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Quantification of the fungal fraction released from various preloaded fibrous filters during a simulated ventilation restart

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Abstract

This study aimed to demonstrate that particles, especially those associated with fungi, could be released from fibrous filters used in the air-handling unit (AHU) of heating, ventilation and air-conditioning (HVAC) systems during ventilation restarts. Quantification of the water retention capacity and SEM pictures of the filters was used to show the potential for fungal proliferation in unused or preloaded filters. Five fibrous filters with various particle collection efficiencies were studied: classes G4, M5, M6, F7, and combined F7 according to European standard EN779:2012. Filters were clogged with micronized rice particles containing the fungus *Penicillium chrysogenum* and then incubated for three weeks at 25°C and 90% relative humidity. The results indicated that the five clogged tested filters had various fungal growth capacities depending on their water retention capacity. Preloaded filters were subjected to a simulated ventilation restart in a controlled filtration device to quantify that the fraction of particles released was around 1% for the G4, 0.1% for the M5 and the M6, and 0.001% for the F7 and the combined F7 filter. The results indicate that the likelihood of fungal particle release by low efficiency filters is significantly higher than by high efficiency filters.

KEYWORDS

air-handling unit, fibrous filter, filtration, fungal aerosol, indoor air quality, particle release

1 | INTRODUCTION

Indoor Air Quality (IAQ) has become one of the main concerns for people's health and safety. Air-handling units (AHUs) in heating, ventilation and air-conditioning (HVAC) systems are used to purify and recycle indoor air. In general, they consist of two filtration stages composed of a pre-filter and a fine filter.¹ However, in some circumstances, HVAC filters can be bio-contaminated and become a pollutant source.^{2,3} In fact, microorganisms can degrade the filters and microbial products, such as allergens, endotoxins, and mycotoxins, can be released and degrade the IAQ.⁴⁻⁷ In particular, fungi are potent sources of allergens⁸⁻¹¹ and epidemiological studies confirm that they may adversely influence respiratory diseases, such as asthma^{7,11} and bronchitis.¹²

Fungal growth in fibrous filters and spore release during filtration were observed in hospitals by Elixmann et al.¹³ Maus et al.¹⁴ showed the viability of the microbial spores over a short time in an unused fibrous filter. Moreover, Maus et al.¹⁵ confirmed that microbial spores, particularly fungal ones, can be viable over a long time (>5 days) and that microbial growth is mainly possible when air stops circulating through the filter. In fact, the impaction and desiccation of outdoor air microorganisms on the surface of the fibrous filters used in AHUs can decrease their cultivability, particularly for bacteria such as *Bacillus subtilis* and *Pseudomonas fluorescens*.¹⁶ However, bacteria like *Staphylococcus* and *Micrococcus*, bacterial spores like *Bacillus* sp., endospores,¹⁷ and fungal spores like *Penicillium* sp., *Aspergillus* sp. and *Cladosporium* sp.¹⁸⁻²¹ are commonly found in indoor air. These are more resistant and can grow on filters mainly due to the presence of

nutrients and humidity. Sources of nutrients on filters include cotton fibers,^{22–24} chemical components added during filter manufacture,²⁵ and particulate cake formed at the filter surface.^{23,24} The effect of relative humidity (RH) on microorganism proliferation has been illustrated in several studies. In static flow conditions, fungal growth on cellulose fibers was observed at RH >70%–80%²² and on used cotton filters at RH > 90% after one month of continuous filtration.⁶ Significant microbial growth was also observed when the HVAC was turned off over the weekend for energy-saving reasons.^{26–28} In dynamic flow conditions, studies showed that the microorganism population decreases at RH <70%–80%.^{6,14,22} Hamada and Fujita²⁷ showed that the particulate cake can accumulate humidity in the filter, which provides suitable conditions for microorganism growth. However, none of those studies quantified the water retention capacity of the filter, only the air relative humidity.

There are few studies reporting the influence of microorganism growth on filtration performances. Verdenelli et al.²⁹ showed that the consumption of some filter material by microorganisms can decrease the efficiency value of the most penetrating particle size (MPPS). They also showed that microbial proliferation is responsible for an increase of 5% in the initial value of the pressure drop. Forthomme et al.² investigated three parameters that may influence microorganism growth in the filter: the relative humidity, the presence of airflow, and the nature of the filter. Concerning fungal growth, they showed that *Penicillium oxalicum* grows only when the RH is about 100%, that the presence of airflow tends to slow the growth and that there is no significant difference between the microbial growth in glass and polypropylene filters. Forthomme et al.² also investigated the microbial release from F7 filters and measured a released fraction of 0.082±0.054% for a glass fiber filter and 0.0075±0.0013% for a polypropylene fiber filter after 168 hour of incubation at 100% RH and 25°C. Jankowska et al.³⁰ studied the release of fungal spores on an F5 and an F8 filter (according to European standard EN779:1993). They observed an increase in fungal spores released from the filters with an increase in air velocity. With the same air velocity during a filter clogging and release test, the fungal fraction released was between 0.009% and 0.33%. González et al.³¹ also studied the microbial release from a two-stage filtration process. The amount of cultivable microorganisms was below the detection limit of the BioSampler (SKC), so the microbial fraction that could have passed through the filters was not quantified. The microbial fractions released from the filters were estimated by assimilating the microorganisms to particles with the same diameter measured with a particle counter (Welas, Palas). These studies show that the detection and quantification of microbial particles released from a filter is difficult and requires a sufficient contamination, which can be obtained with optimal growth conditions. The small amounts of microorganisms released necessitate a measurement method with a low detection limit. Another limitation that hinders the comparison of the different filters in these studies is the various conditions used in terms of initial fungal concentration in the filter, fungal growth, and type of filter.

