex, all purchased from Supelco, Bellefonte, PA, USA) were used. New fibers were conditioned with helium at 260 °C for 5 min prior to use. After each extraction cycle, fibers were automatically kept back inside the SPME needle to prevent possible contamination and were conditioned before re-use with helium at 200 °C for 1 min.

3. Sample preparation and analysis

3.1. Standards

Aqueous mixtures of the reference MVOCs (0.1 μl in 10-ml distilled water) were prepared in the 20-ml SPME vials. Each mixture was analyzed using all three fibers. Vials containing the mixtures were flushed with nitrogen for a few seconds and sealed

with strong metal caps (Microliter Analytical Supplies). The SPME syringe was allowed to penetrate the septum, and the fiber was exposed to the headspace of the vial. Preincubation time was 1 min (at 50 °C), extraction time was 3 min (at 50 °C), and desorption time in the injector of the GC was 2 min (at 220 °C). Sterile, nitrogen-flushed vials (20 ml) containing 10-ml water were used as blanks.

3.2. Subcultivated fungi and building materials

Pieces (approximately 1 cm^2) of agar with visible fungal growth were transferred to 20-ml SPME vials. Sterile agar pieces (DG18, MEA) were used as blanks. Analysis conditions for the three fungal strains were the same as those used for the standard MVOCs except that there was no preincubation and the extraction time was increased from 3 to 5 min. The 70-μm Carbowax-stableflex fiber was used.

A piece (2–4 cm^2, 200–300 mg) of each building material sample under test (the most mold-affected area) was inserted into a 20-ml vial and sealed. The 70-μm Carbowax-stableflex fiber was used and the preincubation time was 1 min (at 70 °C) and the desorption time was 2 min (at 220 °C). The extraction conditions varied (extraction times were 5, 10, 15, and 20 min and extraction temperatures were 45, 50, 60, 70, and 90 °C). Two pieces of gypsum board that were not affected by molds, as judged by stereomicroscopy, were used as blanks.

3.3. GC-MS

A Saturn 2000 ion-trap GC-MS instrument (Varian, Palo Alto, CA, USA) equipped with a fused-silica capillary column (CP-Sil 8CB-MS, 0.25 μm film thickness, 30 m × 0.25 mm i.d.) (Chrompack, Middelburg, The Netherlands) was used. Samples were injected with closed split at 5 psi using a Combi Pal SPME autosampler (Walnut Creek, CA, USA). A Merlin microseals inlet and a glass insert liner (ID 0.8 mm) designated for SPME analysis were used. Helium was used as the carrier gas, and the temperature of the column was programmed to rise from 45 to 280 °C at a rate of 6 °C/min. The temperature of the injector, transfer line, and ion trap was held at 220, 280, and 230 °C, respectively. Samples were analyzed both in EI and CI (isobutane) modes.

4. Results

4.1. Standards

The 70-μm Carbowax-stableflex fiber gave the best overall results—the other two tested fibers gave either lower general peak intensities or, in particular, the 75-μm Carboxen-PDMS fiber, peak tailing at the prevailing injector temperature. In EI, using total ion current (TIC), 3-octanone gave the highest response followed by 1-octen-3-ol, 2-hexanone, and 2-heptanone. 2-Methylpentane and the lower alcohols showed considerably lower intensity (2-methyl-1-propanol the lowest). In general, CI (TIC) gave higher peak intensities than EI, most noticeable for the lower alcohols (except 2-pentanol) (data not shown).

4.2. Fungi present in building materials

S. chartarum was isolated from both gypsum board samples and one of them also contained *Penicillium* spp. (only the *S. chartarum* strain isolated from the first-mentioned sample was available for analysis). *Chaetomium* spp., *Aspergillus* spp., and *Penicillium* spp. were isolated from the masonite, and *A. penicillioides*, *Penicillium* spp, and *Cladosporium* spp. were isolated from the mineral wool.

4.3. MVOCs from subcultivated molds

Subcultures of *S. chartarum*, *A. penicillioides*, and *C. globosum* were analyzed. Several of the reference MVOCs were identified in the chromatograms of the isolated fungi but not the agar blanks (Fig. 1). All molds produced 2-pentanol, 2-heptanone, 3-octanone, 1-octen-3-ol, and 2-methyl-1-butanol. Interestingly, 2-pentanol was only observed in EI mode and 2-methyl-1-butanol was found only in CI mode; the other compounds mentioned were detected both by CI and EI. In EI, but not in CI, it was necessary to focus at diagnostic ions for the individual metabolites. Other metabolic compounds not included among the reference MVOCs were also found but were not identified.

4.4. MVOCs in building materials

Extraction for 20 min (at 70 °C) provided the clearest chromatograms. Peaks obtained from the

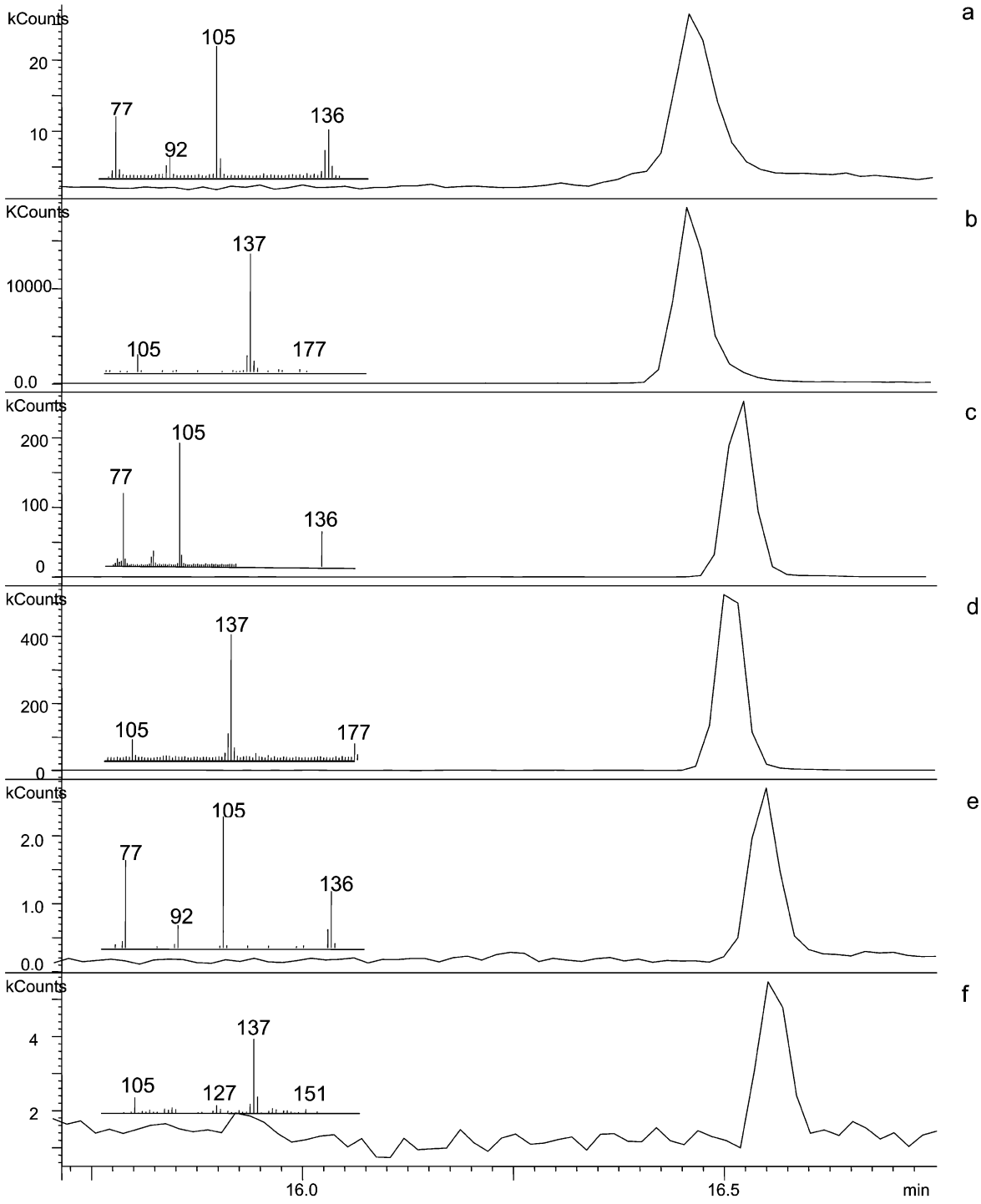

Fig. 1. Chromatograms with EI and CI mass spectra of methyl benzoate focussing at *m/z* 105 and 137, respectively: (i) authentic methyl benzoate diluted in water (a, b); (ii) methyl benzoate in a culture of *S. chartarum* (c, d); (iii) methyl benzoate from gypsum board culture-positive for *S. chartarum* (e, f).

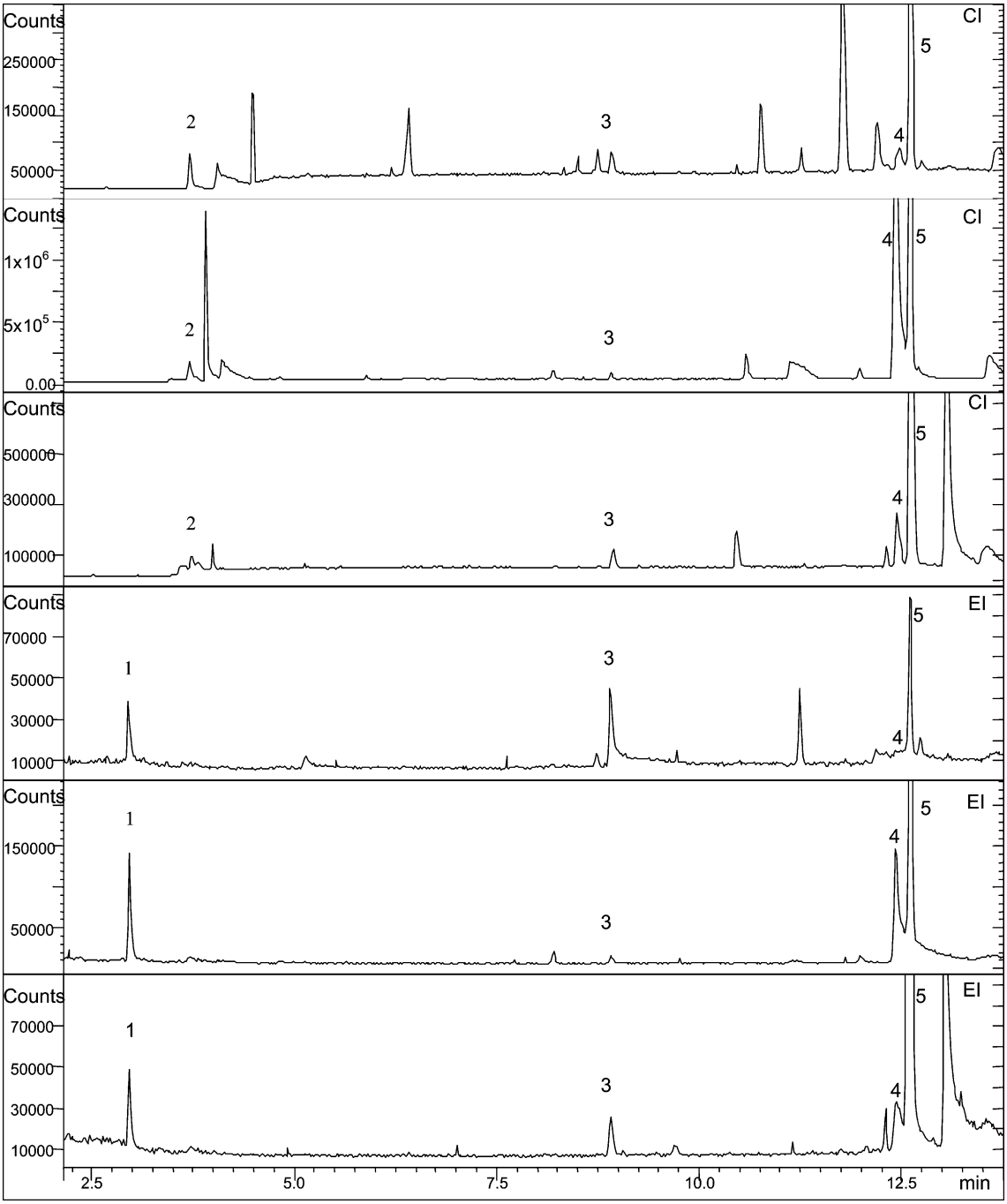

Fig. 2. SPME chromatograms showing MVOCs identified from the isolated fungal strains with both CI and EI modes: *S. chartarum* (upper), *A. penicillioides* (center), and *C. globosum* (lower). Key: (1) 2-pentanol, (2) 2-methyl-1-butanol, (3) 2-heptanone, (4) 1-octen-3-ol, and (5) 3-octanone.

building materials were compared with those of the standard MVOCs and of the cultivated fungi. The most affected gypsum board paper contained 1-octen-3-ol and a trace amount of 3-octanone; the latter was found also in the second gypsum board paper. Both of these samples also contained 2-hexanone and 2-heptanone; these were, however, also observed in the gypsum board blank samples. Both masonite and mineral wool contained 3-octanone, 2-hexanone, and 2-heptanone, although the latter two were found only in trace amounts in mineral wool. Blank samples were not available for mineral wool and masonite. Notably, the described MVOCs could be revealed in the building materials only when using CI mode (data not shown).

