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Inactivation of *Aspergillus niger* spores from indoor air by photocatalytic filters

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ABSTRACT

The effects of UV-A and UV-C radiation on fungal spores were investigated before and after their germination in photocatalytic and non-photocatalytic air filters commonly used in heating, ventilating, and air conditioning (HVAC) systems.

Immediately after the coating of spores on filters, exposure to both types of UV radiation induced the appearance of an inactivation threshold for long durations of exposure probably resulting from the presence of *Aspergillus niger* spores inside the activated charcoal layer. The use of a thin photocatalytic filter without activated charcoal demonstrated a better disinfection efficiency with total inactivation of the spores, due to an optimal contact between spores and TiO_2 coating.

The effects of UV radiation were then assessed on spore germination for both types of filters. The inactivation of spores in illuminated photocatalytic filters resulted in an irreversible inhibition of the fungal germination under UV-A or UV-C radiation. In contrast, fungal spores were able to germinate in non-photocatalytic filters despite previous exposure to both types of UV radiation. The monitoring of ergosterol amounts, the major sterol of fungal membranes, corroborated these results.

Finally, UV-A or UV-C radiation exposure of filters after spore germination had a lesser disinfection efficiency than experiments whereby spores had just been applied onto the filters, due to the absence of contact between the biological pollutants and the photocatalyst coating.

Our results thus demonstrated the interest to use photocatalytic filters ensuring optimal contact between pollutants and TiO_2 coating to lead to a total inactivation of fungal spores in filters of HVAC systems.

1. Introduction

Today, populations in developed countries spend more than 80% of their time in confined indoor environments. Indoor air quality (IAQ) and the potential impacts of indoor air contamination have therefore become of particular concern for public health and safety reasons. Factors affecting indoor environments mainly include relative humidity, temperature, air exchange rate, air movement, ventilation, chemical (gaseous or particles) and biological pollutants (bioaerosols). Increased bioaerosol levels in indoor environments may come from various sources including building materials when sufficient moisture and nutrients are combined or air conditioning or ventilation systems under specific conditions [1–6].

General interest in exposure to bioaerosols has developed over the last two decades, because of their presence in occupational environments and their serious implications on human health. Indeed, many studies have shown a link between the presence of bioaerosols indoors and adverse health issues including infectious diseases, respiratory pathologies, allergic reactions [7,8]. Moreover, many of the medical symptoms due to exposure to biological pollutants are accentuated in confined environments due to the accumulation of specific bioaerosols as a result of poor building ventilation.

HVAC systems are commonly used in residential, commercial or industrial buildings to maintain indoor air quality and ambient temperature. Ventilation allows the removal of interior airborne pollutants through filtration, cooling or heating fresh indoor air at

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the same time. In HVAC systems, traditional filters usually allow the capture of biological particles but do not kill them. Eventually, due to favourable conditions such as high humidity, poor design or maintenance or accumulation of organic compounds collected by filters as source of nutrients, aerosolized microorganisms become able to survive and grow within these filters [6,9–11]. HVAC systems can therefore disseminate airborne microorganisms, toxins, allergens and microbial volatile organic compounds (MCOVs) from contaminated filters into the environment where they are inhaled by building occupants [12–15]. In addition, the accumulation of microorganisms on the filters leads to a decrease in their efficiency to remove pollutants in the long run.

In this context, the recent emergence within the HVAC industry of the photocatalytic process presents a great opportunity to address contamination of air filters concerns. The effectiveness of photocatalysis to inactivate a wide range of harmful microorganisms has been documented in the literature for a number of years [16–22]. If dimensioned appropriately, photocatalysis may represent an effective process adaptable to a number of applications for disinfection of both indoor air and drinking water in various environments such as industrial and health care environments [23,24]. Within the HVAC industry, the photocatalytic process is still an emerging technology, in particular where photocatalytic filters are employed in the purge of airborne microorganisms. Moreover, very few studies have taken an interest in the survival of aerosolized microorganisms trapped into photocatalytic filters [20,22,25].