The main objective of this study was to quantify the amount of particles, abiotic and fungal, that could potentially be released from

Practical implications

- This study demonstrates that indoor air biocontamination through HVAC systems is dependent on the filter exposure to humidity because of possible fungal growth and fungal particle release downstream of the filter. Our results show that the water retention of the filter must be lower than a threshold value (40 g/m²) to limit fungal proliferation. The coarse filter G4 studied with a fungal loading released more particles (3 log) than the fine F7 filter.

preloaded filters containing fungi during an HVAC system restart. The influence of the type of filter on the fungal growth and particle release was investigated. The study was limited to a laboratory-scale investigation to quantify the particles and fungi released. Moreover, the water retention capacity of filters was evaluated to explain the variation in fungal growth observed between them. Filter incubation was carried out under optimal conditions for fungal growth and experiments were performed in the same conditions with each type of filter to compare the fungal growth capacity and fungal release.

2 | MATERIALS AND METHODS

2.1 | Fibrous filters tested

Five fibrous filters, regarding EN779:2012,³² from Camfil® were studied: classes G4 (10 AeroPleat Eco, 1474.38.00, Camfil, France), M5 (Basic-Flo A5, 1341.00.07, Camfil, France), M6 (Basic-Flo A6, 1342.00.05, Camfil, France), F7 (Hi-Flo TM7, 1368.92.00, Camfil, France), and F7 with granular activated carbon (AC) (City-Flo XL, 1612.50.50, Camfil, France). The filters were tested in a flat geometry, whereas the commercial geometry is bag filters except for the G4 pleated filter. The filters selected for the study could correspond to a first pre-filter stage (G4) and a second fine-filter stage (M5, M6 or F7) in HVAC systems often found in office buildings.¹ Table 1 presents the main characteristics of the five filters studied. The composition of each filter was given by the manufacturer. The fiber diameter was estimated from scanning electron microscope (SEM) observations. The thickness (Z) of each filter was determined with a micrometer. The porosity of the whole filter was measured by the mercury intrusion method using an Autopore IV 9500 (Micrometrics, France). Permeability (B) was determined experimentally from filter pressure drop (ΔP) measurements versus filtration velocity (v) considering Darcy's law for a laminar airflow (Eq. (1)):

$$B = (\mu Z v) / \Delta P \quad (1)$$

where μ is the dynamic viscosity of air. Basis weight was determined by measuring the weight of dried filter samples with a known surface area. Filter samples were dried at 70°C (maximal temperature of operation) for a week.

TABLE 1 Characteristics of the studied fibrous filters

Parameters	G4	M5	M6	F7	Combined F7
Materials of the filters	Cotton+polyester fibers	Polypropylene fibers	Polypropylene fibers	Glass fibers	Glass fibers and granular AC
Fiber diameter (μm) ^b	11±2	16±8	22±9	1.0±0.2	9±7
Thickness (mm) ^a	2.8±0.7	2.3±0.3	2.4±0.3	7.2±1.1	4.3±0.4
Porosity (%) ^a	92±1	82±1	81±2	96±2	86±3
Permeability $\times 10^{-10}$ (m^2) ^a	22±4	3.9±0.9	3.1±0.3	1.7±0.5	1.8±0.1
Basis weight (g/m^2) ^a	65±5	210±30	180±30	75±10	210±20

^aAverages and ranges for N=3.

^bAverages and standard deviations for N=50.

The M6 filter is an electret type, with charged fibers. The F7 combined filter is composed of a glass fiber layer and a granular AC layer; the structural properties were measured for the whole filter (both layers), except for the fiber diameter that concerned only the glass fiber layer.

2.2 | Filter clogging and fungi incubation

Samples of the studied filters were clogged using a dust generator (RBG 1000, Palas, Germany), with micronized rice particles naturally containing the wild strain *Penicillium chrysogenum*. This method of clogging generates both the substrate and the fungi in a dry manner. The particle size distribution of the aerosol was characterized with an aerodynamic particle counter (APS 3321, TSI, France), and the mass median aerodynamic diameter was $5.9\pm 0.1 \mu\text{m}$. The micronized rice particles were intended to be representative of outdoor PM_{10} in terms of size distribution but not with regard to chemical or biological composition. The initial concentration of *Penicillium chrysogenum* was 2250 ± 850 CFU per gram of micronized rice particles.³¹ Note that *Penicillium chrysogenum* was the only fungus found in the rice sample.

Clogging was carried out in a vertical cylindrical filtration column (diameter 45 mm) at the nominal filtration velocity of the filters, that is, 0.8 m/s for G4, 0.21 m/s for M5 and M6, 0.13 m/s for combined F7, and 0.3 m/s for F7. The filtration column was supplied by the dust generator (RBG 1000, Palas, Germany), and the flow was controlled with a flowmeter (4000 series, Mass Flowmeter 4043, TSI, France). Filter pressure drop was measured with a differential manometer. Tested filters were set up in a removable filter holder in the filtration column to preserve particulate cake integrity when they were moved. The experiments led to the clogging of six filter samples for each type of filter. Clogging was stopped when the filter pressure drop reached 50% of the maximal pressure drop recommended by the filter manufacturer, that is, 125 Pa for G4, M5, and M6 filters and 225 Pa for F7 and combined F7 filters. The fungal loading of the filters was calculated from the weight of particles collected by the filter samples after the clogging combined with the initial concentration of *Penicillium chrysogenum* in the rice of 2250 ± 850 CFU/g.

After clogging, all the samples in the filter holders were incubated in an airtight container for three weeks at 25°C and 90% RH to ensure optimal fungal growth.² Beakers of ionized water were placed in the airtight container to maintain the level of humidity, and the

RH value was measured every day with a temperature and humidity probe. Growth capacity was calculated based on Eq. (2), to estimate the growth of *Penicillium chrysogenum* in the filters.

$$\text{Growth capacity} = (\text{CFU}_{\text{after incubation}} - \text{CFU}_{\text{initial}}) / \text{CFU}_{\text{initial}} \quad (2)$$

where CFU are colony-forming units, and the fungal concentration is in CFU/cm^2 .

2.3 | Particle release quantification

After incubation, the filter holders containing the tested filter samples were put back in the filtration column to quantify the possible particle release during a simulated restart of ventilation at the nominal filtration velocity of the tested filters. At restart, the airflow was instantly turned on and a preset expansion valve was used to avoid an overshoot of the airflow setpoint. Two kinds of analyses were applied separately to quantify the released particles: firstly, in terms of fungal concentration, and secondly, in terms of total particle concentration and particle size distribution. Each analysis was repeated three times for each type of filter, and the results were expressed as the mean value and the standard deviation.

First, to estimate the released fungal concentration, a HEPA filter (AX3215, Lydall, France), H14 regarding EN1822:2009, was installed downstream of the tested incubated filter in the filtration column to collect all the micronized rice and fungal particles released. Prior to being used, the HEPA filter was dipped in ethanol and then dried for 24 hour at 70°C to prevent any contamination. After the ventilation system was restarted for 14 minutes, the incubated filter sample and the HEPA filter were analyzed by CFU counting to quantify the fungal concentration remaining in the tested filter samples and the concentration released during the test. The release tests were performed for 14 minutes because the last two particle-counting results were below 10 particles per cm^3 with the lowest efficiency filter. Fungi were extracted from the filters according to the method established by Forthomme et al.,² which consists of stirring the filters for 1 hour in 50 mL of extraction solution (MgSO_4 (0.01 mol/L), Tween-20 (0.25%)) then applying ultrasound for 1 minutes. Extracted solutions were diluted and spread on dichloran rose-bengal chloramphenicol agar plates (DRBC, Biokar Diagnostics, France). Finally, plates were incubated for three days at room temperature ($23\pm 2^\circ\text{C}$), and then, the

number of CFU was determined. The enumeration was carried out in duplicate for each extracted solution. The released fungal fraction was calculated from Eq. (3).

$$\text{Released fraction} = \text{CFU}_{\text{released}} / \text{CFU}_{\text{after incubation}} \quad (3)$$

where $\text{CFU}_{\text{released}}$ and $\text{CFU}_{\text{after incubation}}$ are the fungal concentrations in CFU/cm^2 in the HEPA collector filter and the tested filter, respectively. Secondly, the size distribution and the number concentration of the particles released were measured by isokinetic sampling every 2 minutes, in the optical particle diameter range of 0.25–17 μm , with a particle counter (PCS 2010, Palas, Germany). The time variation in the number concentration of particles released from the filters was estimated.

2.4 | Water retention capacity

The water retention capacity is one of the key parameters for the growth of fungi. It was determined for the five unused filters and the micronized rice particles by the following Karl Fisher method:

- First, the dry mass of samples was measured at 70°C. This temperature was chosen to dry the filters without altering their porous structure.
- Samples were then incubated at 25°C and 90% RH in an airtight container until weight stabilization was attained.
- Finally, the water contained in the samples was quantified with a Karl Fisher titrator (870 KF Titrino plus, 803 Ti Stand, 860 KF Thermoprop, Metrohm, France).

For each unused filter and for the micronized rice particles, three samples were analyzed. Water content was expressed as the weight of water as a function of the volume of the solid, that is, fiber volume for filters (filtering surface area \times filter thickness \times (1-filter porosity)) and particle volume for the micronized rice. It was also expressed as a weight fraction of the water retained.

2.5 | SEM observations

Scanning electron microscopy (SEM) observations were carried out for the G4 and F7 filters (lowest and highest efficiency filters out of all the tested filters) to confirm that unused filters were not contaminated before the tests. Moreover, clogged filters were also observed to confirm that *Penicillium chrysogenum* could grow in the filter

samples, particularly inside the filter fibers. For this test, the G4 and F7 filters were partially clogged (deep filtration step) to avoid the formation of a particulate cake and to promote fungal growth mostly inside the fibers. The filters were clogged up to 120% of the initial pressure drop, that is, 27 Pa for G4 and 63 Pa for F7. The clogged filters were incubated at 25°C and 90% RH in an airtight container for 1.5 months and then dried at 70°C to stop fungal growth. For SEM observations (JSM-5800, JEOL Europe, France), samples were coated with gold/palladium for 5 minutes.

2.6 | Data analysis

Concerning the fungal analysis on petri dishes, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on 1 and 10 CFU counted, respectively, on the zero dilution petri dish, corresponding to 31 and 310 CFU/cm^2 . In terms of statistical analysis, experimental data obtained from the different filters were compared using a one-way analysis of variance (ANOVA), which evaluated whether the experimental mean values obtained for the various filters were significantly different. If the null hypothesis for ANOVA was rejected (i.e., if the experimental mean values from the various tested filters were equivalent), the analysis was followed by the post hoc Scheffé test to determine which means actually differed, by comparing all possible combinations of two means. This conservative test was chosen because it is commonly used for unplanned comparisons.³³ The conclusions from the ANOVA and Scheffé test were made at the 0.05 level of significance.

3 | RESULTS AND DISCUSSION

3.1 | Comparison of water retention capacities

Water retention capacities of unused filters and micronized rice are presented in Table 2. The results reveal differences between the water sorbed by the various tested filters and the micronized rice particles. As expected, the results indicate that the combined F7 filter containing activated carbon (AC) has the highest water retention capacity, due to the adsorption of water by the AC. The water retention capacities (expressed in weight of water per volume of solid) of the G4, M5, M6, and F7 filters were compared using ANOVA followed by the Scheffé test. The combined F7 filter was not included in the comparison due to the presence of AC, which makes it highly hydrophilic. First, the G4 filter presenting a significant water retention capacity was tested versus the M5, M6, and F7 filters. The Scheffé test results

TABLE 2 Water retention capacity of unused filters and micronized rice particles in kg of water per m^3 of fiber or particle, and weight fraction of water retained in %

	Unused filters					Micronized rice
	G4	M5	M6	F7	Combined F7	
Water retention capacity ($\text{kg water}/\text{m}^3$ of solids) ^a	90 \pm 8	4.4 \pm 1.4	0.9 \pm 0.5	55 \pm 10	93 \pm 31	548 \pm 56
Weight fraction of water retained (%) ^a	31 \pm 2	0.9 \pm 0.3	0.2 \pm 0.1	20 \pm 4	26 \pm 9	38 \pm 4

^aAverages and ranges for N=3.

support the hypothesis that the water retention capacity of the G4 filter is significantly different from that of the M5, M6, and F7 filters. This result is explained by the presence in the G4 filtering medium of hydrophilic cotton fibers mixed with polyester fibers. The M5 and M6 filters, composed of polypropylene fibers, reveal a lower water retention capacity than the G4 filter in accordance with the literature, which indicates that polypropylene has a poor wetting surface with a contact angle of 92° for a flat surface.³⁴ In general, when a liquid is in contact with the surface of a solid, a contact angle of the first over the second is formed. A liquid will wet a solid easily if the latter has a higher surface energy than the liquid. In fact, the liquid adhesion to the solid surface will be greater than the cohesion of the liquid. When wetting is perfect, the contact angle becomes zero. In this case, the adhesion energy is maximal. The Scheffé test comparison did not support the difference in mean water retention capacity between the M5 and M6 filters. Regarding the F7 filter, its water retention capacity seems to be higher than the M5 and M6 filters, which can be explained by the contact angle of 0° between the glass fiber cloth and water droplets.³⁵ The Scheffé test showed that the mean water retention capacity of the F7 filter is significantly different from that of the M5 and M6 filters. To conclude, the results indicate that for fibrous filters, the nature of the fibers and the presence of adsorbent materials can significantly influence the capacity of water sorption. The water retention capacity of the material can be related to the water activity needed for fungal growth. Hocking and Pitt³⁶ determined the minimal water activity for *P. chrysogenum* growth to be 0.78. Moreover, if there is sufficient nutrient, microorganisms need an RH above 70% to grow in static conditions.^{6,22} Comparing the weight fraction of water retained, the G4, F7, and combined F7 filters adsorb more than 20% of their initial weight in water. Hydrophobic filters (M5 and M6) adsorb

less than 5% of their weight in water. These results indicate that the combined F7 filter, the G4 filter, and, to a lesser extent, the F7 filter constitute favorable media for fungal growth because they are able to hold sufficient water when exposed to moist air.

Finally, a large amount of water is sorbed by micronized rice particles, which means that these can provide the water needed for fungal growth even with hydrophobic filters. In fact, Hamada and Fujita²⁷ observed that the accumulation of dust promotes moisture retention and the growth of fungi. Accumulation of water in the particulate cake may also reduce the impact of the ventilation system on the fungal population decrease by protecting fungi from the airflow. Note that the urban aerosol could have a less significant water retention capacity, depending on the nature of the particles.

3.2 | SEM observation of fungal growth

Figures 1 and 2 show the SEM pictures of unused and clogged-incubated filters. Both figures reveal that the unused filters, G4 and F7, were not initially contaminated by *Penicillium chrysogenum*. However, fungi are clearly identified inside the fibers of the clogged-incubated filters as well as the micronized rice particles, which demonstrates fungal growth in the clogged filters. Moreover, the size of *Penicillium chrysogenum* spores was estimated from 50 analyzed spores from SEM pictures; the mean value and standard deviation obtained was $2.2 \pm 0.6 \mu\text{m}$, which is comparable to the mean diameter of $2.0 \pm 0.5 \mu\text{m}$ measured by Righelato et al.³⁷

A quantitative comparison of fungal growth in G4 and F7 filters is not relevant from SEM observations, but they do enable the size of micronized rice particles, fungal spores and fibers to be compared visually.

FIGURE 1 SEM pictures of unused (left) and clogged-incubated (right) G4 filters

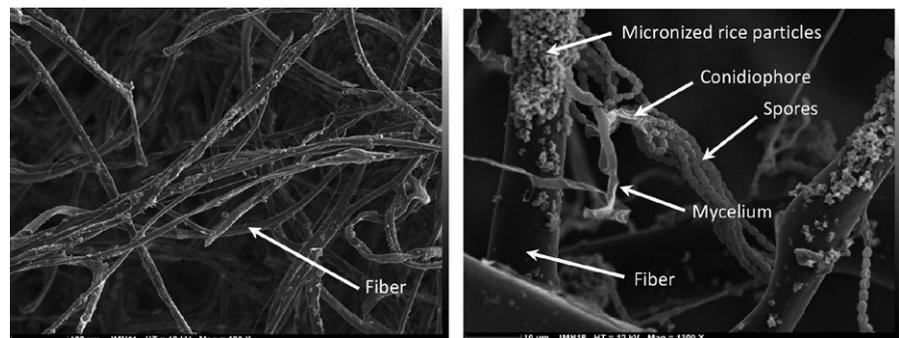
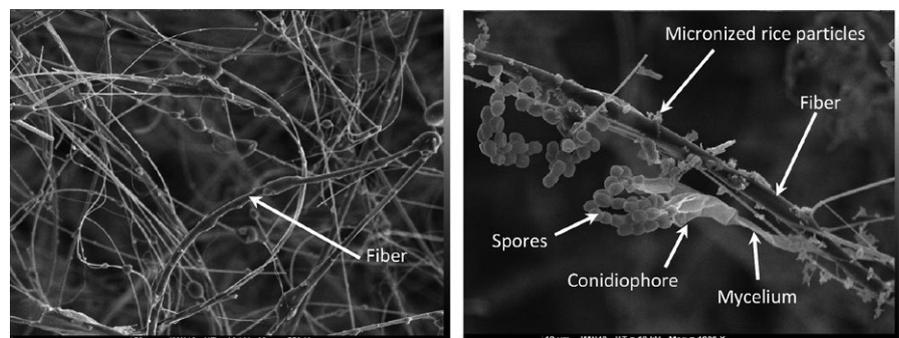


FIGURE 2 SEM pictures of unused (left) and clogged-incubated (right) F7 filters



3.3 | Quantification of fungal growth in filter samples

The weight of micronized rice particles on clogged filters, the initial fungal concentration, the fungal concentration after incubation, and the growth capacity are presented in Table 3. The weight of deposited micronized rice particles and the initial CFU load are different according to the filter because clogging was stopped at 50% of the maximal pressure drop. The initial fungal concentrations and the fungal concentrations after incubation were compared for each filter by the Scheffé test. The results support the hypothesis that the fungal concentrations after incubation are higher than the initial fungal concentrations in the different filters. This means that the incubation step enabled fungal growth in the filters.

Table 3 indicates that the growth capacity (Eq. (2)) is higher than 10^4 for all filters. The growth capacity is also presented in Fig. 3 as a function of the theoretical overall weight of water sorbed by a clogged filter surface. This weight was calculated based on the water sorbed by the clean filter and by the micronized rice particles (Table 2). The results show that, except for the combined F7 filter that contains AC, the growth capacity increased with the amount of water possibly retained until a threshold value of water sorbed. This means that water

retention is a limiting factor for fungal growth until the water retention capacity is equal to or higher than the threshold value. Consequently, knowing the water retention capacity could help to limit fungal proliferation on clogged filters. For the hydrophobic filters, for example, the studied M5 and M6, more than 90% of the weight of water sorbed by the clogged filter surface was associated with the particulate cake. This confirms the hypothesis that the particulate cake can change the hydrophobic property of a filter and enhance fungal growth as shown by Hamada and Fujita.²⁷

Concerning the combined F7 filter, fungal growth capacity was lower than expected based on the weight fraction of water retained. This finding can be explained by the fact that the filter is divided into two layers, one of glass fibers and another of granular AC. Regarding the high particle collection efficiency of the combined F7 filter (c.f. §3.4, Fig. 5), most of the particles, particularly the fungi, are collected by the glass fibers and cannot reach the AC containing water. Thus, the water adsorbed by the AC layer is not available for the fungi. Moreover, a Brunauer–Emmett–Teller (BET) analysis (performed with ASAP 2020, Micrometrics, France), which measures the specific area and the porous volume of sorbent materials, showed that the AC of the F7 filter has a microporous volume of $0.26 \pm 0.07 \text{ cm}^3/\text{g}$ and a mesoporous volume of $0.03 \pm 0.05 \text{ cm}^3/\text{g}$. These data indicate that

TABLE 3 Weight of collected rice particles, initial and after incubation fungal concentration in studied filter samples, fungal growth capacity in the filters, and fraction of *Penicillium chrysogenum* released from filter during ventilation restart

	Filters				
	G4	M5	M6	F7	Combined F7
Weight of collected rice particles (g/m^2) ^a	144±14	75±15	71±13	51±5	135±23
Initial fungal concentration (CFU/cm^2) ^a	33±3	17±2	16±2	12±1	30±4
Fungal concentration after incubation (CFU/cm^2) ^a	$(1.6 \pm 0.1) \times 10^8$	$(2.1 \pm 0.5) \times 10^6$	$(1.3 \pm 0.3) \times 10^5$	$(4.2 \pm 0.9) \times 10^7$	$(5.4 \pm 0.6) \times 10^6$
Growth capacity (–) ^a	$(4.8 \pm 0.5) \times 10^6$	$(1.2 \pm 0.3) \times 10^5$	$(1.4 \pm 0.4) \times 10^4$	$(3.7 \pm 0.9) \times 10^6$	$(1.8 \pm 0.2) \times 10^5$
Fraction of CFU released (%) ^a	1.3±0.4	$(0.74 \pm 0.58) \times 10^{-1}$	$(1.3 \pm 0.2) \times 10^{-1}$	$(2.8 \pm 1.2) \times 10^{-3}$	$(1.2 \pm 0.3) \times 10^{-3}$

^aAverages and ranges for N=3.

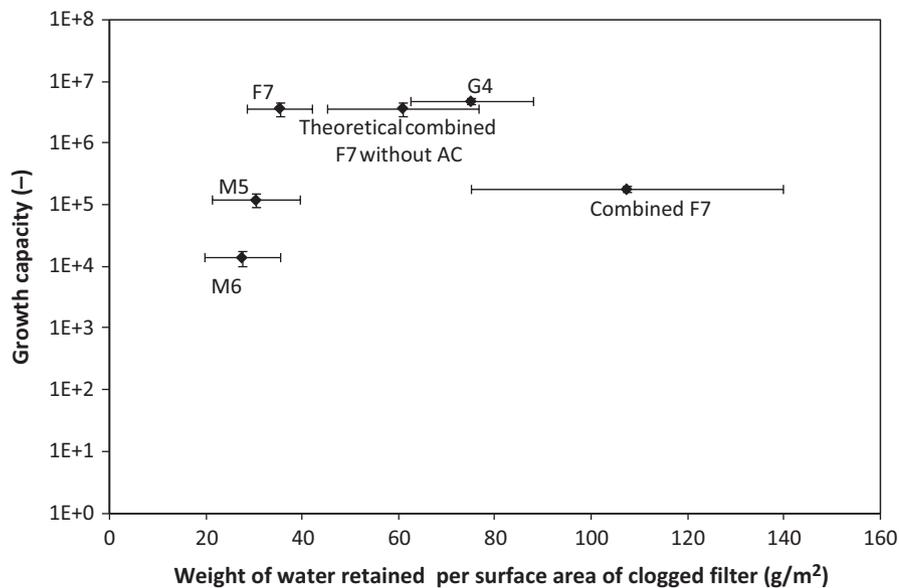


FIGURE 3 Growth capacity as a function of the weight of water retained by filters G4, M5, M6, F7, combined F7, and theoretical combined F7 without AC (averages and ranges for N=3)

the AC is mainly microporous so that the pore diameters available for water adsorption are less than 2 nm.³⁸ Consequently, as the size of *Penicillium chrysogenum* spores is $2.2 \pm 0.6 \mu\text{m}$, the fungal particles that reach the AC in the filter cannot access the amount of water sorbed by it. This means that even if a combined filter sorbs a large amount of moisture, the water is not completely available to the fungi, depending on the porous structure of the AC, which decreases the growth capacity of the filter. Furthermore, if water is sorbed in a large amount in the combined filter, it can induce significant competition with the sorption of gaseous pollutants.

The theoretical weight of water retained by the combined F7 filter was also calculated assuming that it did not include granular AC. This theoretical value is plotted in Fig. 3, which shows that (i) the growth capacity is equal to that of the F7 filter, (ii) the micronized rice particle load is the same as for the combined F7, and (iii) the weight fraction of water contained in the filter is equal to that of F7. Thus, despite the high uncertainty regarding the weight of water retained, the theoretical value calculated for the combined F7 filter without AC follows the same tendency as the other filters that do not contain AC. This observation confirms that (i) the growth capacity of a clogged filter has a threshold value regarding the water fraction and (ii) the water sorbed in the AC is not completely available to the fungi, depending on the porous structure of the sorbent. Furthermore, it is important to note that this study was carried out in specific laboratory conditions, particularly for the filter incubation; that is, the ventilation was stopped, the RH was higher than 90%, and incubation was longer than a week. In the literature, Pasanen et al.³⁹ obtained a maximal growth capacity of 100 after 35 days of incubation at 98% RH, while Forthomme et al.² measured a growth capacity of between 10^2 and 10^3 with F7 filters incubated at 90% RH. The growth capacity of 10^4 obtained in the current study may be explained by the choice of the couple of micronized rice and its endogenous fungi *P. chrysogenum*. In full-scale HVAC, the growth of fungi in filters would not be so high because ventilation stops are shorter. Moreover, the optimum growth conditions would probably not occur, except in particular cases of ventilator

default, moisture damage, an overextended ventilation stop, inappropriate filter storage, extremely wet weather, etc.

3.4 | Quantification of fungal particles released

The concentrations of fungi in the filters after incubation and released after ventilation restart (measured over 14 minutes) are presented in Fig. 4. The results show that a significant amount of fungal particles was released from all the filters. To compare the different filters, the fraction of fungi released was calculated (Eq. (3)) and the results are presented in Table 3. These fractions are quite comparable to those measured in the literature. Forthomme et al.² observed a released fraction for two F7 filters of $0.082 \pm 0.054\%$ for a glass fiber filter and $0.0075 \pm 0.0013\%$ for a polypropylene filter. Jankowska et al.³⁰ observed a released fungal fraction from F5 and F8 filters of between 0.009% and 0.33%, which is the same order of magnitude as the values in Table 3.

The fractions of fungi released from the 5 different filters were compared by ANOVA followed by the Scheffé test. Pairwise comparisons showed that the only statistically significant differences are between the G4 and all the other filters. This result may be explained by the fact that the G4 filter is composed of cotton fibers, which can be used as nutrients by the fungi and thus degrade filtration performance.^{22–24,39} Regarding the M5, M6, F7, and combined F7 filters, the Scheffé test did not indicate significant differences between their microbial fractions released. Two other comparisons (M5 and M6 versus F7 and combined F7; M6 versus F7 and combined F7) supported the same conclusion. These observations indicate that the fraction of fungi released tends to be significantly higher from low efficiency filters.

During filter clogging, *Penicillium chrysogenum* spores or fragments were mostly transported by the fraction of fine micronized rice particles (with a diameter $< 1 \mu\text{m}$) because of their specific surface area. These particles are close to the MPPS range of the filters, between 0.1 and $0.4 \mu\text{m}$, in which the particle collection efficiency is the lowest

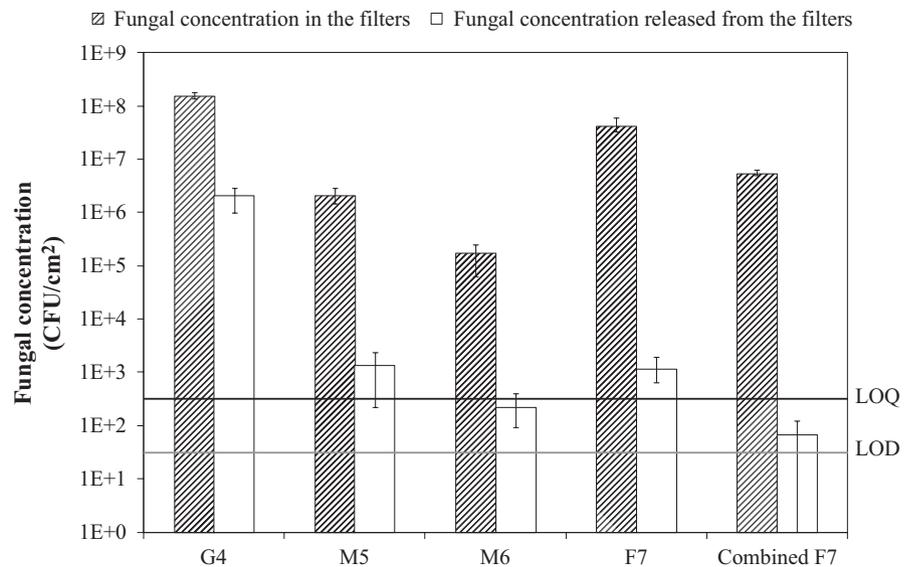


FIGURE 4 Fungal concentration in studied filter samples after incubation and released during ventilation restart (averages and ranges for N=3)

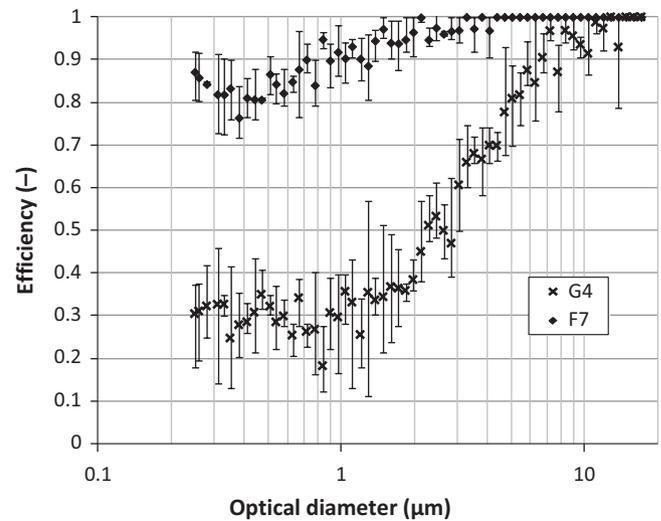
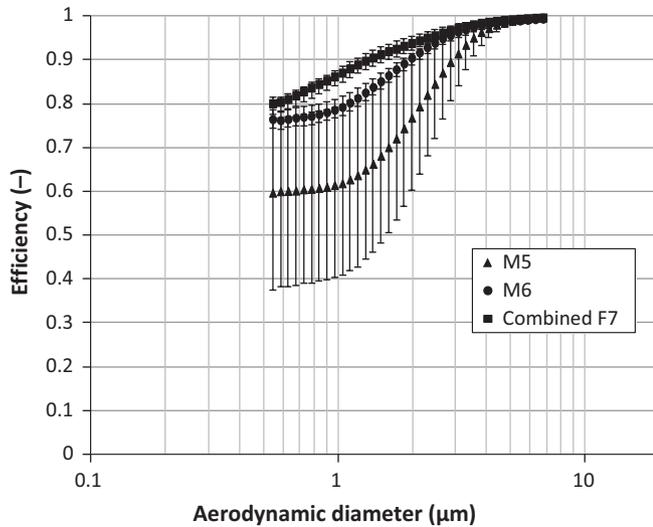


FIGURE 5 Particle collection efficiency during the in-depth filtration stage as a function of particle diameter. Left: M5, M6, and combined F7 filters; right: G4 and F7 filters (averages and ranges for N=3)

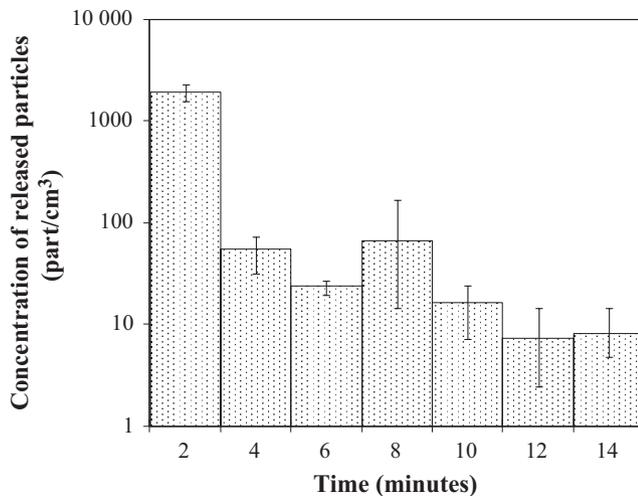


FIGURE 6 Time variation in the particle concentration downstream of the G4 clogged-incubated filters for 14 minutes of ventilation restart (averages and ranges for N=3)

and the particle penetration is the highest. Figure 5 presents the particle collection efficiency of the M5, M6, and combined F7 filters as a function of the aerodynamic particle diameter (from particle sampling and measuring in the range of 0.5–20 μm with an APS 3321, TSI, France), and the particle collection efficiency of the G4 and F7 filters as a function of the optical particle diameter (from particle sampling and measuring in the range 0.25–17 μm with a PCS 2010 Palas). Note that the amount of particles counted downstream of the F7 filter was about 50 000 particles over 2 minutes (about 50 particles/ cm^3), so that the fractional efficiency could be calculated correctly. Efficiency was calculated at the beginning of clogging, that is, during the in-depth filtration stage, at the nominal filtration velocity with micronized rice particles representing PM_{10} (mass median aerodynamic diameter of $5.9 \pm 0.1 \mu\text{m}$). In particular, the results show that the filtration efficiency of the M6 filter was significantly higher than that of the M5, explained

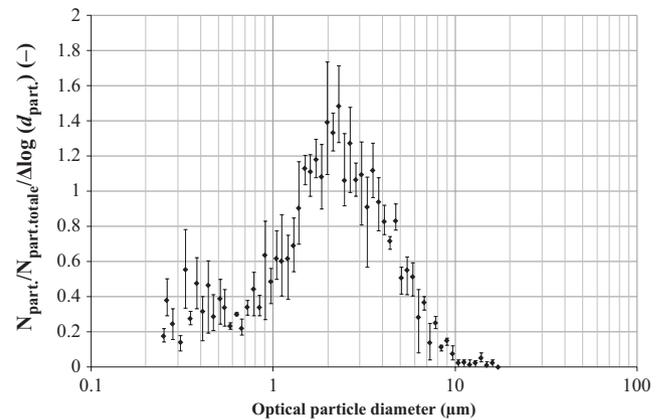


FIGURE 7 Particle size distribution of the particles released from the G4 filter during the ventilation restart (averages and ranges for N=3)

by the presence of electrostatic charges. The figures illustrate that the particle collection efficiency for the MPPS is a function of the filter efficiency, which means that the depth of penetration of fine particles inside the filter depends on the filter efficiency. Thus, fungal particles will proliferate deep inside the filter, and a significant proportion of them will be released at the ventilation restart depending on the filtration efficiency of the fine particles. The lowest filtration efficiency of the G4 filter demonstrated in Fig. 5, in comparison with the other tested filters, is in accordance with the experimental results of fungal particles released.

Note that in this study, filters were over-incubated for three weeks to observe a significant amount of fungi released downstream of the tested filter. This over-incubation may lead to a proliferation of *P. chrysogenum* on the downstream side of the filter. Moreover, the ventilation starting method used in the study corresponded to a pulse of air, whereas in real HVAC systems, the methods may be different, for example, a gradual increase in the airflow rate. These two

experimental conditions may promote the release of fungal particles. In HVAC systems, two filtration stages with a G4 filter (1st stage) and an F7 filter (2nd stage) are commonly found.¹ Based on the previous results, a large amount of fungal particles could be released from the G4 filter, but these should be collected by the downstream F7 filter. Few fungal particles should be released from the F7 filter, depending on the initial load. Consequently, the overall fraction of released fungal particles is expected to be lower in two-stage than in one-stage filtration because the first stage will reduce the fungal concentration in the second stage, which will decrease the final amount of released particles. For example, based on the previous results, if the G4 and F7 filters were implemented in two filtration stages, the fraction of fungal particles released downstream of the process would be less than 10⁻⁴%. This result is in accordance with experiments in a lab-scale AHU performed by González et al.,³¹ which showed that during restarts of ventilation no fungal aerosols were detected downstream of a two-stage filtration system (G4+F7).

3.5 | Quantification of particles released

Figure 6 illustrates the time variation in the particle concentration downstream of the G4 filter during the ventilation restart. A pulse of particles appears for the first 2 minutes (about 790 particles counted) followed by a rapid decrease (about 20 particles counted in the next 2 minutes) before stabilizing at three particles counted, in the optical particle diameter range of 0.25–17 µm. The filtration column was supplied with unfiltered air containing particles, and the remaining concentration (below 10 part/cm³) corresponds to the particles present in the filtration column that were not collected by the filter. Assays were not long enough to confirm the stabilization of the particle concentration downstream of the filter over time.

The particle size distribution of the total released particles over the 14 minutes of the ventilation restart for the G4 filter is illustrated in Fig. 7. The results show a major modal diameter at about 2.0 µm, which could correspond to the fungal spores (identified as 2.2±0.6 µm by SEM). Figure 7 also shows a minor modal diameter, close to 0.5 µm, which could be associated with the micronized rice particles (whose mean diameter by optical counting is 0.6 µm).

Regarding the M5, M6, F7, and combined F7 filters, the particle counter detected no particles, indicating that particle release was below the detection limit. This could mean that the amount of particles released is higher for a low efficiency filter in accordance with the previous result for fungal particle release. These observations were obtained for a specific type of loading. In real conditions, the range of particle sizes is wider, which could modify the particle release.

4 | CONCLUSIONS

Water retention capacities of five unused filters of various efficiencies and of micronized rice particles were quantified. The results indicate the possibility of fungal growth in filters. SEM pictures clearly show the presence of mycelium and spores on fibers as well as particles

after the loading and incubation of the filter, which illustrate fungi proliferation. Moreover, the fungal growth in a clogged filter is linked to the water sorbed in the filter. In fact, a threshold value of water sorbed provides a maximal growth capacity. This threshold value gives information about the amount of water needed for fungal proliferation in the filter. For example, to prevent fungal growth in the filter, thermal treatment could be used to keep the moisture low inside induct AHUs.

Particles, especially fungal ones, were released from the filters pre-loaded with micronized rice particles containing *Penicillium chrysogenum* when the controlled ventilation system was restarted. A pulse of particles was identified by particle counting during the first minutes of the ventilation restart for the least efficient filter. The results indicate that the amount of fungal particles released from the filters during ventilation restart in the laboratory tends to be higher for a low efficiency filter. Moreover, this study serves as a comparison of different types of filter based on the growth capacity of the fungi and on the released fraction in the same experimental conditions. It gives important information about the risk of fungal contamination and the lifetime of the filter. In a real HVAC system, the filter exposure to humidity, which is closely related to fungal growth, and the fungal fraction released could become important parameters to determine when filters need to be changed.

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