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Testing Antimicrobial Efficacy on Porous Materials

M.Y. Menetrez[a] K.K. Foarde[b] V.R. De Jesus[a]

[a]US Environmental Protection Agency, and [b]Research Triangle Institute,
Center for Engineering and Environmental Sciences, Research Triangle Park, N.C., USA

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Abstract
The efficacy of antimicrobial treatments to eliminate or control biological growth in the indoor environment can easily be tested on non-porous surfaces. However, the testing of antimicrobial efficacy on porous surfaces, such as those found in the indoor environment (i.e., gypsum board, heating, ventilating and air-conditioning duct-liner insulation, and wood products) can be more complicated and prone to incorrect conclusions regarding residual organisms and non-viable allergens. Research to control biological growth using three separate antimicrobial encapsulants on contaminated duct-liner insulation has been performed in both field and laboratory testing. The results indicate differences in antimicrobial efficacy for the period of testing.

Introduction

The constant use of heating, ventilating, and air-conditioning (HVAC) systems can cause the adherence of dirt and possible hazardous substances to the surface of the components and duct lines [1]. The degree of surface dirt and debris, as well as the environmental conditions involving HVAC system operation and maintenance which can introduce moisture, may initiate the active growth of microbial organisms [2]. Exposure to the spores and metabolites of these organisms can cause significant health consequences, especially to individuals with pulmonary disease [3]. The installation of interior fibreglass duct liner (FGDL) in HVAC systems is a common industry practice. Simple cleaning of porous materials such as FGDL as part of building remediation has been shown to be ineffective compared to replacing FGDL [4–7]. In addition, the physical abrasions caused by cleaning, FGDL deterioration due to improper installation, and operation and maintenance can cause porous materials to become frayed and to lose fibre into the passing air stream. Encapsulation of the FGDL surface can potentially add structural support to the FGDL surface by bonding the surface fibres to the surface with encapsulating product. Fibre shedding and remaining biological contamination can potentially be withheld from passing into the air stream, and regrowth of contaminants is inhibited.

These polymer coatings are applied by spray or trowel to the duct surface as a thick liquid or mastic compound. There is little information regarding the efficacy of field-applied antimicrobial encapsulants. Laboratory testing of antimicrobial treatments allows increased experimental control and a variety of test conditions unattainable in the field. The research described below uses both field and laboratory testing of antimicrobial encapsulant efficacy.

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Dr. M.Y. Menetrez, US Environmental Protection Agency
Office of Research and Development, National Risk Management Research Laboratory
Air Pollution Prevention and Control Division
Research Triangle Park, NC 27711 (USA)
Tel. +1 919 541 7981, Fax +1 919 541 2157, E-Mail menetrez.marc@epa.gov

Increasing our understanding of indoor air biocontaminants and developing prevention and control techniques for mitigation of these pollutants can be accomplished by evaluating the factors controlling growth and emissions of indoor biocontaminants, developing standard methods for assessment of material susceptibility to microbial growth, and evaluating the effectiveness of prevention and control techniques. Laboratory experiments provide a controlled environment so that reproducible data can be generated, and critical parameters that promote or discourage the germination, growth, dispersion, and death of indoor biocontaminants can be isolated and identified. The critical parameters identified provide a scientific base for evaluation and development of effective prevention and control techniques to reduce the risk of exposure to indoor biocontaminants.

Under a co-operative agreement, Research Triangle Institute is using a static chamber test method to evaluate the impact of moisture, soiling, and temperature on fungal growth potential of FGDL. FGDL is commonly used in both residential and commercial HVAC systems to provide the needed thermal insulation and noise control. Although the popularity of exterior insulation is growing, the use of interior FGDL in residential and commercial buildings is common. Many building investigations have documented biocontamination of such materials, and the appropriateness of their use in high humidity locations has come into question.

A series of experiments were conducted in static environmental chambers to assess some of the conditions that may impact the ability of a variety of FGDL materials to support the growth of fungus. The outputs from this project included: (1) fungal growth data on various FGDL, and (2) test methods to measure the fungal resistance of FGDL. Critical parameters determining the fungal growth on FGDL can be identified by analysing the fungal growth data. Pollution prevention and mitigation techniques and guidelines can be developed by controlling those critical parameters. The test methods can be used by duct-material manufacturers to assist the development of fungal-resistant products. Both static and dynamic chamber experiments were used with new and used building materials both with and without antimicrobial treatment and encapsulents/sealants.

This paper discusses the evaluation of the effect of antimicrobial encapsulants on microbial growth in both laboratory and field investigations of contaminated FGDL taken from the test house. The evaluation of the efficacy was conducted on three commercially available products (coating I was a polyacrylate co-polymer containing zinc oxide and borates, coating II was an acrylic coating containing decabromodiphenyl oxide and antimony trioxide, and coating III was an acrylic primer containing a phosphated quaternary amine complex). The FGDL was cleaned and coated according to manufacturer's specifications with three antimicrobial encapsulants by members of the National Air Duct Cleaners Association. This paper summarises many of the procedures which have been detailed by Foarde et al. [8].

Materials and Methods

Field Study

The field research conducted at the EPA research house involved: (1) sample collection, pre-cleaning, and residential heating and air-conditioning (HAC) system evaluation; (2) HAC duct liner repair and replacement, cleaning, and encapsulant coating; and (3) HAC system operation and sample collection for an 18 month period.

The EPA test house is a 20-year-old, 1,300 ft^2 (~ 121 m^2) single-story ranch, with a centrally located vertically mounted air handler containing cooling and gas heating. An assessment of the house for areas of excess moisture was performed by visual inspection and use of a conductive moisture meter. The only area in the house that was found to contain unwanted excessive moisture was the HAC system internal duct insulation [9]. Samples were collected for microbial analysis using a high-volume small surface sampler [10]. Vacuum samples of 10-cm^2 surface areas of supply and return ducts were collected using a template, and gravimetrically analysed for dust mass [11]. Eluted filter dilutions were plated and colonies counted for viable colony forming units (CFUs) per unit area of sampled duct surface. These surface samples provided the core data used to indicate antimicrobial treatment efficacy. Additional samples were collected to indicate variability in the growth of microbial populations.

Sterile swab samples, bulk material samples of duct insulation, and liquid bulk samples of air-conditioning condensate were eluted into sterile buffer with Tween-80 and plated onto trypticase soy agar (TSA) and Sabouraud's dextrose agar (SDA) to identify fungal and bacterial growth. The samples plated onto SDA were evaluated for fungal growth and TSA plates for bacterial growth. In addition, Mattson-Garvin bioaerosol slit-to-agar samples of indoor and outdoor air were collected for fungal and bacterial viable contaminants.

After cleaning, each of two sections of supply trunk lines was equally divided into four cross sections. Each section was coated with one of the three antimicrobial encapsulants, and one section was used as a control. In addition, sections of the FGDL were removed (and replaced) from the test house for the laboratory portion of the research experiments.

Samples collected from the test house were analysed: (1) prior to coating, (2) after coating, and (3) quarterly for the remainder of the 18-month period. Each FGDL surface microbial evaluation consisted of collecting 3 samples from each of the 3 coated areas, plus 2 samples from the uncoated area, totalling 11 samples.

Laboratory Study

The field study of FGDL coatings in the test house was limited to those conditions found in a controlled residential building. Air travelling through a HVAC or HAC system would experience a drop in

