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Evaluating the potential efficacy of three antifungal sealants of duct liner and galvanized steel as used in HVAC systems

KK Foarde[1] and MY Menetrez[2]

[1]*RTI, 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA;* [2]*Air Pollution Prevention Control Division, National Risk Management Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27709, USA*

Current recommendations for remediation of fiberglass duct materials contaminated with fungi specify complete removal, which can be extremely expensive, but in-place duct cleaning may not provide adequate protection from regrowth of fungal contamination. Therefore, a common practice in the duct-cleaning industry is the postcleaning use of antifungal surface coatings with the implication that they may contain or limit regrowth. However, even the proper use of these products has generally been discouraged because little research has been conducted on the effectiveness of most products as used in heating, ventilating, and air-conditioning (HVAC) systems. Three different coatings were evaluated on fiberglass duct liner (FGDL). Two of the three coatings were able to limit growth in the 3-month study; the third did not. One of the coatings that was able to limit growth was further evaluated in a comparison of FGDL or galvanized steel (GS) under conditions that mimicked their use in HVAC systems. The results showed that both moderately soiled and heavily soiled uncoated FGDL and GS duct material can support fungal growth, but that GS duct material was more readily cleaned. The use of an antifungal coating helped limit, but did not fully contain, regrowth on FGDL. No regrowth was detected on the coated GS.
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Introduction

Heating, ventilating, and air-conditioning (HVAC) systems become dirty to various degrees after a period of use. Depending on the amount of dirt, the material, and the environmental conditions, the HVAC system may become an active growth site for microorganisms and a source of biological contamination. Cleaning alone is ineffective for remediation of microbial growth on porous materials such as fiberglass duct liner (FGDL). Recent experiments confirm field experience that duct cleaning alone may not provide adequate protection from regrowth of fungal contamination on FGDL [7]. Current recommendations for remediation of fiberglass duct materials contaminated with fungi specify complete removal of the materials [13,15,16]. However, removal of contaminated materials can be extremely expensive. Therefore, a common practice in the duct-cleaning industry is the postcleaning use of antifungal surface coatings with the implication that they may contain or limit regrowth. In addition to becoming microbially contaminated, FGDL may become frayed due to poor installation, damage, or deterioration, and release debris into the air stream. Because it is expensive, time consuming, and disruptive to normal building use, replacement of contaminated FGDL is sometimes not acceptable to building owners. Encapsulants/sealants/coatings are used to seal the frayed material and are applied to FGDL systems following cleaning to (1) reduce fiber shedding from surface damage or degradation, (2) isolate remaining contaminants from the air stream, and/or (3) reduce microbial growth or regrowth through the use of antifungals in the coatings.

Correspondence: Karin Foarde, Center for Environmental Technology, RTI, 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA
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Generally, these are polymer coatings that are applied as thick liquids or mastics using brushes, trowels, or sprayers, depending on their properties. With regard to microbial growth, little information is available on the efficacy of these treatments under actual-use conditions.

In an earlier study, a Cary, NC, EPA test house was found to have a biocontaminated FGDL supply trunk [12]. The surface of the FGDL-lined portion of the supply-side trunk duct was contaminated primarily with a yeast and the fungus *Cladosporium* sp. *Penicillium* sp. was isolated from the duct surface samples only at locations distant from the air handler. The first part of this paper describes the results of a project using the contaminated FGDL from the test house to evaluate microbial growth in the presence of coatings on contaminated FGDL in a laboratory test. The objective was to determine whether commercially available antifungal coatings, placed on the newly cleaned FGDL that 1 year previously had been actively growing microorganisms, could prevent growth at a range of constant relative humidity (RH) conditions at a fixed temperature.

The second part of the paper describes a project to determine whether the use of an antifungal coating could effectively limit growth (surface loading) at different levels of soiling of both FGDL and galvanized steel (GS).