The main contribution of this study is to compare the efficiency of commercial photocatalytic and non-photocatalytic filters under UV-A or UV-C radiation to inactivate *Aspergillus niger* (*A. niger*) spores. Fungal spores are commonly detected in indoor environments as well as in filters of ventilation systems [26–28]. Moreover, *A. niger* strains are known for their resistance to any environmental stress conditions, and especially their spores which contain aspergillin, the black fungal spore pigment of *A. niger*, protecting them from UV radiation exposure [29,30]. As a result, they can be considered as representative airborne microorganisms appropriate for the assessment of the efficiency of photocatalytic filters [22,31,32]. In view of this, we investigated the effects of UV radiation exposure: (i) on *A. niger* spores immediately after their coating on filters; (ii) on fungal germination after exposure to UV radiation in filters; (iii) on *A. niger* spores after their germination in filters. To go further in our investigation, we also performed chemical analyses to monitor the amount of ergosterol, the principal sterol in fungal membranes, as a chemical marker of the quantity of both spores and mycelia in filters after UV radiation exposure.

2. Experimental conditions

2.1. Fungal strain and culture conditions

For all experiments, the *A. niger* strain IP 1187.79 was used and maintained on malt extract agar (MAEc medium) supplemented with chloramphenicol to limit bacterial growth (0.5 g/l). Colonies of *A. niger* were grown on MEAc agar plates for 7 days in a climate chamber where temperature and relative humidity (RH) were fixed at 25 °C and 98%, respectively.

2.2. Culture media

An aqueous solution with chloramphenicol was used to recover spores from *A. niger* colonies grown on MEAc agar plates.

A rich medium appropriate for an optimal growth of fungi was used to spray the spores on filters. It was composed of 1 g of KH_2PO_4, 0.5 g of $MgSO_4$, $7H_2O$, 5 g of pancreatic digest of casein, 10 g of glucose, 220 g of glycerol, 0.1 g of chloramphenicol per litre of demineralized water, pH 5.4–5.7.

2.3. Preparation of the initial suspension of A. niger spores

Spores were washed from 7-day MAEc agar plates and then counted in a Thoma counting chamber with an optical microscope (Zeiss Axio ScopeA1 model). The spore concentration was finally adjusted with appropriate dilutions to 2×10^7 spores/ml.

2.4. Light sources

Experiments under UV radiation were performed using two Philips PL-L mercury lamps (18 W), one emitting only UV-A radiation with a peak emission at 365 nm and the other emitting only UV-C radiation at 254 nm. A digital radiometer (VLX-3W, UVItec) was used to determine the radiance intensity of both light sources. It was equipped with 365 nm and 254 nm detectors calibrated in the spectral range of 355–375 nm and 254 nm, respectively. The total radiance intensity at the surface of the filters was of 3.6 mW/cm^2 for UV-A experiments and of 3.35 mW/cm^2 for UV-C experiments. A good homogeneity of the radiance intensity was confirmed on the whole surface of filters samples used for all experiments.

2.5. Filters

We tested three non-woven filter materials supplied by the manufacturer Ahlstrom Corp. Two filters were 2.5 mm thick, multilayered materials, used as gas filters for general ventilation. They were both composed of an inner activated charcoal layer supplied in diameters ranging from 0.25 to 0.60 mm, between two layers of non-woven fibres made of cellulose and polyester. These two filters were named non-photocatalytic AC filter and photocatalytic AC filter for this study. The photocatalytic filter was coated with PC500 TiO_2 by Cristal Global (anatase > 99%, specific surface area: 350–400 m^2/g, crystallites mean size of about 5–10 nm). The TiO_2 coating procedure was developed by the Ahlstrom firm (1069950A1 European patent). Fig. 1 shows the scanning electron microscopic pictures of a photocatalytic AC filter with TiO_2 coating. Accordingly, the diameter of the filter fibres was about 20 μm.

The third filter, called thin photocatalytic filter for this study, 0.76 mm thick, was made of cellulosic and polyester fibres with a PC500 TiO_2 coating but without the activated charcoal layer. For all experiments, filters were cut into pieces of 5 cm × 5 cm.

2.6. Procedures

2.6.1. Controlled coating of the spores on filters

An Iwata Airbrush Eclipse HP-BCS set up with a compressor was used to spray the *A. niger* spores suspension onto the filters. The suspension was sprayed with an airflow rate adjusted to 8.6 ml/min. The suspension was stirred every three tests to ensure a uniform distribution of spores in the suspension and no spore agglomeration, before spraying. The whole device was located under a class II microbiological safety cabinet (MSC) to ensure sterility of the experiments. This initial quantity of fungal spores coated on each filter was determined to be of 5×10^6 spores, after an extraction procedure, by dilution plating on MEAc medium and incubation for 48 h at 25 °C and 98% RH, before counting *A. niger* colonies.

2.6.2. UV radiation exposure

The effects of UV-A (3.6 mW/cm^2) or UV-C radiation (3.35 mW/cm^2) exposure of non-photocatalytic or photocatalytic filters were investigated: