INVESTIGATING THE INFLUENCE OF RELATIVE HUMIDITY, AIR VELOCITY AND AMPLIFICATION ON THE EMISSION RATES OF FUNGAL SPORES

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ABSTRACT

Although a significant amount of work has been done to elucidate the conditions under which fungi will grow on the surfaces of materials, little information is available that quantitatively relates surface concentrations to airborne concentration and, ultimately, exposure. This paper discusses the impact of relative humidity (RH), air velocity, and surface growth on the emission rates of fungal spores from the surface of contaminated material. Although the results show a complex interaction of factors, we have determined, for this limited data set, that emission rates are inversely proportional to RH, but directly related to air flow rate and surface loading.

KEY WORDS

Bioaerosol, chamber study, duct, emissions, microbial contamination

INTRODUCTION

Progress has been made in establishing the conditions in which greater or lesser amounts of fungal growth will occur on surfaces. However, information is lacking that allows us to relate surface levels of fungal contamination to potential respiratory exposure. Quantitative estimates of the actual impact on the indoor environment (e.g., the significance of 100 cm^2 of *Aspergillus versicolor* growing on a wall in an office or a building ventilation duct) have not been possible because there is little information on the dynamics of biocontaminant release from contaminated surfaces. Further, the emission rates and mechanisms for fungal dissemination are poorly understood. Data are needed that quantitatively relate fungal spore emissions from contaminated materials to airborne concentrations indoors, and subsequently to occupant exposure. The quantitative relationship between surface contamination and the concentration of fungal spores in the air has not been determined, nor has the effect of that concentration on occupants. An improved understanding is required of the factors that enable and/or promote emissions, such as activity (translation energy), air flow, and relative humidity (RH).

A series of well-controlled, dynamic chamber studies using miniducts have been undertaken to investigate the impact of RH, air velocity, and amplification on fungal emissions. The two factors that were anticipated to have the most impact on emissions were air velocity and RH. RH controls the release of spores by some fungi [1,2]. High humidity is important for those fungi whose

active spore release mechanisms depend on the rupture of turgid cells, while tissue desiccation in low humidity is important for other types of fungi with another class of spore release mechanisms. The velocity of air flowing over fungal colonies is known to be important to dissemination both as a source of energy for liberation and to enhance mass transport for humidity-driven processes.

In previous experiments, we reported that fungal emissions were influenced by complex interactions among RH, surface growth, and material characteristics [3]. RH had a tremendous impact on the dissemination of both *Penicillium* and *Aspergillus* spores. Under the high RH conditions where growth occurred, there was little or no release of spores. However, as the RH was lowered, spore release was triggered. While the data are limited to these few cases, the potential impact on current control and remediation practices is profound. Controlling uncontrolled humidity by lowering RH is almost always a recommended practice. These data pointed out that during remediation this strategy could lead to increased short-term emissions.

This paper presents data that begin to investigate the relationship between increasing surface growth (loading) in the early stages of contamination and spore emission rates, and then extends the previously reported results on RH for stable populations to include air velocity as an additional parameter. The objectives of these experiments were to: 1) initiate experiments relating surface load or concentration (for new growth) to the emission of fungal spores, and 2) measure the impact of air velocity at a variety of RHs. A ventilation duct was chosen as a specific example of a potentially contaminated site to initiate these studies. Culturable fungal spore emissions were measured.

METHODS AND MATERIALS

Experimental Apparatus for Dynamic Experiments

The experiments were conducted in the Dynamic Microbial Test Chamber (DMTC) [4]. The DMTC is a room-sized test facility designed and constructed to conduct studies on the conditions and factors that influence biocontaminant emissions and dissemination. The chamber, a cube with inside dimensions of 2.44 m, was constructed with stainless steel walls and floor and an acrylic drop-in ceiling. Temperature (18 - 32°C) and RH (55 to 95%) control are provided through an air handler unit (AHU) with an air circulation rate of 1.4 to 4.8 m^3/min.