Methods and materials

Dynamic chamber tests

Experiments were conducted in the dynamic microbial test chamber (DMTC). 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 [18]. The

14.71-m^3 chamber was constructed with stainless steel walls and floor and an acrylic drop-in ceiling. Temperature (18–32°C) and RH (55 to 95%) were controlled, using 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.

Figure 1 is an artist's rendition of the DMTC containing the miniduct apparatus. 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 for the miniducts was chosen to limit the total amount of air required for a single test, allowing multiple tests to be run simultaneously, and to simulate flow conditions in an HVAC duct. For this test, the air velocity over the surface of the duct material was maintained at 250 cm/s (500 ft/min), which is a reasonable duct velocity and has been used in other miniduct experiments [7,8]. The dynamic chamber conditions were controlled at 23.5°C and 95% RH. This provided approximately 94% RH air to the samples in the miniducts (the blower warms the air slightly).

Static chamber tests

The microbial growth or material susceptibility tests were performed following ASTM 6329, *Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers* [1]. Use of this method enabled the generation of a quantitative endpoint for growth in a well-controlled environment with improved repeatability and comparability between tests and materials. This method was developed for evaluating fungal growth (as quantified by culture analysis) on indoor materials as part of a comprehensive research program to apply indoor air quality engineering to biocontamination of buildings and has been used successfully to evaluate the ability of different types of materials to sustain the growth of *Penicillium glabrum*, *Aspergillus niger*, *Aspergillus versicolor*, and *Penicillium chrysogenum* [4,6,9].

Static chambers (32×39×51 cm), modified acrylic-walled desiccators, were used to provide controlled environments for the microbial growth tests. The chambers were placed in a temperature-controlled, Class 10,000 clean room. Each static chamber was equipped with shelving, a bottom tray containing a saturated salt solution, and a hygrometer. Saturated salt solutions are used to maintain known equilibrium relative humidities (ERHs) within each chamber [10].

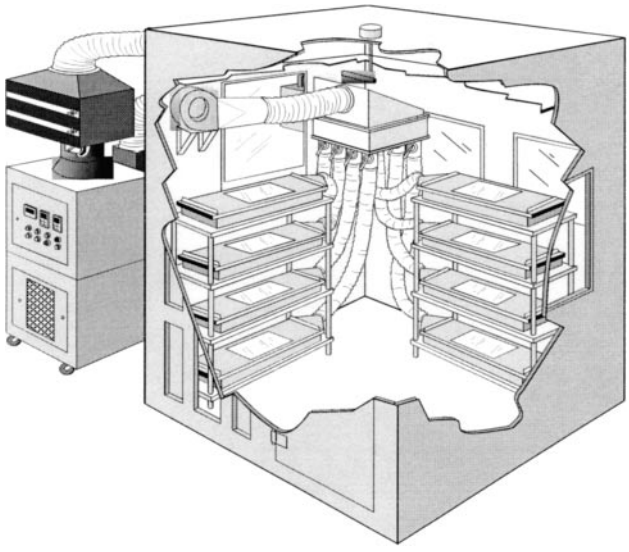

Figure 1 Artist's rendition of DMTC.

Each test required approximately 30 individual pieces (3.8-cm squares) of duct liner or GS. Periodically, usually day 0 and then monthly, at least 3 of the original 30 individual pieces of the filter were removed for quantification of growth. On each test day, sample pieces were removed from the chambers, individually placed in sterile receptacles containing phosphate-buffered saline with polyoxyethylene (20) sorbitan monooleate, and shaken on a wrist-action shaker for 30 min, after which the sample piece/buffer suspension was diluted and plated on Sabouraud's dextrose agar. The plates were incubated at 23±3°C in alternating light and dark conditions. Colonies were counted as moderate growth became visible.

The tests were performed using chambers maintained at ERHs ranging from 70 to 97%. The higher ERHs were chosen to provide a worst-case scenario for the duct materials, when used in poorly designed and inadequately maintained HVAC systems.

