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Microbial Volatile Organic Compound Emission Rates and Exposure Model

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Key Words
Indoor air · Volatile organic compounds · Bio-contaminants · Total volatile organic compounds · Microbial volatile organic compounds · Emission rates

Abstract
This paper presents the results from a study that examined microbial volatile organic compound (MVOC) emissions from six fungi and one bacterial species (*Streptomyces* spp.) commonly found in indoor environments. Data are presented on peak emission rates from inoculated agar plates loaded with surface growth, ranging from 33.5 $\mu g \cdot m^{-2}$ per 24 h *(Cladosporium sphaerospermum)* to 515 $\mu g \cdot m^{-2}$ per 24 h *(Rhodotorula glutinis)*. Furthermore, changes in MVOC emission levels during the growth cycle of two of the micro-organisms are examined. This report also includes a calculation of the impact of MVOC emissions on indoor air quality in a typical house and an application of an exposure model used in a typical school environment.

Introduction

The presence of biocontaminants in heating, ventilation, and air-conditioning (HVAC) systems in indoor environments may be linked to sick building syndrome (SBS), although there is no consensus among indoor environment researchers as to what extent biocontaminants affect occupant health in indoor settings [1–3]. Microbial volatile organic compound (MVOC) emissions in indoor settings may trigger SBS symptoms, such as allergic reactions, e.g., irritated eyes, throat and skin [4]. Studies carried out in Canada [5] and Sweden [6] have speculated on the role of mould and MVOCs in increasing health risks for indoor occupants, yet a causative relationship remains unproven [7]. Gaining knowledge about MVOC occurrence in indoor settings is of particular importance for indoor air quality (IAQ) investigations, since MVOCs are produced during the active metabolism of micro-organisms and are released at varying emission rates [8]. Furthermore, MVOC detection and identification in field surveys may serve as an initial indication of biocontamination, even where it may not be readily identifiable by visual inspection of problem areas.

The purpose of this work was to determine, under laboratory conditions, the identity and amounts of MVOCs emitted by seven common indoor biocontaminants over a period of time in order to relate those emissions to the colonised surface area. This was done in an attempt to develop micro-organism-specific peak emission rates that may be used in conjunction with other IAQ variables (e.g., air exchange rates) to predict a biocontaminant's total contribution to total volatile organic compound (TVOC) levels in a known indoor space.

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Materials and Methods

Seven biocontaminants commonly found in indoor environments were selected for this study: six fungi (including a yeast) and an actinomycete: *Aspergillus glaucus, Aspergillus versicolor, Cladosporium sphaerospermum, Penicillium chrysogenum, Penicillium italicum, Rhodotorula glutinis,* and *Streptomyces* spp. The micro-organisms used were obtained from the Research Triangle Institute's culture collection (Research Triangle Park, N.C., USA).

An inoculum of approximately $5 \cdot 10^6$ colony-forming units was spread on a 100-mm glass dish containing a growth media. Tripticase soy agar (TSA) was used for *Streptomyces* spp., and Sabouraud dextrose agar (SDA) was used for the fungi. TSA was chosen for this investigation since it is the standard nutrient media for bacterial growth. However, fungal culture media that stimulate sporulation (e.g., cornmeal agar) were not used, since this investigation was focused only on MVOCs emitted by actively growing micro-organisms. SDA consists of glucose and peptone, and is one of the most widely used media for cultivating fungi [9]. All experiments described in this report were performed at room temperature in order to mimic typical building conditions. After inoculation, three dishes were placed inside an autoclaved glass chamber with an internal volume of 9 litres, and an airflow rate of 50 ± 5 standard cubic centimetres per minute ($scm^3 \cdot min^{-1}$) was established and maintained throughout the experiment. The air was preconditioned by being passed through a high efficiency particulate air filter, followed by a bed of activated carbon to remove any organic contaminants present. The air also was humidified to a relative humidity of 95% prior to its introduction into the chamber in order to prevent the desiccation of the inocula and/or the growth media. The micro-organisms were allowed to grow until they were mature (7–10 days) and confluent growth covered the surfaces of the dishes. All cultures were examined for contamination by other species throughout the test. The experiment was aborted and the culture discarded if any unwanted contamination was observed.

After confluent growth was observed in every chamber, the airflow inside the chambers was stopped for 24 h to allow the chamber headspace to become saturated with the volatile metabolites. The airflow was then restarted and the headspace sampled for 24 h using two glass tubes containing Tenax®-TA sorbent medium (total sampled volume was 72 litres). Sorbent tubes were placed in series to maximise MVOC collection efficiency; the detected MVOC mass trapped on both sorbent tubes was combined during data analysis to account for the possibility of sample breakthrough on a single sorbent tube. Sorbent tubes were capped and refrigerated at 4 °C until sample analysis.

The analytes adsorbed on the Tenax®-TA tubes were thermally desorbed at 250 °C into a gas chromatograph (GC) equipped with a flow splitter. One portion of the sample flow was separated on a DB-624 megabore column interfaced to a mass selective detector (MSD) for individual compound identification in the total ion chromatogram scan mode. The initial temperature of the GC oven was set to 20 °C for 5 min and ramped to 240 °C at a rate of $3\,°C \cdot min^{-1}$. Ultra-high purity (99.999%) helium was used as both desorption and carrier gas.

The second flow portion was analysed on a flame ionisation detector as toluene. Toluene is the preferred analytical surrogate in IAQ studies, and was used in this investigation to ensure consistency between the results presented in this article and the results of other indoor air researchers. A five-point calibration of compounds previously identified as MVOCs [1] was performed in triplicate with each set of headspace samples, along with the appropriate background and media blanks. Each micro-organism was investigated at least twice, using fresh cultures to ascertain the precision of the experimental results.

Table 1. MVOC Peak emission rates for seven common indoor biocontaminants

Biocontaminant	MVOC peak emission rate $\mu g \cdot m^{-2}$ per 24 h
A. glaucus	205.0
A. versicolor	63.7
C. sphaerospermum	33.5
P. chrysogenum	110.0
P. italicum	437.0
R. glutinis	515.0
Streptomyces spp.	81.5

Results

MVOC Peak Emission Rates

The measured emission rates in this investigation are peak emission rates, since they reflect single-day peak emissions from the tested micro-organisms. In a growth environment, fungi experience an exponential increase in biomass, followed by a period of time where biomass either remains constant or drops because of autolysis [10]. Thus the MVOC peak emission rate variations observed in this article may be a direct result of normal microbial metabolism. It was anticipated that observed emission variations between replicates would be significant. However, the actual MVOC peak emission rates were relatively consistent between replicates, possibly due to the 24-hour sampling time chosen for this investigation. Such long sampling may have minimised the influence of any short-time variations in MVOC emissions inside the chambers. The variation from the mean average for the test micro-organisms as a group was ±28%; the largest single variation was 76 and the lowest was 5.4%.

R. glutinis yielded the highest average peak emission rate throughout this experiment (515 $\mu g \cdot m^{-2}$ per 24 h), whereas *C. sphaerospermum* produced the lowest (33.5 $\mu g \cdot m^{-2}$ per 24 h). The average peak emission rate for all the micro-organisms studied was 206 ± 57.8 $\mu g \cdot m^{-2}$ per 24 h (table 1), based on a total colonised surface area of 1 m^2.

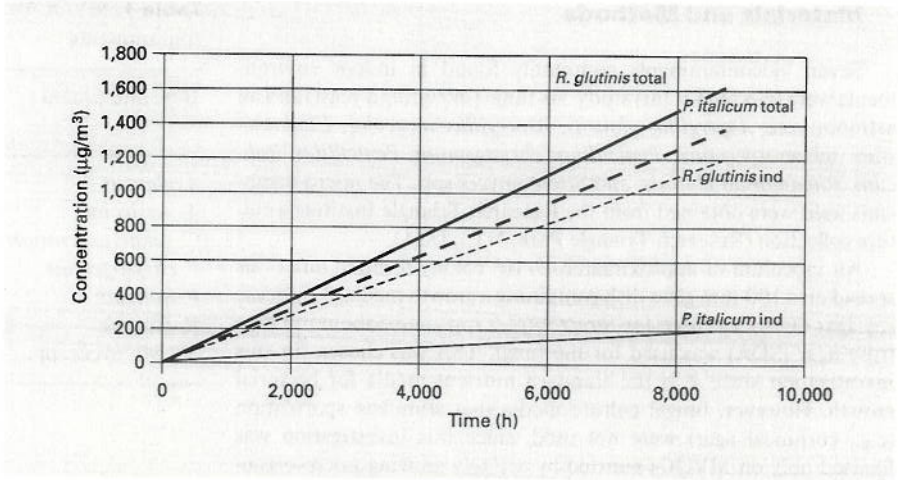

Fig. 1. Total and peak individual MVOC concentrations during scenario 1 (MVOC source present, HVAC on 24 h/day).

Table 2. Classification of MVOC classes from seven common indoor biocontaminants

Biocontaminant	Alcohols/ ketones	Aldehydes	Hydro-carbons	Hetero-compounds	Sequi-terpenes
A. glaucus	88.4	9.8	1.3	ND	0.1
A. versicolor	30.4	52.0	ND	1.7	ND
C. sphaerospermum	5.1	30.6	9.9	ND	21.8
P. chrysogenum	43.2	10.1	7.4	0.5	ND
P. italicum	34.5	4.1	9.8	0.9	37.8
R. glutinis	96.7	0.2	ND	0.6	0.3
Streptomyces spp.	21.8	26.4	3.1	35.6	ND

All figures are percentages of emissions.
ND = Not detected.

Different experiments were performed to evaluate peak emission rate variations over the life of the biocontaminant colonies. New dishes inoculated with *P. chrysogenum* and *Streptomyces* spp. were introduced into the glass chambers, followed by headspace collections as described above. Headspace samples were collected when the colonies were new (2–4 days), matured (6–14 days), and middle-aged (14–18 days). MVOC emissions increased to a maximum at maturity (fig. 1). However, in a real world situation, the periodic formation of new colonies and the subsequent overlap of their life cycles may mask the trends observed in this investigation.

MVOC Identification and Classification

Many researchers [11–13] have attempted to identify an MVOC indoor biocontaminant marker, and for this reason an attempt was made to identify every compound found in the headspace of the growth chamber. Individual MVOCs that could not be positively identified were classified according to their functional group (e.g., terpene, aldehyde), as denoted by their mass spectral fragmentation patterns. In those cases where hydrocarbons were identified, it was considered sufficient to classify branched alkanes as such. Except for *P. italicum,* the primary MVOC emissions from each micro-organism were alcohols and ketones (table 2, 3), confirming results reported in the literature [14–16]. Percentage emissions were calculated by dividing the mass concentration of each MVOC by the total mass quantified from each biocontaminant after background subtraction. Alcohols and ketones, as well as reduced sulphur compounds (e.g., dimethyl disulphide, dimethyl trisulphide), have been previously identified as odiferous compounds associated with biocontaminated indoor environments [17–19]. These compounds account for 32–93% of the total MVOC emissions found in individual samples. The re-

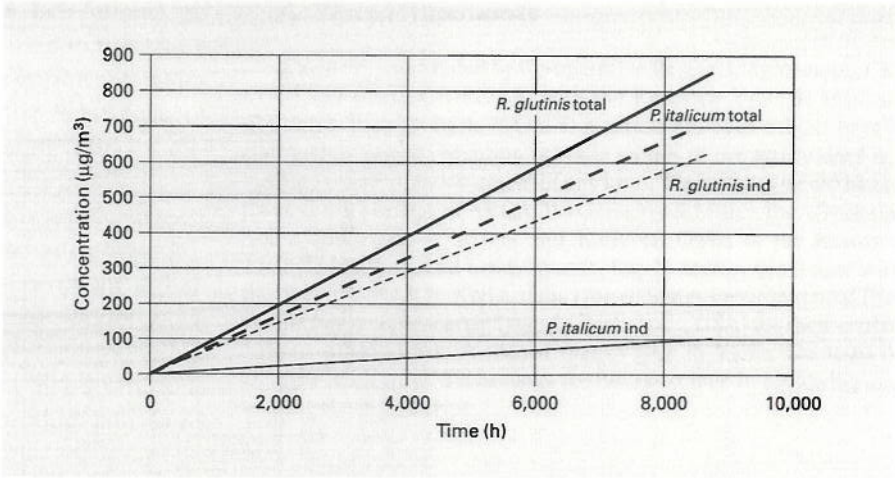

Fig. 2. Total and peak individual MVOC concentrations during scenario 2 (MVOC source present, HVAC on 08:00–17:00 h).

