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Bacteria Removal and Viability Attenuation by Means of an Electrostatic Barrier

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Key Words
Electrostatic filtration · Bacterial control · Ventilation ducting · Viable particles · Epifluorescence microscopy

Abstract
This study is focused on bacterial control through the removal and attenuation of viability by means of a commercial electrostatic air cleaner inserted in the ductwork of a central heating and air-conditioning system. Adapting the system to include an electrostatic barrier resulted, on an average, in removal of 88% of the bacteria in the airflow. In addition, the ratio of viable to non-viable organisms, calculated on the basis of epifluorescence measurements, was changed appreciably by passage through the electrostatic filter. Evaluation of performance was followed by two different strategies of sampling-analysis: a plate count method and epifluorescence microscopy. The system overall was highly efficient in removing the bacteria, since those few that evaded the filter underwent attenuation of around 50% of their viability on passage through it. This work suggests a strong positive effect when an electrostatic barrier is inserted in a ventilation duct.

Introduction

Contaminated central air handling systems can become breeding grounds for biological contaminants and can then distribute them inside a building. In the last twenty years, air quality in confined indoor atmospheres has been given particular attention with regard to the protection of public health. In 1982, the World Health Organisation suggested that indoor pollution of buildings was a possible cause of Sick Building Syndrome (SBS) and Building Related Illness (BRI). Indoor pollution is a consequence not only of the activities that are carried out inside the buildings, but also its construction materials including the decorations employed and the number of people crowding into it. The air in a building may contain a cocktail of pollutants including tobacco smoke, volatile organic compounds (VOCs) and formaldehyde, mineral and other fibres including asbestos and glass, oxides of carbon and nitrogen and ozone. In urban areas outdoor pollutants can add to the problem, particularly through increasing the level of fine particulates (PM_{10}, $PM_{2.5}$) and gases from road traffic. Although the potential impact of most of the indoor pollutants listed on people's health is well recognised, problems related to the presence of dispersed microorganisms is little understood because of the fewer number

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of observations available. The origins of micro-organisms indoors are manifold, a major proportion come from the outdoor air. Many studies have shown evidence of a massive presence of micro-organisms in air conditioning and ventilation systems [1]. It is reported, in recent literature, that various fibrous organic materials in domestic use (felts, wall papers, fabric–non-fabric filters) can be important sources of micro-organisms and that their growth will be sustained if the relative humidity exceeds 70% [2]. Some authors have observed microbial contamination in filters included in a hospital air conditioning system, from which micro-organisms were subsequently released into 'clean air' areas [3]. Growth, release and dispersion into the atmosphere therefore have to be carefully monitored. Within such a framework, this work has looked at the removal of bioaerosols, avoiding the use of filtering materials, by means of electrostatic systems and looked also at the influence of this on bacterial viability.

The Electrostatic Filter Employed

Electrostatic filters collect aerodispersed microparticles by means of electric fields created by applying a high voltage between electrodes. Particles ionised by the electric field follow the generated lines of force and are attracted onto the surfaces of the collecting electrode.

In this research a commercially available electrostatic filter "Femec" with the configuration shown in Figure 1 was used. The filter has two stages of operation: particles are first charged by the discharge electrode (positive) by means of the corona effect (Figure 1) and then the charged particles are attracted to the collection surfaces of the plate electrodes of the second stage [4].

Experimental

The chemical–environmental laboratories of the University of Ancona were selected as a test environment. The aim was to control indoor air quality utilising the existing air conditioning ductwork with the added assembly of aerosol generation and sampling-analysis equipment added according to the schematic depicted in Figure 2.

Bioaerosol Generation

Bioaerosol generation was carried out following two different operating modalities. The first sampled air directly from the air conditioning network was taken as a source of bioaerosols and inorganic particles (Figure 2, point 1). This air was taken into an equalisation volume, inserted at the end of the sampling ductwork, to allow the concentration of bacteria to obtain as uniform a distribution as possible. However, the bacterial population in the ventilation ductwork of the 'working building' turned out to be variable because of factors such as the external climatic conditions, extent of classrooms occupation and replacement of filters in the ventilation system (system maintenance), hence it was found necessary to carry out the tests under new and more reproducible operating conditions. The second operating modality generated bacteria starting from a controlled culture solution of *Escherichia coli*. This solution was aerosolised by means of a commercial ultrasonic humidity generator (Figure 2, point 2) (Criofog 3000 P, [Criofog di Pierini, Ancona, Italy] frequency 2 MHz) [5]. The humidifier was fed with a solution containing 2×10^7 *E. coli* per millilitre of a physiological solution. Once aerosolised, water quickly evaporated from the generated drops, leaving solid residues containing bacterial cells and suspended particles. The aerosol generation and sampling scheme is outlined in Figure 2.

Sampling-analysis

Bioaerosols and total aerosols samples were analysed by the following different analytical methods.

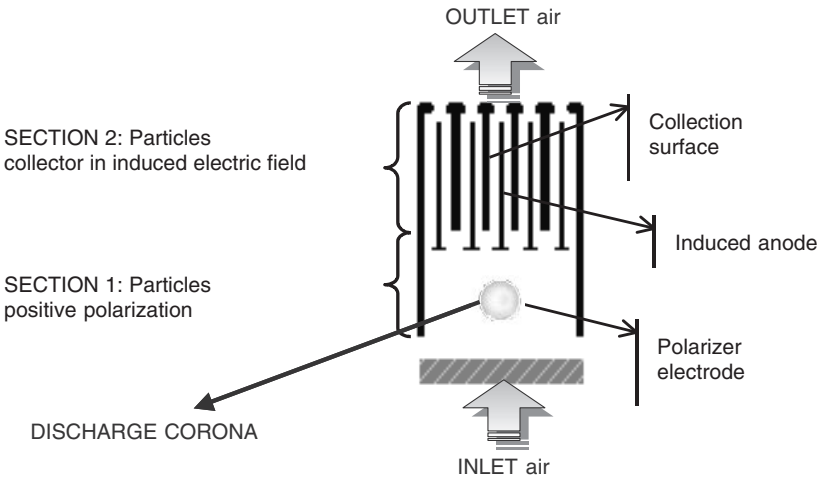

Fig. 1. Electrostatic filter scheme.

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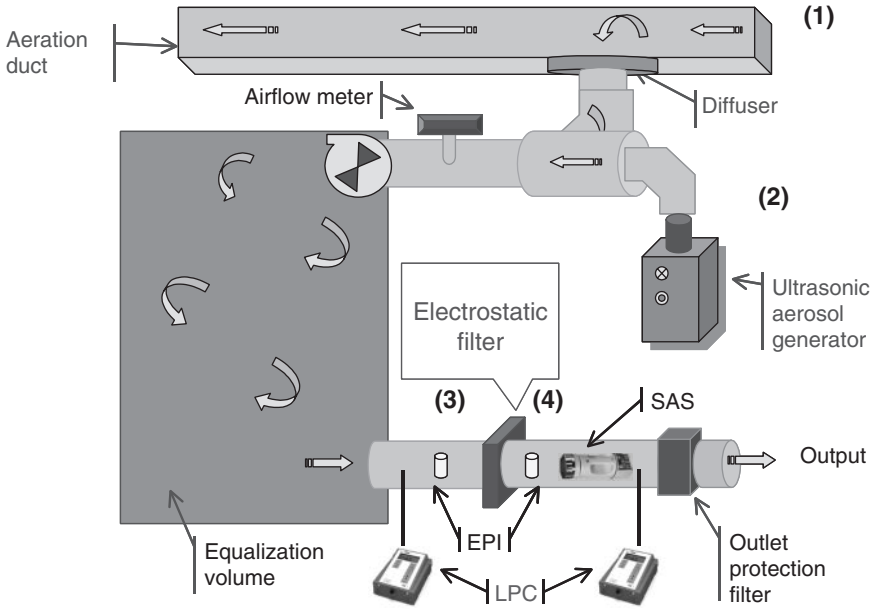

S.A.S. : "Surface air system", an active sampler for plate count method (method 1)
EPI. : Epifluorescence microscopy, for bacterial count and bacterial viability control (methods 2 and 4
L.P.C.: "Laser particle counter", for total aerosol count (method 3)
(1) (2): Particles sources ; (3) (4): Sampling points

Fig. 2. Generation-sampling scheme.

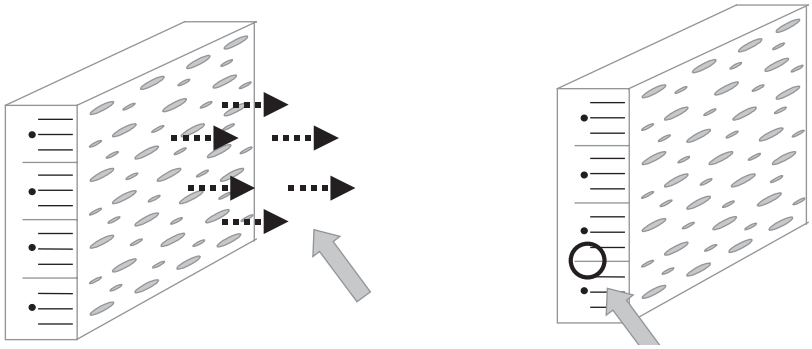

Fig. 3. Sampling strategy.

Method 1: *Plate Count Method*. Bacterial removal was evaluated by sampling the air leaving the electrostatic filter by means of an active sampler, SAS, placed in direct contact with the surface of the filter (Figure 2, point 4; Figure 3(a)), respectively under conditions of filter ON and filter OFF. The air volumes sampled ranged from a minimum of 60 L up to a maximum of 2700 L. The sampler used Rodac dishes [PBI International] with a specific cultural medium for total bacteria determination, Plate Count Agar (PCA). The dishes were thermostated at 37°C for 48 h after which colonies were counted and the results expressed as colony forming units per cubic metre ($CFU \cdot m^{-3}$).

Method 2: *Bacterial Count by Means of Epifluorescence Microscopy*. The second analytical approach used polycarbonate cassettes (diameter 25 mm) for bacteria sampling. Depending on the needs of the experiment, cassettes were equipped with black nucleopore polycarbonate filters (porosity 0.4 µm) or anodisc filters (porosity 0.2 µm). Cassettes were connected to an air pump (flow $14\,L \cdot min^{-1}$) which allowed samples to be collected under isokinetic conditions. These were placed inside the ductwork, upstream (Figure 2, point 3) and downstream (Figure 2, point 4) of the electrostatic barrier. Air volumes sampled ranged from 14 to 210 L. After sampling, filters were fixed and stained with a solution containing Acridine Orange (a nucleic acid stain) and formaldehyde, then placed on slides. Examined under an epifluorescence microscope, the slides allowed a direct count of bacteria [6].

Method 3: *Total Aerosols Count*. Laser light scattering counters (DUSTTRACK TM Aerosol Monitor Model 8520 LPC, [TSI Incorporated]) were used to measure the total aerosol concentration before (Figure 2, point 3) and after (Figure 2, point 4) the electrostatic barrier [7].

Method 4: *Bacterial Viability Control*. Two further strategies were adopted to verify, besides the total particle removal efficiency, the effects of the filter on bacterial viability. The first, based on plate count methodology, took samples directly from the internal surfaces of the electrostatic filter (Figure 3, point (b)). An amount of 200 µL of liquid culture medium (Brain Heart infusion agar) containing *E. coli* was poured onto four

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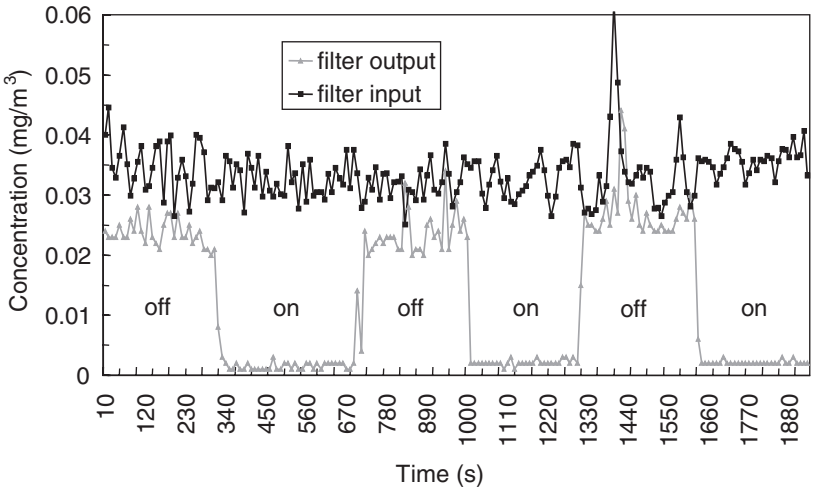

Fig. 4. Particles concentrations monitored upstream and downstream of the electrostatic filter (power OFF and ON).

disks (diameter 25 mm) placed symmetrically at a distance of 1 cm from each other on the surfaces of the first charging stage thus obtaining four representative samples. At the end of the test, the disks were transferred into test tubes containing physiological solution, so allowing the samples to re-solubilise after which an aliquot of each solutions was transferred to Petri dishes.

The second strategy was the same as described in Method 2, but making use of a double-staining system with Sybr Green I, (SG, Molecular Probes, Eugene, Oregon, USA) and Propidium Iodide, (PI, Sigma St. Louis, Montana, USA) and examining the result under the epifluorescence microscope. Both stains bind to the DNA of micro-organisms. The difference between the two stains lies in their ability to cross plasma membranes. The stain SG can enter both living and dead cells, while PI penetrates only damaged or dead cells. Therefore, under the microscope, living cells show green fluorescence, while dead cells show red [8].

Results and Discussion

The numerical counts obtained by the LPC are evidence that the electrostatic filter used removes total particles with good efficiency, as shown in Figure 4, where particles concentrations upstream and downstream of the electrostatic filter were monitored under conditions of filter OFF and ON. In the tests with the synthetic aerosol generation, results demonstrate an efficiency of 89%, while with particles arising from the ventilation duct the efficiency decreases slightly to 85% (Table 1) . The smaller dispersion with the results, obtained with synthetic aerosol generation, underlines the necessity to work under controllable and reproducible conditions.

Two different analytical strategies were adopted to verify bacterial removal. The first used microbiological analysis by a plate count method using PCA. Sampling the air outlet from the electrostatic filter showed an efficiency of around 90% with particles from the ventilation duct and of around 91% with synthetic aerosol generation (Tables 2 and 3). Actually, since inlet and outlet sampling are not simultaneous, these efficiency values must be considered as informative only because they are numerically the result of the statistical evaluation performed. Instead, removal efficiency can be correctly evaluated by means of epifluorescence microscopy because of the simultaneous sampling this method entails. Epifluorescence microscopy is a more up-to-date methodology for counting bacteria, which enables direct observation of cells without necessarily inducing bacterial growth. Furthermore it allows us to avoid all limitations, which make the plate count method unsuitable for estimating total bacteria number in environmental samples. In fact, the use of the plate count method can underestimate the real bacterial concentration because it does not take account of cells which are damaged during sampling or which are not subsequently presented with adequate growth conditions (temperature, culture medium). Also, in the presence of several bacteria types some could inhibit the growth of others. Epifluorescence microscopy is highly sensitive and therefore more suitable than an incubation method even if the methodology is substantially more complex and requires specialist knowledge and expensive instruments. The removal efficiency measured by this method of 85% or higher appears in good agreement with previously reported values (Table 4).

To verify the influence of the electric field on bacterial viability, the epifluorescence analytical approach using dual staining with SG and PI, was adopted. The viable to non-viable cells ratio ($R = V/NV$), obtained from sampling downstream of the electrostatic filter under conditions of filter OFF and ON, allows a comparison index. The

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Table 1. Total particles removal efficiency (Laser light scattering count method).

	Count	Variance	Standard deviation	Efficiency (%)			
				min.	max.	range	average
Synthetic aerosol	15	18	4	83	96	16	**89**
Duct aerosol	15	26	5	72	99	27	**85**

Table 2. Removal efficiency in the case of ambient particles collected from ventilation duct (Plate count method).

	Off	On	Efficiency (%)
Count	13	13	13
Average	223	62.4	**89.6**
Variance	1.50E+05	1.44E+04	194
Standard deviation	388	120	13.9
Minimum	0	0	50
Maximum	1.01E+03	394	100
Range	1.01E+03	394	50

Table 3. Removal efficiency of bacteria from synthetic aerosol generation (Plate count method).

	Off	On	Efficiency (%)
Count	19	19	19
Average	372	47.5	**90.9**
Variance	116691	9538	45.5
Standard deviation	342	97.7	6.7
Minimum	77.8	2.2	78
Maximum	1278	439	98
Range	1200	437	20

different R values obtained are summarised in Table 5. The number of living cells was cut by half in the presence of the electric field, with average R values of 2.7 OFF and 1.4 ON.

On the other hand the plate count method approach allows a further examination of the bacteria sampled. Unlike the previous sampling strategy, *E. coli* bacteria were placed directly on the discharge electrode surfaces. In the absence of the electric field bacterial growth was evident, while with the same fluid dynamics conditions, the presence of the electric field reduced their growth as shown in Figure 5. These results suggest a bactericidal effect of the applied electric field, an aspect that further differentiates the electrostatic filter from filtering systems based on felted or other textile filters.

Table 4. Removal efficiency in the case of ambient particles collected from ventilation duct (Epifluorescence microscopy method).

	Off	On	Efficiency (%)
Count	8	8	8
Average	516644	230478	**84.9**
Variance	2.86E+11	4.13E+10	144
Standard deviation	534911	203166	12
Minimum	14100	10600	65
Maximum	3.61E+06	949500	98.5
Range	3.59E+06	938900	33.5

Table 5. Viable/non-viable ratio under conditions of filter OFF and filter ON (Epifluorescence microscopy method).

	Off	On
Count	6	6
Average	**2.7**	**1.4**
Variance	3.3	2.0
Standard deviation	1.8	1.4
Minimum	1.3	0
Maximum	5.6	4.1
Range	4	4.1

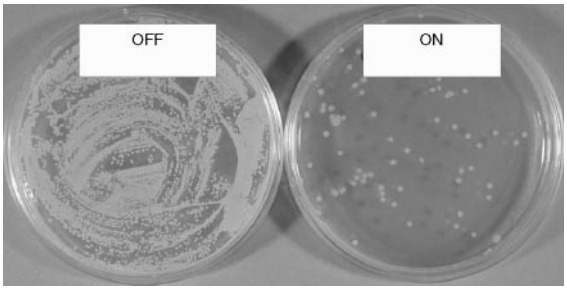

Fig. 5. Sampling on the electrostatic filter collection surface. Bacteria showing different growth patterns under conditions of filter OFF and filter ON.

Conclusions

The electrostatic filter examined in this work was shown to remove efficiently most of the total particle load in an airflow, in agreement with literature reports. Also, viable cells appear to be removed with the same efficiency as other particles. A study of biological

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contamination using either 'natural' atmospheric particulates or a synthetic aerosol generated from an *E. coli* culture, shows in both cases a reduction in the level of bacteria. The average removal, verified through statistical comparison and through direct filter inlet/outlet comparison turned out to be 88%. The undesirable eventuality, always possible with all filtering systems, that the bacteria already captured in the system re-disperse into the atmosphere is prevented by the bactericidal electrostatic action. This in particular shows the advantage of using the electrostatic filter rather than fabric filtration, where some reports describe a net growth of bacteria on the filter. Bactericidal action was demonstrated by either measuring the viable/non viable ratio in the air leaving the filter or by the growth suppression of bacteria plated onto internal surfaces of the filter. The monitoring method preferred used epifluorescence microscopy and although this demands specialist knowledge and expensive instruments, it appears more sensitive and therefore more capable of giving reliable results. The results from this work are so clear cut that they make a case for further work with the objective of establishing, not just the filter efficiency, but its effectiveness for the protection of indoor air quality.

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References

1 Cosentino S, Pisano PL, Palmas F: Comparison of indoor climate and microbial contamination in two office buildings with different ventilation systems. L'Igiene moderna 1990; 93:749–763.
2 Cheng-yan Li, Zhi-ting Zeng: A report on indoor bacterial contamination in air and the effect of room ventilation and purification. Proceedings of Indoor Air 1990, Toronto; 2:55–59.
3 Elixmann JH, Schata M, Jorde W: Fungi in filters of air conditioning systems cause the building related illness. Proceedings of V International Conference Indoor Air Quality Climate 1990, Toronto; 1:193–202.
4 Bontempi L, D'Errico M, Fava G: Abbattimento della carica batterica totale aerodispersa mediante barriera filtrante di tipo elettrostatico. Ingegneria Ambientale 2001; XXX (1):44–51.
5 Battistoni P, Bernetti R, Fava G: Filtri elettrostatici a doppio stadio. Descrizione matematica e verifica dell'efficienza di rimozione di particelle respirabili. Ingegneria Ambientale 1993; XXII (1):16–22.
6 Bontempi L, Dottori M, Fava G, Ruello ML: Batteri nelle condotte di ventilazione. Rimozione ed attenuazione di vitalità mediante barriera filtrante di tipo elettrostatico. Proceedings of 43° International Conference AICARR 2002, Milano; 1:369–381.
7 ASHRAE: Method of testing air-cleaning devices used in general ventilation for removing particulate matter, NY, American Society of Heating and Air-Conditioning Engineers (ASHRAE standard 52–76, 1976).
8 Mezzanotte V, Citterio S, Ghiani A, Labra M, Sgorbati S: Determinazione mediante citometria di flusso della carica batterica residua in reflui trattati. Ingegneria Ambientale 2000; XXIV (7/8):364–369.

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