Selection of test organisms and inoculation

Two fungi, capable of growth at differing ERHs, were selected for testing. Under optimal nutrient conditions at 28°C, *A. versicolor* was able to grow with the ERH as low as 74% [2]. *P. chrysogenum* was able to grow with the ERH at 78% at 25°C [2]. *A. versicolor* was selected because its growth on fiberglass materials is well documented, and because it is a toxigenic fungus [3]. The *A. versicolor* was isolated from a contaminated duct liner. *P. chrysogenum* was selected because it is one of the most frequently isolated molds from the air, dust, and surfaces of indoor environments [11]. This organism has also been isolated from a number of air-conditioning systems in environments where patients were suffering from allergic disease [17]. 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 maintained in the University of Texas Medical Branch Fungus Culture Collection as UTMB3491. The organisms were prepared for inoculation by gently stroking a sterile swab wetted with sterile water across the surface of a petri dish to collect the growth. The material collected on the swab was eluted into sterile water (to minimize introduction of extraneous nutrients) until a reading of 15% T at 520 nm (Milton-Roy Spec 20D) was achieved (approximately 1×10^7 colony-forming units [CFU]/ml) [5].

The suspension was mixed well and 10 μl was pipetted onto each block of test material for a final inoculum of approximately 1×10^5 CFU per sample. Uninoculated blocks were used as controls. Test blocks (inoculated and uninoculated) were placed in the appropriate static chamber maintained at the specific test RH for incubation.

To quantify fungal growth, triplicate inoculated and duplicate uninoculated blocks were removed from each chamber for analysis, usually on days 1, 7, 14, 21, 28, 35, and 42. Following removal, the sample blocks were weighed on a Fisher Scientific A200-DS balance to 0.001 g (0.01% of the mean block mass), and placed in sterile receptacles containing phosphate-buffered saline with 0.1% Tween 80. The blocks in buffer were shaken on a wrist-action shaker for 30 min, then the block/buffer suspension was diluted and plated on SDA. Plates were incubated at room temperature for

at least 1 week. Colonies were counted shortly after visible growth was first noted and again as moderate growth became apparent.

Coatings

All three coatings were designed for use on HVAC system components and/or interior surfaces of lined and unlined duct systems. Coating I was a polyacrylate copolymer containing zinc oxide and borates. Coating II was an acrylic coating containing decabromodiphenyl oxide and antimony trioxide. Coating III was an acrylic primer containing a phosphated quaternary amine complex. The coatings were selected by members of the National Air Duct Cleaners Association (NADCA) as those commonly used in the industry. The used FGDL was cleaned and coated by members of NADCA. RTI's Microbiology Laboratory (RTI-ML) personnel coated the new FGDL and GS. All coating followed manufacturer's instructions found on the labels of the products. The final coverage rate of the material was determined gravimetrically. Four passes over the material surfaces were required for all coatings to achieve maximum coverage. The coverage rates were: coating I — 0.5 l/m^2, coating II — 0.47 l/m^2, and coating III — 0.28 l/m^2.

Artificial soiling of duct materials

The materials were artificially soiled, and then inoculated in an aerosol deposition chamber. Sieved (250 μm), autoclaved duct dust obtained from a local duct cleaner was used to soil the samples. Material samples were placed around the periphery of the deposition chamber floor. Duct dust was injected using an air injector, mixed in the chamber, and allowed to settle on the test material samples [7]. The test organism suspension was nebulized into the aerosol deposition chamber utilizing a six-jet BGI-Collison nebulizer (BGI, Waltham, MA) at 138 kPa (20 psi) for at least 2 h and allowed to settle on the duct material pieces [7]. Two levels of soiling were used: moderately soiled (approximately 100 mg dust/100 cm^2) and heavily soiled (approximately 1000 mg dust/100 cm^2). These levels were selected with reference to the 1.0 mg dust/100 cm^2 definition of cleanliness given in NADCA Standard 1992-01 [14]. This standard is intended only for nonporous surfaces, but its definition of the amount of soil that may remain in a cleaned duct provides the only quantitative benchmark available. Therefore, moderately soiled, as targeted in this research, was about 100-fold higher than the standard, and heavily soiled was about 1000-fold the amount in the NADCA 1992-01 standard.