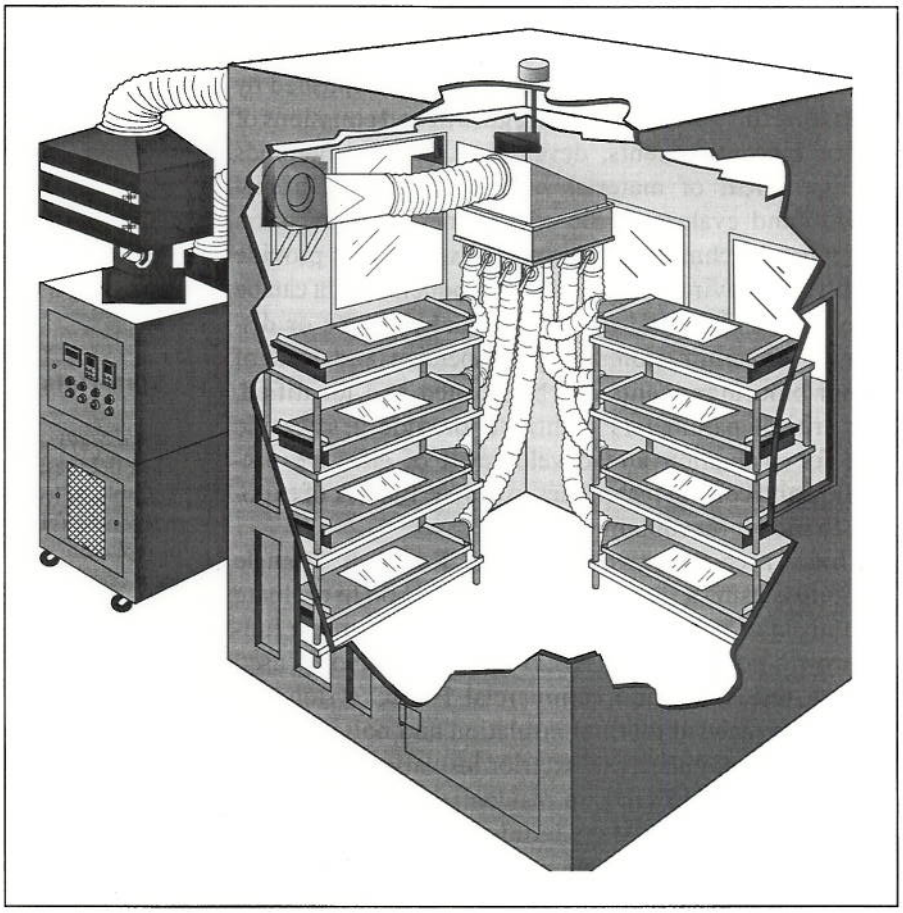

Fig. 1. The dynamic microbial test chamber (DMTC).

humidity, from saturation near the cooling coils to lower levels further along in the duct system. To expand the limitations of environmental control to evaluate the microbial growth impact of high humidity and soiling, dynamic chamber and static chamber experiments were evaluated.

Dynamic Chamber. To allow for experiments which involve air movement over contaminated surfaces and the release of biocontaminants, research was conducted in the dynamic microbial test chamber (DMTC) depicted in figure 1 [12]. The stainless steel chamber is a room-sized 2.44 m^3 cube, an air-handler unit (AHU) which kept the chamber at a temperature of 18–32 °C, a controlled relative humidity (RH) ranging from 55 to 95%, and an air circulation rate of 1.4–4.8 $m^3 \cdot min^{-1}$. A separate AHU forces conditioned DMTC air through a high efficiency particulate air (HEPA) filter and eight miniducts [12]. Eight miniducts were tested simultaneously to simulate HVAC duct use with single pass air velocity rates of 250 $cm \cdot s^{-1}$ [13]. The DMTC was operated at 23.5 °C and 95% RH, testing three encapsulant coatings applied to: one set of three soiled FGDL samples, one set of three unsoiled FGDL samples, and two control FGDL samples, for a total of eight miniduct samples. Conditioned, HEPA-filtered air passed over the surface of the FGDL samples, travelling horizontally within the miniduct apparatus, exited the duct and returned to the external air-handler for filtration and conditioning.

Static Chamber. The static microbial test chamber was tested using ASTM 6329-98 [14]. Fungal growth in the (32 × 39 × 51 cm) static chamber was evaluated on 2.5- to 3.8 cm^2 sections of FGDL. The method was previously developed for this purpose as part of ongoing indoor air biocontaminant research [15, 16]. Chambers are constructed from acrylic sheeting with shelves for samples and a saturated salt solution on the chamber bottom to control the equilibrium relative humidity [17].

Test Organism and Soiling

Aspergillus versicolor was chosen as the test organism to be used on FGDL because of the historical data on its use [18], and because it is a toxigenic mould commonly found in the indoor environment. Sections of FGDL were inoculated with *A. versicolor,* using an aerosol deposition chamber, and allowed to equilibrate overnight [13]. Prior to inoculation, FGDL materials were artificially soiled with duct dust which was injected into the chamber, kept airborne with a mixing fan, and allowed to settle over the materials [13]. A target deposition amount was 100 mg dust · 100 cm^{-2}.

Encapsulants Tested

The antimicrobial encapsulants tested were manufactured for use in HVAC system components and duct surfaces. Coating I was a polyacrylate copolymer containing 9% barium metaborate and

0.16% iodo-2-propynylbutylcarbamate; coating II was an acrylic coating containing decabromodiphenyl oxide and antimony trioxide, and coating III was an acrylic primer containing phosphoric acid compounds with a phosphated quaternary amine complex [diethanolamine N-coco alkyl derivatives; 2,2′-(N-coco alkylimino)bis-ethanol].

Dynamic Chamber Test Method

After preparation involving autoclave sterilisation, cooling, and inoculation, the eight 30.5 × 91.4 cm (1 × 3 ft) pieces of FGDL test material taken from the test house were placed in the dynamic chamber miniducts. One sample piece of FGDL was coated with one of each coating (I, II, and III) and one piece was used as a control. The same set of four FGDL pieces were duplicated, then soiled by the procedure described above and placed in the miniducts. All eight FGDL pieces were incubated at room temperature and sampled by the vacuum surface sampling method described above. Filters were eluted into sterile buffer with Tween-80 and plated onto TSA and SDA to identify fungal and bacterial growth.

Static Chamber Test Method

Square pieces of FGDL (2.5–3.8 cm) test material were inoculated and kept at a constant temperature (23.5 °C) in the constant-humidity static test chambers maintained at 70, 85, 90, and 94% RH. After being exposed to chamber growth conditions, triplicate samples were suspended in sterile phosphate-buffered saline containing Tween-80 and agitated for at least 5 min. Diluted aliquot samples of the suspension were plated on SDA and allowed to grow at room temperature. Colony forming units were counted and calculated per area of sample. This analysis was performed on the 1st day, and repeated monthly for 3 months.

Results and Discussion

Field study measurements of FGDL surfaces prior to coating averaged approximately 1,000 CFUs/10 cm^2 of fungal contamination. This concentration of mould contamination did not substantially change during the experiment. Part of the reason for this was attributed to the fact that after 4 months of monitoring, the cooling coils were found to be leaking condensate into the supply duct and needed to be replaced. The conveyance of moisture into the adjacent duct may have been the reason for the initial growth of mould. After replacing the defective cooling coils only background concentrations of fungal contamination were found. The reduced moisture level created by the new cooling coils lessened the potential for the growth of mould and effectively terminated the test house field experiment before differences in antimicrobial efficacy could be demonstrated. After monitoring the test house for 7 months (a complete cooling season) without evidence of fungal concentration exceeding background levels the experiment was stopped.

Table 1. Growth of *A. versicolor* on the surface of FGDL maintained at 94% RH dynamic chamber

FGDL sample	Biological growth, log CFUs·10 cm^{-2}			
	start	month 1	month 2	month 3.5
Untreated	0	3.8	4.40	4.2
Coating I	0	0.9	0.7	0.1
Coating II	0	2.9	3.1	3.3
Coating III	0	1.0	1.1	0

Table 2. Growth of *A. versicolor* on the surface of soiled FGDL maintained at 94% RH dynamic chamber

FGDL sample	Biological growth, log CFUs·10 cm^{-2}			
	start	month 1	month 2	month 3.5
Untreated	0	2.9	4.1	3.8
Coating I	0	1.5	1.6	1.0
Coating II	0	3.3	3.8	4.3
Coating III	0	1.5	0.8	0

The results of the dynamic chamber experiments are shown in tables 1 and 2, for sections of FGDL that were removed from the test house. Surface samples indicated that the population of *A. versicolor* on untreated FGDL increased by 3 orders of magnitude (1,000-fold) by the end of the 1st month (table 1), and then remained approximately constant through the 3.5-month study.

Table 1 shows a slight variation (increase followed by a decrease) in *A. versicolor* population for coatings I and III on FGDL, for the period of 3.5 months. This was in contrast to the increased populations observed in samples with coating II. The increase in fungi population for Coating II was comparable to that observed in the untreated samples, as compared with successful limiting of growth accomplished by coatings I and III.

Soiled FGDL experiments resulted in similar populations of *A. versicolor* for untreated and coating II samples, as shown in table 2. The results were again similar for coatings I and III, in which variations in fungi populations were observed to increase in month 1, and then decrease. The results indicates that antimicrobials can remain effective with moderate dust loading.

A comparison of the results obtained with dynamic and static chamber testing is shown in table 3. The addi-

Table 3. Change in log CFUs · 10 cm^{-2} of *A. versicolor* during the 3-month study

Treatment	Static chamber				Dynamic chamber 94% RH
	70% RH	85% RH	90% RH	94% RH	
Not Soiled					
Untreated	BDL	1.4	4.4	4.4	4.2
Coating I	BDL	BDL	BDL	<1.0	<1.0
Coating II	<1.0	1.9	3.4	4.3	3.3
Coating III	<1.0	BDL	BDL	BDL	<1.0
Artificially soiled					
Untreated	BDL	<1.0	4.4	5.1	3.8
Coating I	<1.0	<1.0	BDL	<1.0	1.0
Coating II	BDL	3.6	4.7	4.7	4.3
Coating III	<1.0	BDL	BDL	BDL	<1.0

BDL= Below detection limit.

tional static chamber testing was performed at 70, 85, 90, and 94% RH, for comparison with the 94% RH dynamic chamber testing. Table 3 indicates that static chamber results at 94% RH are in good agreement with dynamic chamber results at 94% RH. Studies also demonstrated that static chamber fungi growth decreases with decreasing RH. Both methods indicate greater effectiveness in controlling growth with coatings I and III.

Fine particulate matter is a significant pollutant both indoors and outdoors. Measurements of indoor exposures are needed to evaluate total long-term personal exposures to the fine fraction (<2.5 μm) of particulate matter, originating from both ambient outdoor and indoor sources [19]. Bacteria, fungi, viruses, and allergens are important components of outdoor and indoor aerosols. Desiccated, non-viable fragments of these organisms are also ubiquitous. These fragments can remain pathogenic, toxic, or allergenic (depending upon the specific organism or organism component).

Under favourable conditions, biocontaminants are able to grow and multiply on a variety of building materials and indoor surfaces. Manufacturers' claims to prevent this should be the basis for testing the efficacy of antimicrobials. Potential biocontaminants and their metabolites that are claimed to be controlled should be tested on the appropriate materials and under the appropriate conditions of use. Methods of testing for antimicrobial efficacy are incomplete and may be misleading if: (1) testing is performed only on nonporous materials, (2) testing involves a limited number (one or two) of microbial species, and (3) testing of microbial fragments is not included. Analytical methods are available to accomplish a comprehensive determination of antimicrobial efficacy and should be made part of the evaluation process.

The overall goal of antimicrobial efficacy research should be to contribute to developing better methods of understanding indoor air bioaerosol contamination and to enhance the ability to prevent and control indoor exposure to all forms of biocontaminants. Specific objectives include: (1) establishing standardised test methods and procedures for evaluation, (2) developing techniques and equipment to conduct test evaluations, and (3) identifying materials, microbial populations, and constituent biological entities (viable and non-viable metabolites and microbial volatile organic compounds to be tested with antimicrobial treatments.

Conclusions

Three common HVAC (HAC) antimicrobial encapsulants were evaluated for their use on FGDL in both laboratory and field application experiments. Although the field test was inconclusive and truncated, the laboratory chamber experiments showed differences in degrees of efficacy for three antimicrobial coatings. Two antimicrobial coatings limited fungal contamination for the duration of testing. The effective coatings (coatings I and III) contained zinc oxide/borates and a phosphated quaternary amine. The ineffective coating (coating II) contained decabromodiphenyl oxide and antimony trioxide. Although all three antimicrobials are registered with the EPA, they were not equally effective, nor should they be expected to perform equally in field use.

Methods of testing antimicrobial efficacy are needed to evaluate differences in products having or seeking EPA registration. The series of experiments described above are adequate tests for viable mould. However, in addition to viable mould antimicrobial efficacy, additional testing may be needed for viable bacteria (bacteria cells and spores), non-viable mould (mycotoxins) and bacteria (endotoxins), as well as viruses and some forms of allergens (e.g., dust mites), to determine whether the manufacturers' claims apply.

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