duced sulphur compounds are classified as heterocompounds in this article, which also include the nitrogen-containing compounds identified throughout the experiment (e.g., 2-acetylthiazole, methylpyrazine). Predominant emissions from *P. italicum* were identified as sesquiterpenes, consistent with the many sesquiterpene compounds identified by Fischer et al. [20], Larsen and Frisvad [21] and Ezeonu et al. [22]. The identification of α-humulene (table 3) in the headspace of *C. sphaerospermum* suggests its possible role as a microbe-specific growth indicator, yet the emission of this sesquiterpene by *C. sphaerospermum* growing on building materials needs to be ascertained before it is identified as such.

Use of an IAQ Model to Predict the Impact on IAQ

To understand the implications of the emission rates measured in these experiments better, an IAQ model was used to estimate concentration and potential exposure, as has been done with other indoor air contaminants. The goal was to estimate the potential indoor concentration levels by using a typical emission rate from these experiments and reasonable ventilation parameters. The model used was RISK IAQ Model for Windows, which is a completely mixed-room model incorporating source/sink behaviour that can generate concentration and exposure estimates as functions of time [23]. The ventilation flows and pollutant emission rates can be set as desired for each modelled room.

For this study, a school was modelled with 10 rooms distributed over a floor area of 371.6 m^2, 2.44-meter ceilings and served by one HVAC system. The total HVAC airflow to the area was 56.6 $m^3 \cdot min^{-1}$ (2,000 cfm). The school was assigned three separate HVAC scenarios: (1) MVOC source with the HVAC system always on and no outdoor air (fig. 1); (2) MVOC source with the HVAC system on from 08:00 to 17:00 h, 9-hour duty cycle, and no outdoor air (fig. 2), and (3) the same 9-hour duty cycle as scenario 2, with the addition of an outdoor air intake level of 5% of the recirculating airflow to the HVAC sys-

Table 3. Predominant compound identifications and percent emissions by weight for seven common indoor biocontaminants

Biocontaminant	Identified MVOCs	Emissions
A. glaucus	3-octanone	46.8
	1-octen-3-ol	20.2
	2,4-pentadione	6.8
A. versicolor	1,3-dimethoxybenzene	46.9
	1-octen-3-ol	28.5
	3-octanol	1.8
C. sphaerospermum	?-humulene[1]	13.0
	tetramethyl tetrahydrofuran	10.9
	α-humulene	8.1
P. chrysogenum	1-octen-3-ol	30.2
	3-octanone	7.3
	tetradecene	3.5
P. italicum	cedrene	20.4
	methyl butenone	14.9
	cedrol	14.5
R. glutinis	3-methyl-2-butanol	74.3
	phenyl ethyl alcohol	18.4
	2-methyl-l-propanol	2.8
Streptomyces spp.	dimethyl disulfide	24.5
	bornyl methyl ether	20.0

Figures are percent emissions by weight.
[1] Partial identification.