Procedure

Coatings assessment on FGDL: For both the dynamic and static chamber experiments, the used FGDL was removed from the EPA test house, cleaned and coated by NADCA members, and transported to RTI's ML within 5 days. Once at RTI, the FGDL was cut into 30.5×91.4-cm (1×3-ft) pieces for the dynamic chamber experiments or blocks (2.5- to 3.8-cm squares) for the static chamber experiments. Half of the test materials were artificially soiled, and all of the pieces were inoculated with *A. versicolor*.

The larger pieces for the dynamic chamber testing were placed in the miniducts. Surfaces samples (10 cm^2) were collected at day 0 and again at monthly intervals for 3 months. Surface samples were obtained by the vacuum method and analyzed in duplicate each time from each duct material [7]. The plates were incubated at 23±3°C. Colonies were counted when moderate growth was observed.

The parallel static chamber experiments were conducted by placing small square blocks (2.5- to 3.8-cm squares) of the test material, inoculated with *A. versicolor*, in the constant humidity chambers at 70%, 85%, 90%, and 94% RH.

Contact vacuuming with and without coating on FGDL and GS: Initial growth was established on the newly purchased FGDL and GS (26 gauge) in the dynamic chamber. Both test materials were purchased from an HVAC contractor as representative of the type commonly used locally. First, the pieces were artificially soiled to the heavily soiled level with the autoclaved dust in the aerosol deposition chamber. The dust was allowed to settle overnight. The pieces were then inoculated, using an aerosol suspension of the test organism, while still in the aerosol deposition chamber. The test organism suspension was allowed to settle overnight. The test pieces were then moved to the DMTC and placed in the miniducts.

Once mature growth had become established (3–4 weeks), the pieces were cleaned in place by contact vacuuming. A Minuteman model C82906-03 HEPA vacuum cleaner (Minuteman International, Addison, IL) operating at 2.7 m^3/min (95 CFM) was used. The vacuuming pattern consisted of four passes over each surface crosswise and four passes lengthwise. The antifungal coating was applied to four of the eight pieces (two FGDL and two GS) following the manufacturer's instructions by RTI-ML personnel in the DMTC. Surface samples (10 cm^2) were collected after mature growth was established but before contact vacuum cleaning and

Table 1 Comparison of *A. versicolor* growth on used FGDL within 3 months $\log_{10}$ change in CFU (mean±SE)

Coating	Moderate soiling	Static chamber				Dynamic chamber 94% RH
		70% ERH	85% ERH	90% ERH	94% ERH	
None	No	BDL	0.8±0.9	4.4±0.9	4.4±0.9	4.2±0.2
	Yes	BDL	BDL	4.4±0.9	4.5±0.8	3.8±0.3
Coating I	No	BDL	BDL	BDL	BDL	0.2±0.4
	Yes	BDL	BDL	BDL	BDL	1.0±0.1
Coating II	No	BDL	BDL	3.4±0.9	4.3±0.5	3.3±0.1
	Yes	BDL	BDL	4.4±0.3	4.6±0.1	4.3±0.4
Coating III	No	BDL	BDL	BDL	BDL	BDL
	Yes	BDL	BDL	BDL	BDL	BDL

BDL: below detection limit (100 CFU/10 cm^2).
Shading: growth (defined as 1 $\log_{10}$ increase within the 3 months over the baseline on day 0).
$\log_{10}$ change in CFUs between day 0 (the inoculum) and 3 months, n=3.
Coating occurred within 3 days of cleaning.

again after contact vacuuming or after vacuuming and coating. Duplicate samples were analyzed each time from each duct material.