The chamber was adapted to contain eight miniducts [3]. An artist's rendition of the DMTC containing the miniduct apparatus is shown in Figure 1. The blower forces the conditioned DMTC air into a High Efficiency Particulate Air (HEPA) filter, from which the air for the eight miniducts is obtained. The channel design was chosen for the miniducts to limit the total amount of air required for a single test, allowing multiple tests to be run simultaneously, and simulate flow conditions in a heating, ventilating, and air-conditioning

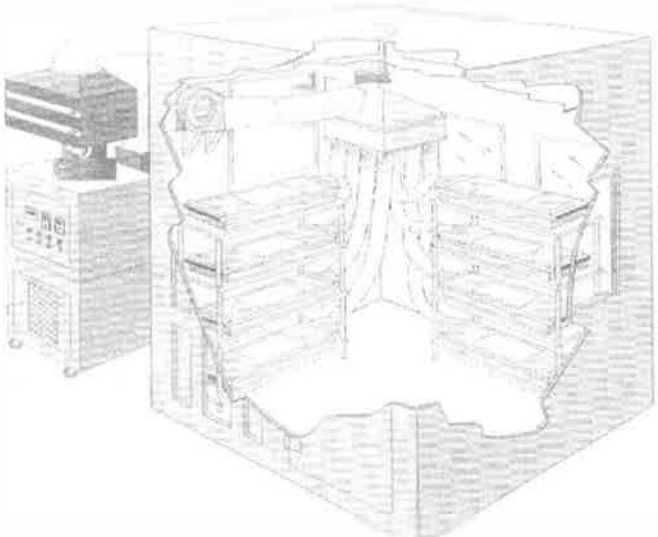

Figure 1. Artist's rendition of DMTC.

(HVAC) duct

Duct Materials Tested

Three newly purchased (from local commercial vendors) common duct materials were studied: two fiberglass duct liners (FDL-A and FDL-B) and one fiberglass ductboard (FGD). The compositions of the fiberglass materials compiled from the Material Safety Data Sheets have been reported previously [3]. Both FDL-A and FDL-B were nominally 2.5 cm thick, and were classed as 24.0 kg/m^3 (1.5 lb/ft^3) in density. In appearance, these duct liners were very similar, with an uncoated surface intended to be attached to a rigid duct material and a polymer coated surface intended to be in contact with the moving air in the duct. FDL-B contained a permanent (bound) antimicrobial in the coating of the airstream surface. The FGD was classed as a 72 kg/m^3 (4.5 lb/ft^3) material, with a reinforced foil outer coating and a dense but uncoated duct interior surface.

Selection of Test Organisms and Inoculation

Penicillium chrysogenum and *Aspergillus versicolor* were selected as the test organisms for these studies. *P. chrysogenum* has been reported as one of the most frequently isolated molds from the air, housedust, and surfaces of indoor environments, and has been isolated from a number of air-conditioning systems in environments where patients were suffering from allergic disease. *A. versicolor* was selected because its growth on fiberglass materials is well documented [5], and because it is a toxigenic fungus. The data for the two test organisms, *P. chrysogenum* and *A. versicolor*, are reported separately.

The particular *P. chrysogenum* strain selected for these studies was isolated from a contaminated building material by RTI and cultivated for use in the laboratory. The culture is being maintained in the University of Texas Medical Branch Fungus Culture Collection as UTMB3491. The *A. versicolor* was isolated from contaminated ductliner. The organisms were prepared for inoculation as previously described [3].

Artificial Soiling of Duct Materials

The materials were artificially soiled first, and then inoculated, in an aerosol deposition chamber as described previously [3]. Sieved (250 μm) duct dust obtained from a local duct cleaner was used to soil the samples. The targeted amount of dust for deposition was approximately 100 mg dust / 100 cm^2. This level has been considered moderately soiled in previous experiments and was selected to relate these data to previous experimental results. All of the artificially soiled material pieces were autoclaved after soiling but before inoculation on a short cycle for better adhesion of the HVAC dust. The short autoclave cycle, though not sufficient for sterilization, significantly reduced the normal flora.