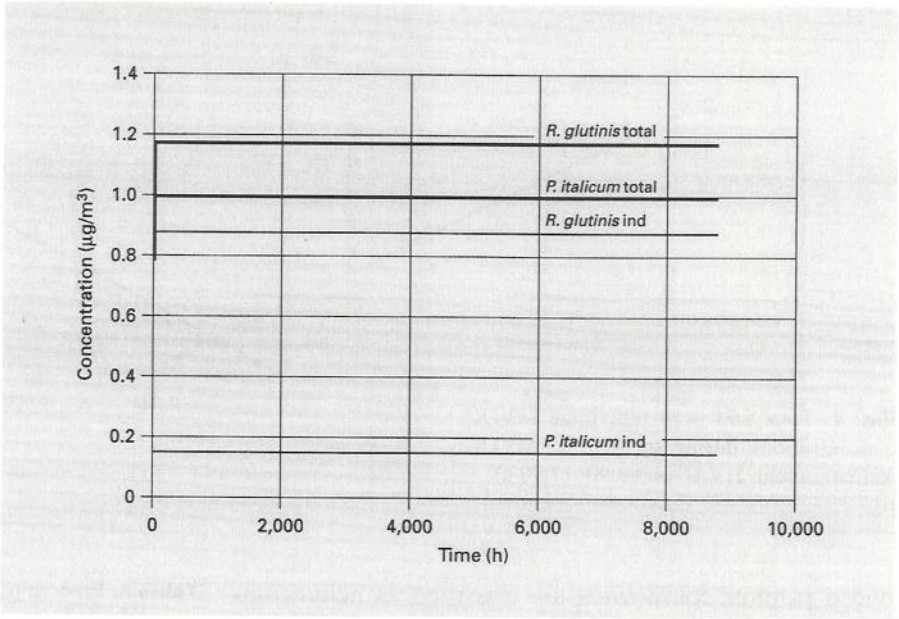

Fig. 3. Total and peak individual MVOC concentrations during scenario 3 (MVOC source present, HVAC on 08:00–17:00 h and taking in 5% outdoor air).

Table 4. Estimated impact of biocontaminant MVOCs on indoor air by microorganisms

Biocontaminant	Calculated MVOC contribution concentration, $ng \cdot m^{-3}$
A. glaucus	29.5
A. versicolor	94.9
C. sphaerospermum	15.5
P. chrysogenum	50.9
P. italicum	202.0
R. glutinis	238.0
Streptomyces spp.	37.7
Biocontaminant average	95.5

tem, as is the case in a typical school (fig. 3). The source of MVOC emissions was calculated from an area of mould contamination of 9.29 m^2 (100 ft^2), selected as representative of comparable emission rates.

Concentration profiles were run, and the exposure levels were calculated for an average person (breathing at 0.83 $m^3 \cdot h^{-1}$ and, for simplicity, staying in the room 24 h a day) for all scenarios. Various surfaces within a building, as well as components of a HVAC system may support microbial growth. The area of contamination was located within the school building, which was served by the HVAC system as defined by each of the three scenarios, modelled, and the results are shown in figure 1–3. The levels of total MVOC and the peak individual MVOC for *P. italicum* and *R. glutinis* were modelled in figure 1–3, based on emission data taken from table 3, 4. The MVOC concentrations were greatest in scenario 1 (fig. 1), in which total MVOC concentrations exceeded 1,500 $\mu g \cdot m^{-3}$ for *P. italicum*. When HVAC operation was limited to 9 h/day (scenario 2; fig. 2) MVOC concentrations decreased to almost half of those shown in figure 1. When 5% of the recycled air is outdoor air and the HVAC system remains on (scenario 3; fig. 3), the results indicate levels below 1.2 $\mu g \cdot m^{-3}$, a low concentration compared to scenarios 1 and 2. The results from the modelling exercises are fairly different, exemplifying the need and benefits of providing ventilation air.

Initial emission modelling for the different scenarios demonstrated concentrations of MVOCs similar to those reported in the literature for known problem buildings. These results, while preliminary, suggest that, once microbial emissions are well enough understood, models may be useful in predicting exposure to and eventual risk from individual micro-organisms. The emission rates, when used in RISK, produced room concentrations consistent with field observations reported from the literature.

Discussion

Volatile metabolic by-products from seven common indoor biocontaminants have been studied in an attempt to establish MVOC peak emission rates from each indi-

vidual micro-organism and to evaluate their impact on overall IAQ. Many of the MVOCs identified in this study have been reported; however, identification of α-humulene and ?-humulene (table 3) in the headspace of *C. sphaerospermum* suggests their possible role as microbe-specific growth indicators.

The modelling results presented in this article suggest that, as more MVOC emissions are identified and quantified, modelling might serve in the future as an aid in assessing the impact MVOCs may have on indoor environments. Modelling provides field investigators with a tool that can be used to assess the potentially deleterious effect of microbiological metabolic by-product emissions to overall IAQ.