Impact of soiling on coated FGDL and GS: The contact-vacuumed/coated test pieces described above were also used in this study. For the static experiments, the 30.5×91.4-cm (1×3-ft) pieces were cut into 3.8×3.8-cm (1.5×1.5-in.) pieces. They were artificially soiled to either the moderately or heavily soiled level, but not re-inoculated, and placed in the 85%, 90%, 94%, or 97% RH static chamber.

For the dynamic chamber experiments, the 30.5×91.4-cm (1×3-ft) pieces were removed from the miniducts and transferred to the aerosol deposition chamber where they were artificially soiled to either the moderately or heavily soiled level. They were not reinoculated, but returned to the miniducts in the DMTC and maintained at 94% RH. Surface growth was monitored for 4 months.

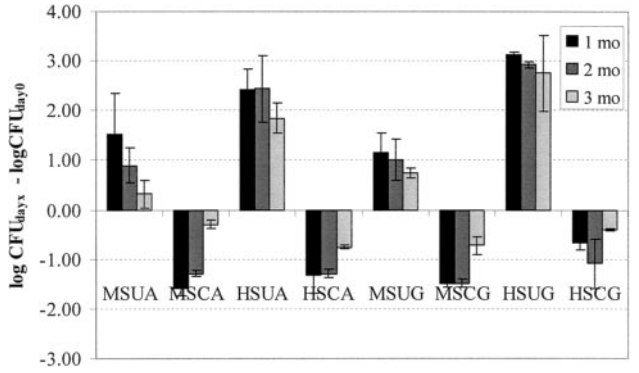

Figure 2 Regrowth of *A. versicolor* on FGDL and GS at 94% RH over the course of the 3-month experiment. Each data point gives the difference between the mean CFUs on the test day (day *x*) to the mean CFUs on day 0. Day 0 sampling was conducted immediately after contact vacuuming, the application of the coating (if coated), and the artificial soiling. First two letters, MS or HS, denote moderate or heavily soiling. U or C stands for uncoated or coated. The final letter is the designation for the material: A is used for FDGL and G for GS.

Results and discussion

Coatings assessment on FGDL

Table 1 shows the results from the static and dynamic chamber experiments with the used FGDL from the test house. Half the samples were soiled to provide an evaluation of the antifungal efficacy after the coatings became soiled.

The dynamic chamber experiment using miniducts was performed to evaluate growth under very high humidity (94% RH) or worst-case conditions. The static chamber testing was performed to extend the amount of humidity to which the materials were exposed. Static chamber experiments were performed at 70%, 85%, 90%, and 94% ERH. There was good agreement between the results of the 94% static chamber testing and the 94% dynamic chamber testing. Table 1 shows that two of the three coatings limited the growth of *A. versicolor*.

Contact vacuuming with and without coating on FGDL and GS

While the results in Table 1 showed that use of some antifungal coatings may limit growth for at least 3 months under "worst-case" conditions even when moderately soiled, a number of questions still needed to be answered.

Two of those questions are: (1) Can the application of an antifungal coating limit growth on cleaned, but previously contaminated, GS and FGDL? (2) Can an antifungal coating reduce the impact of soiling (to different levels of soiling) on previously contaminated, but cleaned, GS and FGDL?

A series of experiments was undertaken using coating III to address these two questions. Coating III was selected as representative of coatings that could potentially limit growth for 3 months. New FGDL and GS were contaminated in the laboratory by artificially soiling as described earlier, inoculating each with test organisms, and maintaining the pieces at 94% RH for 3 weeks until stable, mature growth occurred (no change in surface concentration of the test organism). All of the pieces were then cleaned by contact vacuuming. After vacuuming, half of the GS and half of the FGDL pieces were coated with coating III.

Table 2 shows the reduction in surface loading of *A. versicolor* after cleaning of the FGDL and GS with and without the application of coating III. Student *t* tests were performed to determine the significance of the differences between the reductions in CFUs for each material and treatment. The reduction calculated for FGDL without the coating was -2.29 $\log_{10}$ and with the coating was -3.46 $\log_{10}$ (Table 2). The *p* value calculated for the difference by the *t* test was 0.0004. In other words, there is a

Table 2 Mean (±SE) of *A. versicolor* before and after cleaning with and without the application of coating III with the resulting reduction ratio (n=4)

Material	Coated	Before cleaning CFU($\log_{10}$)/10cm^2	After cleaning CFU($\log_{10}$)/10cm^2	Reduction ($\log_{10}$)
FGDL	No	6.8±0.1	4.5±0.2	−2.29±0.01
	Yes	6.6±0.1	3.2±0.3	−3.46±0.06
GS	No	6.7±0.09	3.9±0.3	−2.78±0.39
	Yes	6.8±0.02	2.9±0.08	−3.89±0.06

Reduction was calculated by subtracting the mean number of CFUs ($\log_{10}$)/10 cm^2 isolated from the surface before treatment from the number of CFUs($\log_{10}$)/10 cm^2 isolated from the surface immediately after treatment.

Table 3 Log change in *P. chrysogenum* after coating within 4 months (mean±SE) on coated and uncoated FGDL and GS (n=3)

Material	Coated	Soil*	ERH			
			85%	90%	94%	97%
FGDL	No	M	1.0±0.4	2.3±0.3	2.6±0.2	2.0±0.2
		H	0.08±0.3	2.5±0.2	1.2±0.3	1.1±0.4
	Yes	M	BDL	BDL	BDL	1.1±0.3
		H	BDL	BDL	BDL	3.1±0.6
GS	No	M	BDL	BDL	BDL	BDL
		H	BDL	BDL	BDL	BDL
	Yes	M	BDL	BDL	BDL	BDL
		H	BDL	BDL	BDL	BDL

BDL: below detection limit (100 CFU/10 cm^2).
Shading: growth (defined as 1 $\log_{10}$ increase within the 4 months over the baseline on day 0).
*M: moderate; H: high.

99.96% chance that there is a difference in using the coating on FGDL. For GS, the reduction ratio calculated without the coating was -2.78 $\log_{10}$ and with the coating was -3.89 $\log_{10}$. The p value for the difference was 0.12. While this p value is not statistically significant at $p<0.05$, the reduction ratios are noticeably different. Both of these results indicate that the application of coatings can further reduce surface contamination beyond the reduction accomplished with contact vacuuming alone.

Even more interesting were the results of the t test comparing the reduction of the two materials (FGDL vs. GS); first without the coating, and then with the coating. The p values were 0.05 when comparing the uncoated materials, and 0.02 when comparing the coated. These results demonstrate the impact that surface characteristics can have. They suggest that GS is more readily cleaned than FGDL, and furthermore, that coating GS is even more effective than coating FGDL in reducing the surface load of organisms.

Impact of soiling on coated FGDL and GS

After the cleaning and/or coating described above, the materials were resoiled to either the moderate or heavy level, but were not reinoculated. Figure 2 shows the results of a 3-month experiment in the DMTC miniducts to quantify regrowth of *A. versicolor* on the duct materials after contact vacuuming, coating, and soiling.

Figure 2 shows that the CFUs on all of the coated materials decreased below the day 0 level and remained there for the full 3 months of the study. The uncoated materials recovered to at least precleaning levels by the first month and remained at similar levels until the study was terminated.

To extend the dynamic chamber results to additional humidities, static chamber tests were performed. Table 3 shows the results for *P. chrysogenum*, and Table 4 shows the results for *A. versicolor*. The static chamber experiments were performed at 85%, 90%, 94%, and 97% ERH. The dynamic chamber experiments (Figure 2) were performed only at 94% RH.

A. versicolor (Table 4) recovered on both the moderately and heavily soiled uncoated FGDL and GS. *P. chrysogenum* (Table 3) recovered only on the uncoated FGDL, not on the GS. These differing results for the two organisms illustrate how factors, such as substrate requirements, can be important and may reflect what happens on materials during normal usage. These results also demonstrate the importance of test organism selection and the need for testing with multiple organisms.

Table 4 Log change in *A. versicolor* after coating within 4 months (mean±se) on coated and uncoated FGDL and GS ($n=3$)

Material	Coated	Soil*	ERH			
			85%	90%	94%	97%
FGDL	No	M	0.8±0.5	2.0±0.2	1.9±0.2	1.4±0.1
		H	0.8±0.2	3.1±0.2	2.4±0.2	2.1±0.1
	Yes	M	BDL	BDL	BDL	BDL
		H	BDL	BDL	BDL	1.9±0.7
GS	No	M	0.2±0.1	1.6±0.2	0.8±0.2	1.7±0.2
		H	−0.1±0.3	1.0±0.5	1.8±0.3	1.5±0.3
	Yes	M	BDL	BDL	BDL	BDL
		H	BDL	BDL	BDL	BDL

BDL: below detection limit (100 CFU/10 cm^2).
Shading:growth (defined as 1 $\log_{10}$ increase within the 4 months over the baseline on day 0).
*M: moderate; H: high.

On the coated GS, there was no regrowth of either organism, regardless of the level of soiling or ERH. Even at 97% ERH and high levels of artificial soiling, the test organisms were not able to reestablish themselves after 4 months.

The results for the coated FGDL differ. Both test organisms were able to reestablish themselves within the 4-month study, but at a limited level compared to the uncoated FGDL. *A. versicolor* regrew on the heavily soiled FGDL at 97% ERH, but not on the moderately soiled. On the other hand, the *P. chrysogenum* regrew at 97% RH on both the moderately and heavily soiled FGDL.

Conclusions

Three commonly used HVAC coatings were evaluated at a range of humidities under controlled static and dynamic conditions. Two of the three coatings limited the growth of fungal contamination, at least in the short term (3 to 4 months). The third did not.

An effective assessment of antifungal efficacy on a material should include a use test. Standard efficacy testing of an antifungal is usually performed only on the active ingredient itself, and not on the antifungal on or in the product as it is going to be used. When the efficacy testing of the actives used in this study was performed for their EPA registration, the results showed that the active ingredient itself was able to kill or inactive many microorganisms.

Only two of the three antifungal coatings inhibited the growth of the test organism. These results confirm the results of a field study of treated FGDL that showed some but not all sealants to be effective in limiting fungal regrowth up to 3 years [19]. Clearly, it is of benefit to industry and to consumers to have information on how a particular product/material could be expected to perform under the conditions that the material will be subjected to for a particular application.

The results also show that application of a coating reduced surface loading beyond that achieved with contact vacuuming alone on both FGDL and GS. Furthermore, GS was more readily cleaned than FGDL when contact vacuumed, and the coating was more effective on the hard surface, GS, than the porous surface, FGDL, at reducing the levels of CFUs after contact vacuuming.

In a recently completed study of antifungal-treated air filters, we observed a masking of the antifungal treatment by dust loading the filters [9]. Dust provided a physical barrier between the antifungal and the organism. However, only fully dust-loaded filters were evaluated. Partially dust-loaded filters were not included. Of interest has been the impact of partial dust loading on antifungal efficacy. The results of this current study suggest that antifungal coatings can remain effective with partial dust loading at least on duct materials. In other words, the dust does not fully mask the antifungal. Some antifungals may be effective, at least in the short term before soiling masks the active ingredient, in continuing to suppress fungal growth under favorable environmental conditions.

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