The chromatograms of the *Stachybotrys* strain contained a large peak eluting at approximately 16.5 min. Its CI spectrum was dominated by an ion of *m*/*z* 137 (M + 1) and its EI spectrum contained an abundant peak of *m*/*z* 105. By using extracted ion current profiling focussing at *m*/*z* 105 (EI) and *m*/*z* 137 (CI), this compound was also identified in *A. penicillioides*, although in much lower amounts than in *S. chartarum*, but not in *C. globosum*. In addition, it was found in the two mold-infested gypsum board samples (but not in the controls), in masonite and in mineral wool (trace amounts only). The mass spectral characteristics were found to correspond to methyl benzoate (Fig. 2). As shown, the retention time and mass spectra of authentic methyl benzoate are identical to those of the studied metabolite.

5. Discussion

Identification of MVOCs may be a useful method for detection and identification of microbial contamination (Korpi et al., 1987; Sunesson et al., 1996), but there exist no conclusive data on species-specific production (Wilkins et al., 2000; Fischer et al., 1999). Headspace-SPME represents an excellent, solventless analysis technique that has been applied to identify VOCs, e.g. in blood and viscera samples (Wolfram et al., 2001; Tranthim-Fryer et al., 2001), urine (Graham and Walker, 2000), and food (Elmore et al., 2000). Various types of commercially available SPME fibers are recommended for analytes of different volatility and polarity. Cross-linking makes the fiber solvent resistant and suitable for trace level analysis (Graham et al., 1999). Headspace-SPME, using 95-μm PDMS and 85-um polyacrylate fibers for manual injection, was previously applied to extract VOCs emitted from various *Penicillium* spp. Headspace vapors over a liquid culture of the molds were flushed through an SPME fiber for 30–50 min at room temperature followed by thermal desorption in the GC, and this technique was found to represent a valid, fast alternative to the conventional use of Tenax adsorption/desorption. 1-Octen-3-ol, 3-octanol, 2-methylisoborneol, geosmin, and 3-octanone were identified as fungal metabolites (Nilsson et al., 1996).

In the present study, different fibers designed for automated injection were compared as regards detection of 11 previously and frequently reported MVOCs (Wilkins et al., 2000; Elke et al., 1999; Fischer et al., 1999). Best performance was achieved with the 70-μm Carbowax-stableflex fiber. 3-Octanone and 1-octen-3-ol were detected with highest sensitivity and 2-methyl-1-propanol was detected with lowest sensitivity. Differences in the affinity between the fiber materials and the analytes due to their respective polarity, differences in the vapor pressures, and the analyte's differences in their signals in the MS could explain this. The use of Merlin microseals inlets is recommended for automated SPME injection.

The molds isolated from the materials studied in this investigation have all been encountered in previous indoor environmental research. *S. chartarum* and *C. globosum* have strong cellulolytic capacity which results in loss of wood stability. *S. chartarum* has been frequently isolated from damp building materials, especially wetted gypsum boards, whereas *C. globosum* may be found in cellulose containing materials, such as plant remains and papers (Korpi et al., 1999; Flannigan et al., 1991). A number of studies have identified *S. chartarum* and *A. versicolor* in damp and sick residences as linked to serious health conditions (Etzel et al., 1998; Hodgson et al., 1998). In the present study, several MVOCs of the studied strains were identified both with EI and CI. 2-Methyl-1-butanol, however, was identified only when using CI, and 2-pentanol was detected only when using EI. EI and CI should therefore be regarded as complimentary ionization methods.

Extraction at 70 °C/20 min was used when analyzing the MVOCs from building materials. Lower temperatures and shorter extraction times led to lower

sensitivity, and higher temperatures or longer extraction times did not reveal any new diagnostic peaks. In general, a high extraction temperature increases the rate of diffusion for the analytes at the fiber–gas interface but may also result in higher background (Cai et al., 2001). Several closely eluting compounds were found when EI mode was used that led to a high background; thus, none of the reference MVOCs was detected in the building materials when using EI. With CI, 1-octen-3-ol and small amounts of 3-octanone, 2-hexanone, and 2-heptanone were identified in the gypsum board papers; the latter three were also identified in masonite and mineral wool.

A striking finding in the present study was the identification of methyl benzoate in two of the three studied mold strains. *S. chartarum* produced this compound in abundance, whereas *A. penicillioides* produced only trace levels. In most published studies, MVOCs are collected at room temperature. This might not be relevant to detect methyl benzoate that has a much lower vapor pressure than many other MVOCs. In the present study, cultures were heated at 50 °C for collecting the volatiles. Methyl benzoate was also identified in the four used building materials, both in CI and EI modes. Previously, methyl benzoate was identified, in trace amount, as an emittant from *Stachybotrys* (Wilkins et al., 2000).

In conclusion, automated SPME combined with GC-MS is useful to identify MVOCs. The technique shows a great promise as a tool for detecting microbial contamination of building materials, and further work on this aspect is merited.

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