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References

1 Bayer CW, Crow SA, Noble JA: Productions of volatile emissions by fungi; in Proc Conf IAQ 1995, Denver, vol 1, pp 101–109.
2 Miller JD: Fungi and the building engineer; Environments for Healthy People; in Proc Conf IAQ 1992, San Francisco. Atlanta, ASHRAE, 1992, pp 147–159.
3 Samson RA, Flannigan B, Flannigan MF, Verhoeff AP, Adan OCG, Hoekstra ES: Health Implications of Fungi in Indoor Environments. Elsevier, Amsterdam, 1994.
4 Wessén B, Schoeps KO: Microbial volatile organic compounds: What substances can be found in sick buildings? Analyst 1995;121: 1203–1205.
5 Lawton MD, Taylor RF: Moldy houses: Why they are and why we care. Final report to Canada Mortgage and Housing, Research Division, Report No. 1952070.00. Ottawa, Morrison Hershfield Consulting Engineers, 1995.
6 Ström G, West J, Wessén B, Palmgrem U: Health Implications of Fungi in Indoor Environments. Amsterdam, Elsevier, 1994.
7 Batterman SA: Sampling and Analysis of biological volatile organic compounds; in Burge HA (ed): Bioaerosols. Boca Raton, Lewis, 1995.
8 Korpi A, Kasanen JK, Alarie Y, Kosma VM, Pasanen AL: Sensory irritation potency of some microbial volatile organic compounds (MVOCS) and a mixture of five MVOCS. Arch Environ Health 1999;54:347–352.
9 Davis BD, Dulbecco R, Eisen HN, Ginsberg HS, Wood WB: Microbiology. New York, Harper & Row, 1967.
10 Cooke R, Whipps J: Ecophysiology of Fungi. Oxford, Blackwell Scientific Publications, 1993.
11 Bjurman J, Nordstrand E, Kristensson J: Growth-phase-related production of potential volatile organic tracer compounds by moulds on wood. Indoor Air 1997;7:2–7.
12 Pasanen AL, Korpi A, Kasanen JP, Pasanen P: Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. Environ Int 1998;24:703–712.
13 Sunesson AL, Vaes WJ, Nilsson CA, Blomquist G, Andersson B, Carlson R: Identification of volatile metabolites from five fungal species cultivated on two media. Appl Environ Microbiol 1995;61:2911–2918.
14 Elke K, Begerow J, Oppermann H, Kramer U, Jermann E, Dunemann L: Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID – A new feasible approach for the detection of an exposure to indoor mould fungi? J Environ Monit 1999;1:445–452.
15 Homer WE, Morey PR, Worthan AG: Microbial VOC sampling in a mouldy building investigation; in Proc Eng Solutions Indoor Air Qual Probl. Research Triangle Park, 1997, pp 593–601.
16 Schleibinger HW, Böck R, Möritz M, Richter A, Rüden H: MVOC produced by micro-organisms growing on different air filter media under different climatic conditions; in Proc Healthy Build IAQ 1997. Bethesda, 1997, pp 575–580.
17 Korpi A, Pasanen AL, Pasanen P: Volatile compounds originating from mixed microbial cultures on building materials under various humidity conditions. Appl Environ Microbiol 1998;64:2914–2919.
18 Wessén B, Ström G, Schoeps KO: Indoor Air – An Integrated Approach. Amsterdam, Elsevier, 1995.
19 Wilkins K, Larsen K, Simkus M: Volatile metabolites from mould growth on building materials and synthetic media. Chemosphere 2000; 41:437–446.
20 Fischer G, Schwalbe R, Moller M, Ostrowski R, Dott W: Species-specific production of microbial volatile organic compounds (MVOCs) by airborne fungi from a compost facility. Chemosphere 1999;39:795–810.
21 Larsen TO, Frisvad JC: Characterization of volatile metabolites from 47 Penicillium taxa. Mycol Res 1997;99:1153–1166.
22 Ezeonu IM, Price DL, Simmons RB, Crow SA, Ahearn DG: Fungal production of volatiles during growth on fiberglass. Appl Environ Microbiol 1994;60:4172–4173.
23 Sparks LE: IAQ Model for Windows, RISK Version: 1.0, User Manual, EPA-600/R-96037 (NTIS PB96–501929), Research Triangle Park, 1996.