

# Antimicrobial Effect of Silver Particles on Bacterial Contamination of Activated Carbon Fibers

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Even though activated carbon fiber (ACF) filters have been widely used in air cleaning for the removal of hazardous gaseous pollutants, because of their extended surface area and high adsorption capacity, bacteria may breed on the ACF filters as a result of their good biocompatibility; ACF filters can themselves become a source of bioaerosols. In this study, silver particles were coated onto an ACF filter, using an electroless deposition method and their efficacy for bioaerosol removal was tested. First, various surface analyses, including scanning electron microscopy, inductively coupled plasma and X-ray diffraction were carried out to characterize the prepared ACF filters. Filtration and antimicrobial tests were then performed on the filters. The results showed that the silver-deposited ACF filters were effective for the removal of bioaerosols by inhibition of the survival of microorganisms, whereas pristine ACF filters were not. Two bacteria, *Bacillus subtilis* and *Escherichia coli*, were completely inhibited within 10 and 60 min, respectively. Electroless silver deposition did not influence the physical characteristics of ACF filters such as pressure drop and filtration efficiency. The gas adsorptive ability of the silver-deposited ACF filter, as represented by the micropore specific surface area, decreased by about 20% compared to the pristine filter because of the blockage of the ACF micropores by silver particles. Therefore, the amount of silver particles on the ACF filters needs to be optimized to avoid excessive reduction of their adsorptive characteristics and to show effective antimicrobial activity.

## 1. Introduction

Bioaerosols are airborne particles of biological origins, including viruses, bacteria, fungi and all varieties of living materials. In suitable hosts, bioaerosols are capable of causing acute or chronic diseases, which may be infectious, allergenic, or toxigenic (1). Numerous engineering solutions are commercially available for the removal of bioaerosols, and others are under development such as air filtration, ultraviolet germicidal irradiation (UVGI), negative air ionization, electrostatic precipitation, photocatalytic oxidation, air ozonation, etc. (2, 3).

Indoor air bioaerosols accumulate in large quantities on filters of heating, ventilating, and air-conditioning (HVAC) systems, where they are able to multiply under certain conditions; especially if high amounts of moisture are present

on the filter (4). Moreover, the organic or inorganic materials deposited on the filter medium after air filtration contribute to microbial growth. This inevitably leads to decreased filter efficacy and, probably, to deterioration of the filters, with the eventual release of microorganisms. Volatile organic compounds produced by microbial metabolism (MVOs) can be emitted from the contaminated filters (5).

Activated carbon fiber (ACF) filters have been widely used for air cleaning by the removal of hazardous gaseous pollutants, such as volatile organic compounds (VOCs), because of their extended surface area and high adsorption capacity. However, bacteria preferentially adhere to solid supports made of carbon materials, indicating that ACF filters have high biocompatibility. Bacteria may multiply on ACF filters, and thus, ACF filters can themselves become a source of bioaerosols (6, 7). To avoid such a problem, antimicrobial ACF filters are required.

As an effective antimicrobial agent, silver is believed to interact with elements of the bacterial membrane, causing a structural change, dissipation of the proton motive force and finally to cell death (8, 9). Because of the antimicrobial activity of silver, many silver-containing materials have been developed for antimicrobial applications (10–13). In this study, ACF filters containing silver particles were prepared using an electroless deposition method, and their efficacy for bioaerosol removal was tested. The electroless deposition method has previously been used for depositing metals onto nonconductive supports such as activated carbon (7, 14).

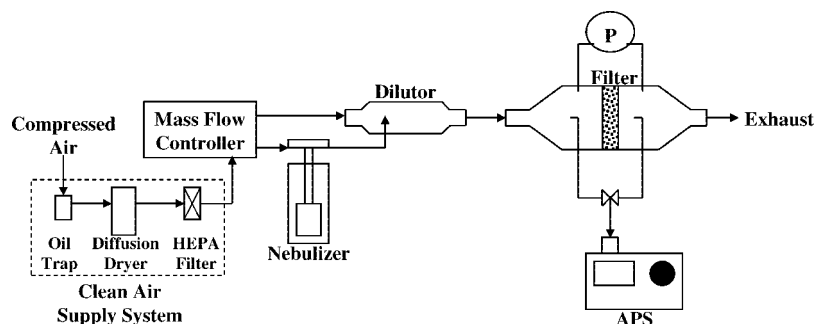
## 2. Materials and Methods

**2.1. Preparation and Characterization of Silver-Deposited ACF Filters.** Silver was coated on the ACF filters (KF-1500, Toyobo, Japan) using an electroless deposition method. The samples denoted as ACF/Ag-10, ACF/Ag-20, and ACF/Ag-30 were prepared without the use of electric current at deposition times of 10, 20, and 30 min, respectively, with silver metal solution. Before the electroless silver deposition, the ACF filters were catalytically activated by immersion for 5 min at 25 °C in an aqueous solution containing palladium to maximize the autocatalysis (14). The prepared filters were characterized using field-emission scanning electron microscopy (FESEM; JSM-6500F, JEOL, Japan), inductively coupled plasma (ICP; Elan 6000, Perkin-Elmer, U.S.), and X-ray diffraction (XRD; D/MAS-Rint 2000, Rigaku, Japan).

The main reason for the use of an ACF filter was its high ability for the adsorption of gaseous pollutants; therefore, maintenance of the adsorptive performance was important, even though silver particles might deposit directly into the pores of the ACF filter. The nitrogen adsorption isotherms of ACF samples were measured using a porosimeter (ASAP 2010, Micromeritics Ins. Corp., U.S.) to characterize the adsorptive ability of the prepared ACF filters. The results of the adsorption isotherms analyses were used to determine the specific surface areas and pore volumes by the BET (Brunauer, Emmett, and Teller) and BJH (Barrett, Joyner, and Halenda) methods, respectively.

**2.2. Preparation of Bacterial Suspensions.** *Escherichia coli* (ATCC 11775) and *Bacillus subtilis* subspecies *spizizenii* (ATCC 6633) were selected as model Gram-negative and Gram-positive bacteria, respectively. For each species, a bacterial suspension was prepared by culturing 0.1 mL of an overnight culture, inoculated in 15 mL of nutrient broth, for 18 h. The nutrient broth was prepared by dissolution of 5 g of peptone and 3 g of meat extract in 1000 mL of sterilized deionized water and sterilization of the solution with an autoclave.

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**FIGURE 1.** Schematic diagram of experimental setup for the filtration tests. Bioaerosols were generated using a nebulizer and measured using APS at upstream and downstream of the test filter. The pressure drop across the filter was measured using a differential pressure gauge. Flow rates of clean air were controlled using mass flow controllers.

**2.3. Filtration Tests.** Figure 1 shows a schematic diagram of experimental setup for the filtration tests. For bioaerosol generation, the prepared bacterial suspensions were washed three times with sterilized deionized water using a centrifuge (VS-1500N, Vision Scientific, Korea) at 6000 rpm for 15 min, to remove the residual particles including the components of the nutrient broth. The washed suspensions were then diluted with sterilized deionized water to give suspensions with an optical density of 0.01 at 600 nm as measured using a spectrophotometer (SP-300, Optima, Japan). A bacterial mixture of *E. coli* and *B. subtilis* (1:1 ratio) was nebulized using a Collison-type nebulizer (1 jet, BGI Inc., U.S.) at a flow rate of 2 L/min and was introduced into a dilutor. Dispersed bioaerosols were diluted and introduced into the test duct where a prepared ACF filter was installed. The face velocity was controlled from 0.1 to 0.5 m/s using a mass flow controller. The air used in this study was dried and cleaned using a clean air supply system that consisting of oil trap, diffusion dryer, and HEPA (high-efficiency particulate air) filter.

The pressure drop across the filter was measured using a differential pressure gauge. The number concentrations of the bioaerosols were measured upstream and downstream of the filter using an aerodynamic particle sizer (APS; model 3321, TSI Inc., USA). The overall particle filtration efficiency ( $\eta$ ) is defined as

$$\eta = C_{\text{down}}/C_{\text{up}} \quad (1)$$

where  $C_{\text{down}}$  and  $C_{\text{up}}$  are the number concentrations of the bioaerosols measured downstream and upstream of the filter, respectively.

**2.4. Antimicrobial Tests.** Antimicrobial tests on the silver-deposited ACF filters were performed using the disk-diffusion method (15). Nutrient agar, made by dissolution of 5 g of peptone, 3 g of meat extract, and 15 g of bacto agar in 1000 mL of deionized water, was used to culture the bacteria after autoclave. 0.1 mL of the prepared bacterial suspension for *E. coli* or *B. subtilis* was spread on the nutrient agar plate. The test filter was placed on the lawn of bacteria and incubated overnight. The antimicrobial activity was observed by visually inspecting the diameter of the inhibition zone around the filter.

The time-dependent antimicrobial characteristics were investigated using the colony counting method;  $10^6$  cells were inoculated onto the test filter and incubated for the contact times of 0, 2, 5, 10, 60, 120 and 240 min, at room temperature, and 70% relative humidity. After the contact time had elapsed, the test filter was immersed in 50 mL of sterilized deionized water in a sterilized plastic bag (BagLight, Interscience, France), with the bag then stroked by a BagMixer (400VW, Interscience, France) for 3 min, at a speed of 9 strokes/s. The bacteria separated from the test filter were then spread on a nutrient agar plate and incubated for 24 h, and a colony analysis performed.

All experiments were conducted nine times, with the results represented as the mean and standard deviation values.

### 3. Results and Discussion

SEM images of the pristine and silver-deposited ACF filters are shown in Figure 2. The results showed that the pristine ACF filter (ACF/Ag-00) had a smooth surface; whereas the silver-deposited ACF filters had rough surfaces because of the coating of silver particles. In the case of ACF/Ag-10, silver particles of about 1  $\mu\text{m}$  were formed, but those on the surface of ACF/Ag-20 were denser, and silver completely covered the surface of ACF/Ag-30. The total amounts of silver on the ACF filters were measured using ICP analysis, and mean crystal sizes were determined according to Scherrer equation using the results of the XRD analysis. The ICP and XRD results are summarized in Table 1. The amounts and mean crystal sizes of silver particles increased from 8 to 35  $\mu\text{g/g}$  and from 12.13 to 15.52 nm, respectively, with the increasing deposition time from 10 to 30 min.

Table 2 shows a summary of the properties related to the adsorptive characteristics of ACF filters, which were determined from the raw data of the nitrogen adsorption isotherms according to the BET and BJH methods. The total specific surface areas (TSSA), micropore specific surface areas (MSSA), total pore volumes (TPV), micropore volumes (MPV), and average pore diameters (APD) of the silver-deposited ACF filters decreased with increasing deposition time. This phenomenon was caused by blockage of the micropores of the ACF by the deposited silver particles. Since decreases in these properties implies a possible loss of active sites for adsorption by the ACF filters, the silver content supplied for antimicrobial purposes needs to be optimized.

The pressure drops across the filters were measured, and as shown in Figure 3, the deposition time did not affect the pressure drop. Figure 3 also shows that the increases in the face velocity, from 0.1 to 0.5 m/s, caused an increased pressure drop from 2 to 9 mmH<sub>2</sub>O. The filtration efficiencies of the ACF filters were calculated using the number concentrations measured upstream and downstream of the filters, using an APS, with the results shown in Figure 4. The size distribution of test bioaerosols generated by the nebulizer is also shown in Figure 4. The aerodynamic mode diameter of the test bioaerosols was 0.78  $\mu\text{m}$ . The size range from 0.67 to 0.89  $\mu\text{m}$ , where the bioaerosols of more than 50% of total number concentration were found, was used to calculate the filtration efficiency. Regardless of the deposition time, the filtration efficiency was about 0.8 at a face velocity of 0.1 m/s, which decreased to about 0.6 at 0.5 m/s. In this study, a flat panel-type filter was used for filtration test to verify the effect of silver deposition on the filtration efficiency and pressure drop of the filter. In real situations, however, air filters having folded filter media have been used to enlarge the filtration area. By enlarging the filtration area, the filtration