Experimental Procedure

After artificial soiling and inoculation, the 30.5 x 91.4 cm (1 x 3 ft) pieces of test material were placed in the miniducts. The temperature and RH throughout the miniducts were maintained at 23.5°C and 94%, respectively. The air velocity through the miniducts was 2.5 m/s (500 ft/min). At the start of an emission rate determination, the chamber RH was lowered from 94% to the test RH (i.e., 64%) by lowering the RH setting on the AHU. The RH change was effective within minutes. Surface sample and air sample collection were performed as described previously [3]. Isokinetic, 1 hour air samples were collected using the Mattson-Garvin slit to agar sampler for culturable bioaerosols. The sampler was turned on immediately. After 1 hour, it turned off automatically. The 150-mm agar plate was changed and the sampler restarted. The sequence was

repeated sequentially for six 1-hour samples. At the completion of each sixth hour sample, chamber RH was returned to the maintenance RH of 94%. No experiments were performed within 2 days of any other to allow the surface of the materials to recover from the decreased RH and to make certain the starting point for all emission measurements was the same. Surface samples were collected at weekly intervals at least 1 day after the bioaerosol sample was collected. The sampler plates were incubated at room temperature. Colony-forming units (CFUs) were counted shortly after visible growth was first noted and again as moderate growth became apparent.

Calculation of Emission Rates

To calculate the emission rates for the organisms, the CFUs on the sampler plates were identified and enumerated, and the CFUs/min were determined. The value was adjusted for the total flow rate, and divided by the area of the emitting surface.

RESULTS AND DISCUSSION

Emissions and Surface Concentration

Figure 2 shows the results of an 8-week experiment to investigate and quantitate the impact of surface load during log-phase growth on the emission rates of fungal spores from the surface of the duct material using *A. versicolor* as the test organism. The test material, FDL-B, was inoculated with two different concentrations of *A. versicolor*. Emissions were measured at 64% RH.

The columns represent surface growth and are referenced to the left axis as Log_{10} CFU/10 cm^2. The two levels of inoculum used are denoted as lower and higher. The lower inoculum was approximately 10^2 CFU/10 cm^2, and the higher inoculum was approximately 10^3 CFU/10 cm^2. Error bars are included only for weeks 0 and 1 to show that no significant difference was measured between the levels of surface growth after the initial inoculation (day 0). The lines represent the spore emission results and are plotted on a log scale against the right axis as CFU/m^2/min.

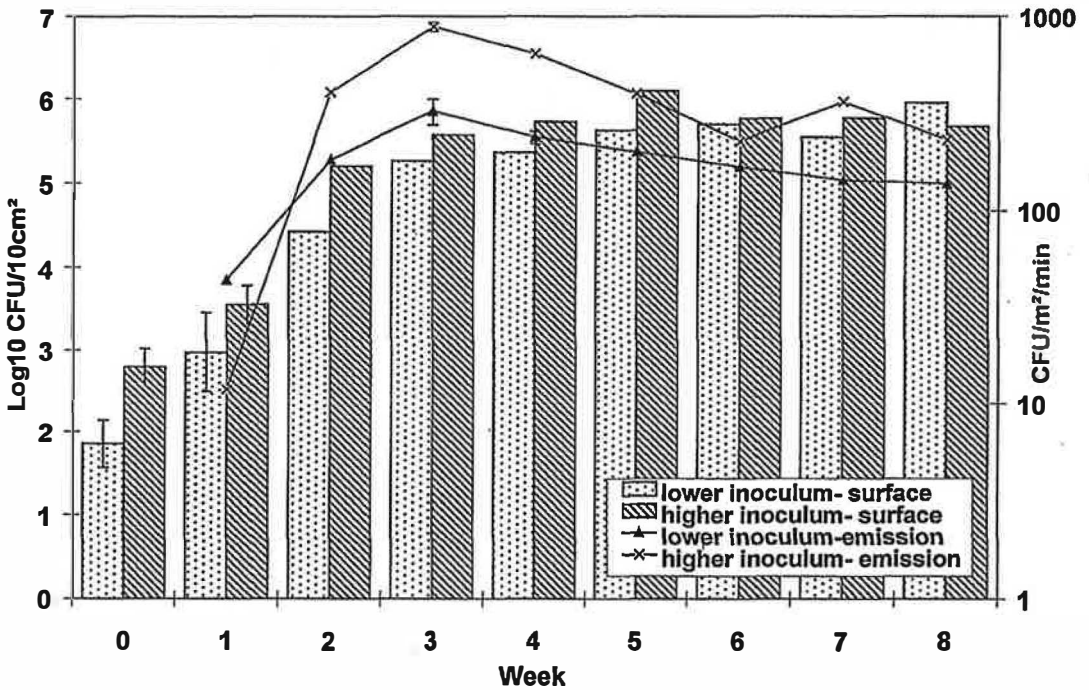

Figure 2 Surface growth vs. spore emissions for *A. versicolor* at lower and higher inoculum levels at 64% RH.

