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# **SFGP 2007 - Microbial Growth onto Filter Media Used in Air Treatment Devices**

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# SFGP 2007 - Microbial Growth onto Filter Media Used in Air Treatment Devices

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## Abstract

This work deals with microbial growth onto filter media and focuses on the ability of microbial communities to proliferate onto filter media. Two microorganism types are studied: microorganisms from activated sludge of wastewater treatment plant (SM) and a toluene specific consortium (TSC). The filter media considered for this study contain activated carbon fibres (ACF), combined volatile organic compounds (VOC), particles treatment purposes, activated carbon fibres felt (ACFF) and activated carbon and cellulose fibres felt (AC<sub>2</sub>F<sub>2</sub>). Using a static growth procedure during 10 days under 100 % relative humidity, artificially contaminated filters are submitted to microbial colonisation. The final concentration of microorganisms per gram of filter have been assessed using a method developed in the lab, based on filter protein content assay. The average surface charge of inocula and filter's fibres are measured to assess the influence of microorganisms adhesion on contamination. The influence of soot particles on TSC proliferation onto AC<sub>2</sub>F<sub>2</sub> filter is then studied. Zeta measures enable the assessment of the implication of soot in microorganisms adhesion onto filter fibres. Consequences of microbial contamination on filter permeability and downstream particles released have then been assessed in a filtration device. Results demonstrate a better resistance of AC<sub>2</sub>F<sub>2</sub> to microbial colonisation. However, SM have more difficulties to proliferate on ACFF than TSC, whereas SM colonise easier AC<sub>2</sub>F<sub>2</sub> than TSC. Charge surface assay has defined an optimal electrostatic compatibility for TSC and AC<sub>2</sub>F<sub>2</sub> and a minimum for SM and ACFF. When soot is added to TSC solution before introduction in AC<sub>2</sub>F<sub>2</sub>, high contamination shapes were observed whereas only a slight one occur without soot addition. Zeta potential measures show favourable charge conditions for adhesion of soot on AC<sub>2</sub>F<sub>2</sub> fibres and TSC on soot particles. The soot may thus have played an interface role in microbial adhesion onto media. This means that electrostatic compatibility between particles is a good approach for assessing microbial adhesion onto filters but could not explain the whole mechanism of microbial proliferation. Other parameters like nutrition

preferences are certainly involved. The contamination have induced filter characteristics modification. Permeability have decreased until 20 % with microbial concentration. Colonised filters have released up to 450 microbial particles/cm<sup>3</sup>. Such a release was only observed when AC<sub>2</sub>F<sub>2</sub> was contaminated with fungi using spore as the reproduction vector (inoculum containing TSC and soot).

**KEYWORDS:** air filter, bioaerosols, contamination assess, permeability, biological particle release

## 1. INTRODUCTION

Airborne microorganisms are associated with numerous health hazards from minor asthma to various cancers (Douwes et al., 2003). The risk level is related to exposure, that is why sources management is critical to the prevention and control of biocontamination (Herr et al., 2003).

An increase of complaints from long-period building residents focused the interest on the so called “sick building syndrome” (Shoemaker and House, 2006). The exact causes of the symptoms remain unknown but different studies have revealed the implication of bioaerosols (Wilson and Straus, 2002; Ahearn et al., 2004). Increase of airborne biological particles concentration in buildings could be due to microbial release from contaminated moist materials like walls, carpet or curtains but the main part of indoor bioaerosols come from outdoor. In consequence, the ventilation system is the major way to control indoor concentration because its filter retains the main part of microorganisms flow from the outside (Möriz et al., 2001; McLarnon et al., 2006) with an efficiency close to non-biological particle efficiency (Maus and Umhauer, 1997). Both biological and non-biological particles accumulate onto the filtering media. Since some microorganisms remain viable after impacting on the filter surface (Maus et al., 2001) they are able to grow when the environment provide favourable climatic conditions (relative humidity > 90 %, temperature close to the atmospheric one) using other particles as nutrients (Chang et al., 1996). Hence, HVAC filter can become an important source of bioaerosols by releasing produced microorganisms and metabolites (Jankowska et al., 2000; Möriz et al., 2001).

Moreover, some researches assume that microbial proliferation onto filters can lead to a reduction of their performances because the structural integrity of the media is modified (Simmons et al., 1997). It had thus been observed, for a microfibre glass acrylic filter, an increase of the most penetrating particle size (MPPS) and resistance to the flow (pressure drop through the filter) respectively up to 500 % and 35 % (Verdenelli et al., 2003).

In this context, the aim of the following study is to evaluate the colonisation level of two air filters (non-woven felts containing activated carbon fibres (ACF); a commercial activated carbon fibre felt (ACFF) and a activated carbon and cellulose fibres felt (AC<sub>2</sub>F<sub>2</sub>) prototype), both contaminated in laboratory conditions. Permeability changes and the microbial particles release downstream of the filters are studied in order to assess the influence of colonisation level on the evolution of filtration performances. A focus on charge compatibility between filter fibres, microbes, and soot particles enable to formulate hypothesis about microbe adhesion and nutrition mechanisms onto fibres.

## 2. MATERIAL AND METHODS

### 2.1 Filters characteristics

Two filter media are considered here:

- Activated Carbon Fibres Felt (ACFF). A 100 % ACF felt (Actitex<sup>®</sup> FC1201) previously studied in laboratory for volatile organic compounds (VOC) adsorption (Lorimier et al., 2005).
- Prototype Felt composed of Activated Carbon and Cellulose Fibres (AC<sub>2</sub>F<sub>2</sub>) which contain 50 % of ACF and 50 % of cellulose fibres for combined treatment of VOC and particles (Rochereau et al., 2006).

Maximum water retention of a filter is considered to be the weight difference between a dry filter and the same saturated with water. This parameter is then used to fill the media with a volume of microbial suspension close to its maximum water holding capacity.

An estimation of organic carbon (OC) and inorganic carbon (IC) released in water by the filters has been performed using total organic carbon analyser (SHIMADZU<sup>®</sup> TOC-5000A). Samples are obtained from media immersed 8 days in milliQ water under agitation. Solutions were then filtered using a glass fibre filter (Whatman<sup>®</sup> N<sup>°</sup> 1825 025) in order to eliminate fibres before measurement. This consideration enables to define the ability of media to provide carbon, used during microbial growth.

Table 1. Water retention capacity of filters and carbon release into milliQ water

	Water holding capacity (g milliQ water/g filter)	Relative dissolved organic carbon (ROC) (mgC/g filter)	Relative dissolved inorganic carbon (RIC) (mgC/g filter)
ACFF	10.20 +/- 0.28	0.01 +/- 0.07	0.00 +/- 0.07
AC <sub>2</sub> F <sub>2</sub>	10.70 +/- 0.53	0.41 +/- 0.13	0.05 +/- 0.07

### 2.2 Inocula characteristics

Two types of microbial consortia are considered in this study:

- The first consortium (SM) corresponds to activated sludge microorganisms from a wastewater treatment plant cultivated in non specific nutrient broth (tryptone, 10 g/L; meat extract, 5 g/L; sodium chloride, 5 g/L), centrifuged (2000 times the atmospheric pressure) during 15 minutes and washed (MgSO<sub>4</sub>; 0.01 M) two times to eliminate the dissolved organic fraction.

- The second consortium (TSC) is prepared from refinery activated sludge and has been specified for aromatic compounds degradation through several growths with toluene as sole carbon source in a minimum nutrient solution (MNS: 0.18 g/L of  $\text{NH}_4\text{Cl}$ ; 0.045 g/L of  $\text{KH}_2\text{PO}_4$ ).

Each microorganisms consortium is then mixed with a concentrated minimum nutrient solution ( $10^{10}$  CFU/L; 1.1 g/L of  $\text{NH}_4\text{Cl}$ ; 0.3 g/L of  $\text{KH}_2\text{PO}_4$ ). The two suspensions are used as contamination inoculum. However, the main element needed for growth is carbon. Experiments with and without soot particles (1 g/L) within the inocula allow the evaluation of filter ability to provide carbonaceous nutrients and the faculty of microorganisms to consume soot carbon.

### 2.3 Static microbial growth simulation

The objective of this step is to simulate microbial growth onto filters in controlled conditions (batch reactor). First, inoculum is introduced in the sterile fibrous media sample. The inoculum volume corresponds to the maximum water holding capacity of each filter to simulate 100 % hygrometry conditions (table 1). Then, the sample is introduced in a sterilised glass reactor as shown in figure 1. A constant temperature of 25 °C is maintained during 10 days using a climatic chamber. Once the experiment completed the contaminated filter is placed in a sterile Petri dish to be dried at 50°C during 48 hours.

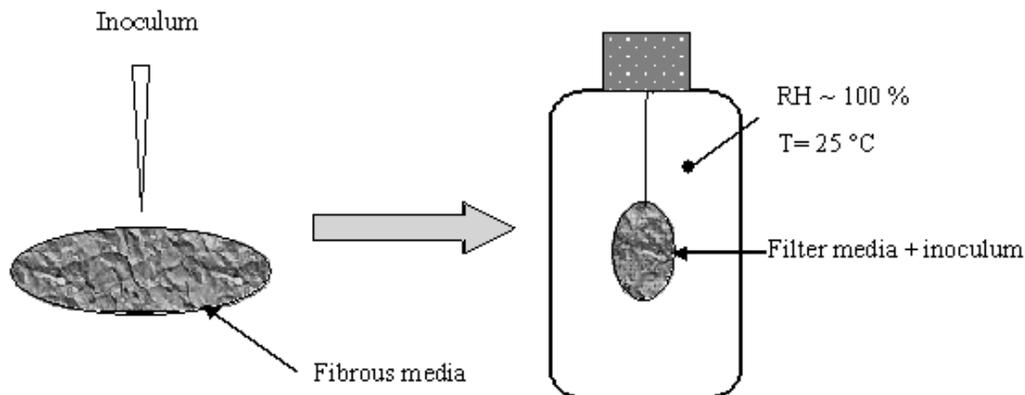


Figure 1. Static growth procedure

## 2.4 Contamination assessment

For a rapid quantification of microbial proliferation onto dried filter media, an indirect measurement method has been elaborated. The objective is to avoid usual cultural methods (Nester et al., 1995) which first necessitate an extraction of microorganisms from the medium to a suspension when applied to air filters. A spate of dilutions of this suspension is realised and each of them is spread out and cultivated onto nutrient agar Petri dishes to form visible colonies.

By assessing the number of colonies on the Petri dish, knowing the volume of inoculated suspension and the dilution, the cultivable microorganisms concentration of the extracted suspension can be defined. This concentration is expressed in colony forming unit (CFU) per mL. This takes into account the fact that microorganisms are usually in agglomerate form and consequently that a colony does not come from a single microorganism growth but rather from a conglomerate.

However, the method contains several limitations, like the time necessary to obtain the result, the restriction to cultivable microorganisms assessment, the microbial death during extraction or the extraction efficiency. Moreover, it cannot be exploited in such a study because the same filter has to be used for the assessment of filtration parameters and colonisation level. As the contamination assessment is destructive for the sample, filtration test has to be performed first but is lethal for microorganisms (introducing an error on the CFU measure).

The method proposed allows to assess the quantity of microorganisms inside a filter using a measure of filter protein content, which is independent of the microbial cultivability and can be performed after a filtration test. To express the result in microorganisms per cubic meter of contaminated filter, a calibration curve has been built giving a correlation between the microorganisms concentration of microbial suspensions (measured using traditional CFU count (B method of the figure 2)) and their protein content (A method on figure 2). This curve shows a linear evolution with a slope of  $4.6 \times 10^7$  CFU/ $\mu\text{g/ml}$ .

Then, to measure filter microbial contamination, 3 samples (1 cm diameter disc) are taken. They are dived in 1 ml of sodium chloride (1 M) to dissolve their protein content. The suspension is then filtered (Sartorius<sup>®</sup>, 0.45  $\mu\text{m}$ ) to eliminate filter fibres and particles in suspension which can come from the filter sample and could interfere with the protein assessment. Proteins in solution are then assessed using the commercial colorimetric method used in the “A” method presented on figure 2 (BSA protein assay kit - Pierce<sup>®</sup>). The average among the three samples is considered to be the filter surface mean concentration of proteins. Finally, the microbial concentration onto the filter is deduced from the calibration curve and thus expressed in CFU/m<sup>2</sup>.

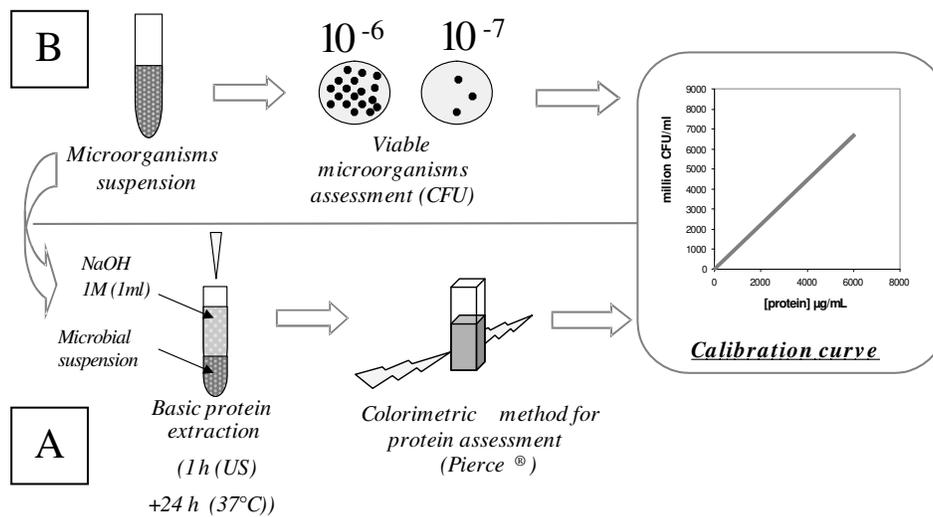


Figure 2. Protocol of CFU (B) and protein (A) concentration correlation.  
*US: ultrasonic irradiation (20 kHz)*

Details on the method can be found in our previous work (Bonnievier et al., 2007).

## 2.5 Zeta potential analysis

Considering the static growth protocol (figure 1), the slide of microorganisms from the surface of the filtering media to the bottom of the reactor is possible if microbes do not adhere rapidly to the fibres. Zeta potential can give information on the electrostatic attraction between microorganisms and filters. To measure a stable surface charge potential, suspensions of fibres, microbes and soot are suspended into the MNS and agitated during 48 h. Surface charge is assessed using a Zetaphotometer (CAD Instrumentation® - Zetaphotometer 4.30).

## 2.6 Filtration test

The filtration device presented in figure 3 allows the measurement of filter permeability changes and microbial release when a filter sample is colonised. The device allows clean air to pass through the contaminated filter at a given flow rate, which can be regulated from 0 to 3000 L/h. This flow corresponds to a 0 to 0.52 m/s air velocity upstream the filter and is in agreement with the ASHRAE 52.1 american standard (1992) which proposes a front air velocity between 0.5 and 2 m/s during filtration test. Moreover, the air velocity range experimented is representative of the one usually used in buildings filtration devices.

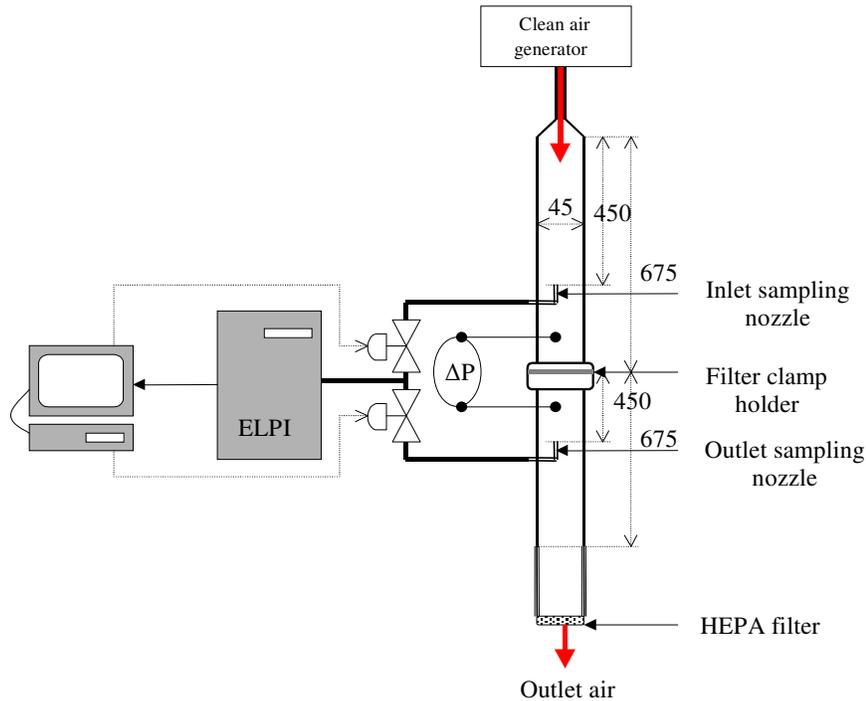


Figure 3. Filtration device

### 2.6.1 Permeability measurement

The filtration device is equipped with two static pressure gauges. One in the inlet and the second in the outlet of the filter holder clamp in order to assess the pressure drop through the tested filter. By applying various flow rates, corresponding to linear laminar regime, Darcy equation (1) enables to calculate the filter permeability ( $k$ ) in  $m^2$ .

$$k = \eta \cdot \frac{Q}{S} \cdot \frac{\Delta x}{\Delta P} \quad (1)$$

### 2.6.2 Microbial release from contaminated filters

Microbes released by contaminated filter are quantified in the filtration device described on figure 3. The procedure consists in generating a flow ramp of clean air through the filter from 0 to 3000 L/h in 30 seconds. The flow is then maintained at 3000 L/h until the particle count remains stable. An isokinetic sampling of air downstream of the filter is performed using a sampling nozzle. The sampled air is analysed in an electric particle counter (DEKATI<sup>®</sup>ELPI 4.0) which measures the quantity and the size distribution of sampled particles.

### 3. RESULTS

#### 3.1 Permeability changes

The permeability change caused by the microbial colonisation of the filter (for an initial microorganism loading concentration of  $(1.0 \pm 0.2) \times 10^8$  CFU.m<sup>-2</sup>) is represented here by the ratio between the permeability of contaminated filter ( $k$ ) and clean filter one ( $k_0$ ), called dimensionless permeability ( $k/k_0$ ). The results summarised on figure 4 were obtained without the addition of soot particles and show the dimensionless permeability as a function of surface microbial concentration for the two media considered. It reveals a decrease of the dimensionless permeability with the contamination level up to 20 %. An explanation could be the pore obstruction when microorganisms colonise the filter medium. Considering the influence of the filter medium, ACFE seems to be more easily colonised than AC<sub>2</sub>F<sub>2</sub> by the two consortia.

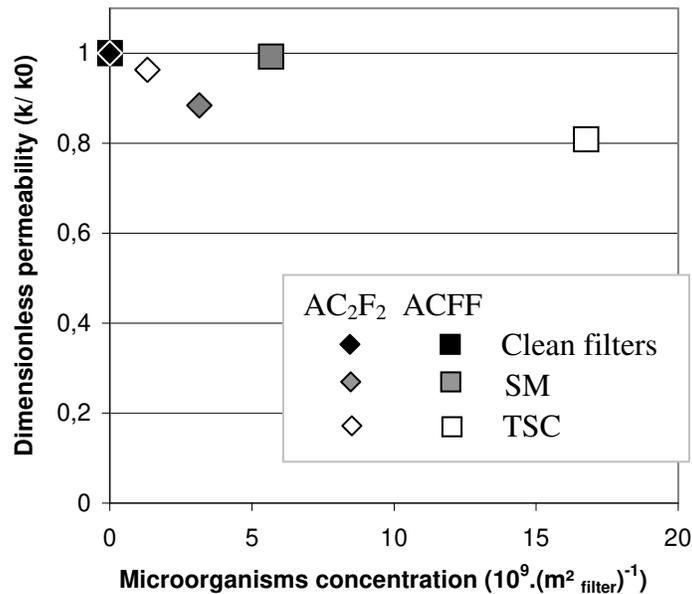


Figure 4. Influence of filter contamination level on dimensionless permeability

Nevertheless no tendency of the inoculum influence is shown because the AC<sub>2</sub>F<sub>2</sub> medium is more colonised by the SM consortium whereas it is the TSC which developed preferentially in the ACFE medium.

## 3.2 Influence of soot particles

### 3.2.1 Influence on contamination level

Considering TSC consortium and AC<sub>2</sub>F<sub>2</sub> medium, static growth tests with and without soot particles within the inoculum were conducted in triplicate to evaluate soot influence on microorganisms proliferation. The surface microbial concentrations measured are presented on table 2.

Table 2. Influence of soot within TSC inoculum<sup>a</sup> on AC<sub>2</sub>F<sub>2</sub> colonisation

	Without soot	With soot (1 g/L)
Contamination level (CFU.m <sup>-2</sup> )	$(1.3 \pm 0.6) \times 10^9$	$(7.2 \pm 1.3) \times 10^{11}$

<sup>a</sup>For an initial microorganisms loading concentration of  $(1.0 \pm 0.2) \cdot 10^8 \text{ m}^{-2}$

A weak growth of TSC is observed on AC<sub>2</sub>F<sub>2</sub> when no soot particles are added to the nutrition mixture, whereas an important colonisation appears in the presence of soot particles on each face of the media.

### 3.2.2 Influence on microorganisms adhesion

To explain the role of soot in filter colonisation, two hypotheses could be taken into account:

- Soot provides necessary carbon source for microbial growth
- Soot allows microbes adhesion onto fibres

The first hypothesis is eliminated because TSC is able to grow on ACFF media without soot addition. This means that ACF has provided enough carbon to permit its proliferation. ACF fibres are included in AC<sub>2</sub>F<sub>2</sub> so at least a small contamination should had been observed on AC<sub>2</sub>F<sub>2</sub>. Moreover, the pattern of ACFF contamination made of few extended colonies (figure 5) supports the assumption of a slide of inoculated microorganisms because, considering the quantity of microorganisms introduced, the colonisation should cover the surface without colony shapes.

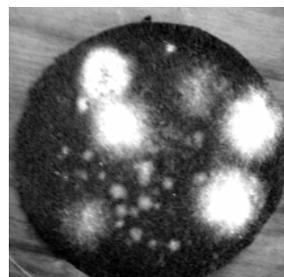


Figure 5. Growth pattern of TSC on ACFF

To test the second hypothesis, Zeta potential measurement is used to see the charge compatibility between microorganisms, fibres and soot. The surface charge potential of soot, ACFF and AC<sub>2</sub>F<sub>2</sub> fibres, TSC and SM microbes are presented in table 3.

Table 3. Surface electrical charge of materials involved in microorganisms adhesion onto filter media

Sample	Zeta potential (mV) <sup>b</sup>
TSC	-3 ± 5
SM	-9 ± 8
AC <sub>2</sub> F <sub>2</sub>	-8 ± 7
ACFF	-13 ± 12
soot	26 ± 13

<sup>b</sup> Measured within the MNS

The Zeta potential of AC<sub>2</sub>F<sub>2</sub> and TSC are slightly negative but close to neutrality whereas the soot charge is markedly positive. Soot surface charge can improve adhesion between TSC and AC<sub>2</sub>F<sub>2</sub> by acting as a “binder” due to its positive surface charge, attracting fibres and microbes. However, surface charge influence on proliferation has to be moderated because a growth is observed in 10 days for each filter contaminated with each microorganisms, even if their surface potential tends all to a slight negativity. Moreover, TSC should proliferate more easily than SM on AC<sub>2</sub>F<sub>2</sub> considering surface charge affinity whereas a greater proliferation of SM is observed.

### 3.3 Release from contaminated filters

Contaminated media were then studied in the filtration device to see if microorganisms that proliferate onto the filter can be released in the downstream air. Table 4 summarises tested filters, contamination levels and shows if the release was significant or not.

Table 4. Microbial release from contaminated filters

Filter	Inoculum	Soot	Colonisation (UFC.m <sup>2</sup> )	Release
ACFF	SM	none	$1 \times 10^{11} \pm 0.25 \times 10^{11}$	-
ACFF	TSC	none	$2 \times 10^{11} \pm 0 \times 5.10^{11}$	-
AC <sub>2</sub> F <sub>2</sub>	TSC	1 g/L	$7 \times 10^{11} \pm 1.3 \times 10^{11}$	+

Among the three filters considered, contaminated AC<sub>2</sub>F<sub>2</sub> is the only one which presents a significant release. Figure 6 shows the evolution of the number of particles measured downstream this filter. Background particle profile is measured before the airflow starting (vertical double line).

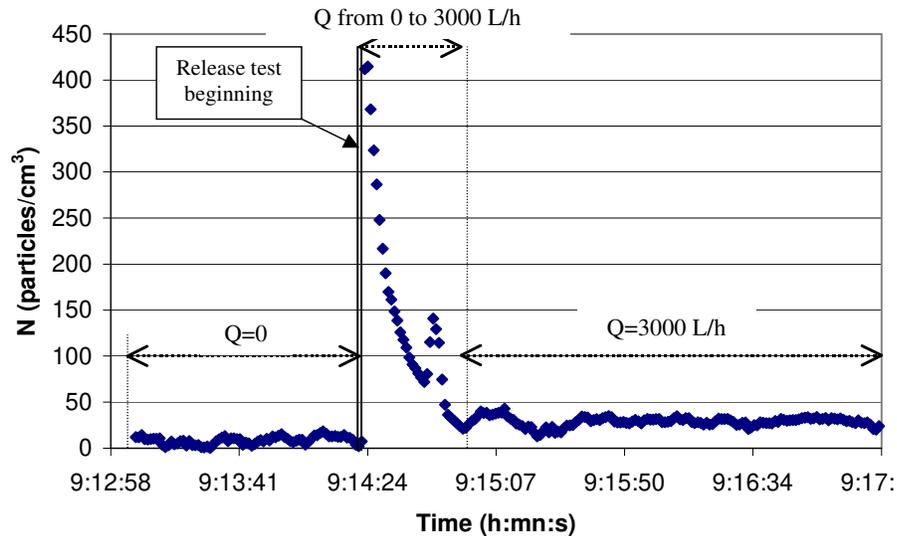


Figure 6. Particles detected in the downstream of the contaminated AC<sub>2</sub>F<sub>2</sub>

A release of particles is observed which rapidly decreases from 450 to around 25 particles/cm<sup>3</sup> after 30 seconds.

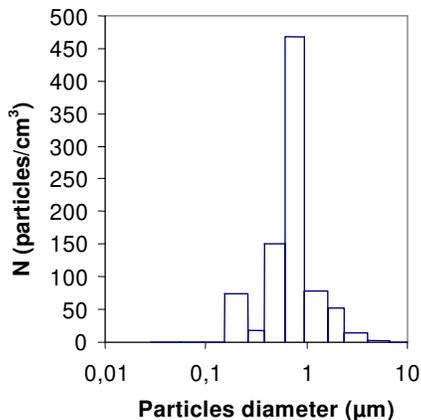


Figure 7. Particle size distribution

The particle size distribution of the released particles, presented on figure 7, is centred on 0.85 µm which could correspond to the fungi spore size observed on SEM analyses (figure 8) and does not tally with soot particle size distribution (not presented here). Thus, it seems that the observed release contains mainly fungal spores. Microscopic observations reveal why microbial release is significant when AC<sub>2</sub>F<sub>2</sub> is contaminated with TSC and why it is not the case for TSC proliferation on ACFF or SM on ACFF, although the level of contamination is high in each case.

On figures 8 and 9, AC<sub>2</sub>F<sub>2</sub> and ACFF invasive microorganisms are different, even if they come from the same inoculum (TSC). AC<sub>2</sub>F<sub>2</sub> microbe is a fungus (*Penicillium oxalicum*) which produces great quantity of spores to be dispersed in air (figure 8 shows fungus reproductive apparatus with chains of produced spores). At the opposite, ACFF invasive fungus (*Engyodontium album*) does not reproduce through great spore dispersion but rather by hyphal growth, which are his constitutive “wires” (figure 9). Its proximity with filter fibres confirms the hypothesis of a weak puff of the fungus in the downstream air.

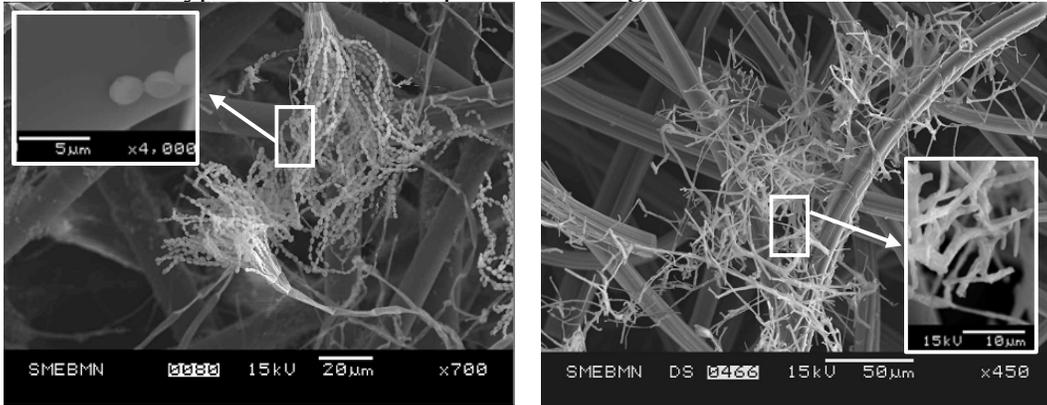


Figure 8. Proliferation of microorganisms from TSC onto AC<sub>2</sub>F<sub>2</sub> with Soot (1g/L)      Figure 9. Proliferation of microorganisms from TSC onto ACFF

#### 4. INTERPRETATIONS AND CONCLUSIONS

This study demonstrated the ability of microorganisms to grow on unused filter media and validate a new method for assessing filter contamination level. For suitable conditions (high humidity and temperature close to 25 °C), visible growth occurred 10 days after inoculation. In a first set of experiments, no carbon sources were added to support microbial growth, which means that microbes consumed carbon from fibres in order to proliferate. However, as filters provide no (ACFF) or a slight (AC<sub>2</sub>F<sub>2</sub>) quantity of dissolved carbon, microbes probably consume the carbonaceous content of fibres to grow. Different rates of contamination and invasive microorganism types are observed when the filtering medium composition varies, although the inoculum is the same. Moreover, considering one filter medium, the study has shown that proliferation rate also depends on microbial inoculum. However, TSC grows more rapidly than SM on ACFF whereas AC<sub>2</sub>F<sub>2</sub> was preferentially contaminated by SM (without soot addition).

This underlines the influence of a global affinity between the microorganism and the filter material. On the other hand, the addition of soot particles when the medium and the inoculum present no particular affinity for

each other (TSC on AC<sub>2</sub>F<sub>2</sub>) seems to enable proliferation rate increase. Zeta potential tests incriminate surface charge incompatibility between TSC microorganisms and AC<sub>2</sub>F<sub>2</sub> fibres which limits their adhesion. Soot particles may play an interface role in microbial adhesion onto fibres as a binder. However, Zeta measurements show better charges attraction of TSC than SM on clean AC<sub>2</sub>F<sub>2</sub> and, for instance, could not explain why SM colonisation of AC<sub>2</sub>F<sub>2</sub> is more important than TSC proliferation. Thus, other explanations, like nutrition preferences, must be involved in colonisation mechanism and have to be taken into account.

This work also revealed filter permeability decrease with the microbial concentration per cubic meter of filter, probably caused by the obstruction of pores during proliferation. A consequence of this could be a premature decrease of volume treatment capacity.

Finally, samplings of downstream air of a contaminated filter submitted to a flow ramp have shown a release of fungi spores up to 450 cm<sup>-3</sup>. This concentration rapidly decreases to reach a stable value around 25 cm<sup>-3</sup>, 30 seconds after air flow starting. However, release has only been observed when the medium was contaminated with fungi which reproduce via great quantity of spores dissemination. The release observed can explain the role played by filtration system in infectious pathology propagation that has been observed in some hospitals (Arnou et al., 1991).

## NOTATION

$k$	permeability (m <sup>2</sup> )
$Q$	flow rate (m <sup>3</sup> /s)
$S$	pipe section (m <sup>2</sup> )

### Greek letters

$\Delta x$	filter thickness (m)
$\Delta P$	pressure drop (Pa)
$\eta$	dynamic viscosity (Pa.s)

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