None of the differences between the emission rates were significant except at week 3 (error bars shown only for week 3). When comparing the surface and the emission data, it is apparent, for *A. versicolor* under these conditions, that surface load directly influences the emission rate. In other words as the surface concentration of organisms increased during log-phase growth, the emission rate increased until a stable population was reached and then the emissions appeared to remain essentially constant.

Air Velocity and RH

Tables 1 and 2 present the first hour emission rates for *P. chrysogenum* and *A. versicolor*, respectively. The surface concentration was monitored to document that a stable population of organisms had been established on the surfaces of all the materials for at least 12 weeks prior to collection of emission data.

The first column lists the three different materials tested, and the second column lists the four RHs at which the emissions were measured. The final four columns show the emission rates for the two test organisms at the four air flows used in the study. In a number of cases (e.g, FDL-B, 64% RH at 2.5 m^2/s), multiple values, separated

Table 1. Emission (CFU/m^2/min) of *P. chrysogenum* spores at four different air flow rates and four different RHs.

MATERIAL	RH, %	AIR FLOW, m/sec.			
		0.5	1.0	1.5	2.5
FDL-B	64	9	13	409	804; 2023; 1249
	70	5; 1	28	228	972; 1480
	84	1	1	59	150
	94	2	5	124; 127	81
FDL-A	64	610	155	465	813; 2392; 1501
	70	27; 7	30	229	710; 1385
	84	0	25	234	287
	94	26	7	145; 75	69
FGD	64	13	20	88	333; 854; 267
	70	8;1	6	17	335; 162
	84	1	0	50	12
	94	17	0	0	47

Table 2. Emission (CFU/m^2/min) of *A. versicolor* spores at four different air flow rates and four different RHs.

MATERIAL	RH, %	AIR FLOW, m/sec.			
		0.5	1	1.5	2.5
FDL-B	64	1	11	45	170; 100; 190
	70	0; 1	1	25	60; 20
	84	0	1	0	0
	94	2	0	0; 0	11
FDL-A	64	24	44	52	43; 15
	70	11; 2	26	17	8; 14
	84	1	0	10	3
	94	0	0	0; 7	20
FGD	64	0	4	138	567; 151; 543
	70	0; 0	5	66	242; 182
	84	1	0	0	22
	94	2	0	0; 0	0

by a semicolon, are reported. These values are replicate measurements from different experiments. Overall, the variability is within normal bounds for this sort of measurement.

As shown previously, the emission rates were inversely related to the RH at air flows of 2.5 m/s. In other words, as the RH decreased, the emission rate increased. The current experiment generally confirms that relationship at the lower air flow rates. However, once the emission rate becomes too low, the natural variability overwhelms our ability to measure a difference.

When the data from the different air flows are compared, it can be seen that the emission rate is related to the flow rate across the contaminated surface. As the flow rate increases, so do the emission rates. This was seen at all four humidities included in the study. Overall, the emission rates were notably lower for the *A. versicolor* than the *P. chrysogenum* regardless of RH or flow.

While additional research will be required to relate these data to exposure, the emission rates measured in these experiments support those measured in our previous paper [3]. In that paper we employed an indoor air quality (IAQ) Model (RISK) to estimate concentration and occupant exposure to better understand the implications of the measured spore emission rates on IAQ. The emission rates, when used in RISK, produced room concentrations consistent with field observations reported from the literature.

CONCLUSIONS

As a first step in attempting to quantitatively relate surface concentrations of fungal growth to potential respiratory exposure, we initiated a series of experiments to help elucidate the relationship between fungal growth on surfaces and the emission rates of fungal spores from those surfaces. The results show a complex interaction of factors. For a limited data set, we have determined that emission rates are inversely proportional to RH, but directly related to air flow and surface loading.

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