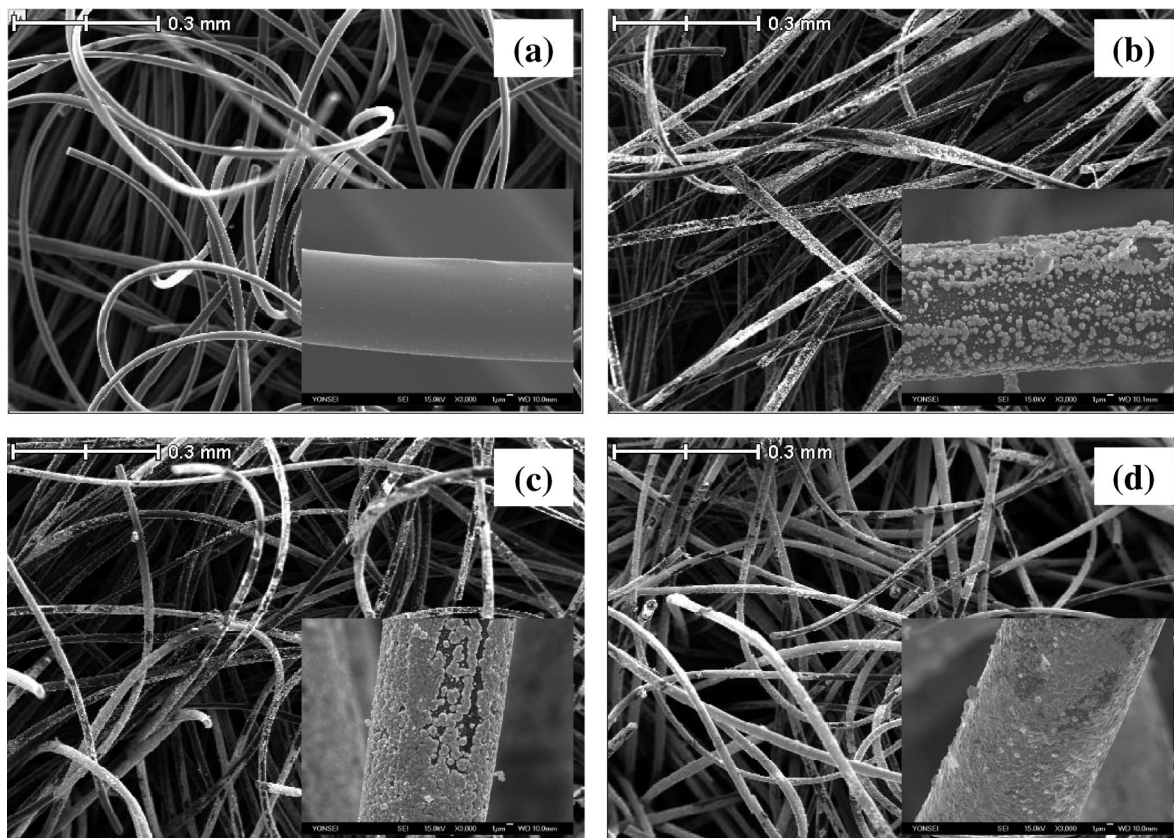


FIGURE 2. SEM images of (a) ACF/Ag-00, (b) ACF/Ag-10, (c) ACF/Ag-20, and (d) ACF/Ag-30.

TABLE 1. Amounts and Crystal Sizes of the Silver Particles Deposited onto Three ACF Filters

	ACF/Ag-10	ACF/Ag-20	ACF/Ag-30
amount of silver ( $\mu\text{g/g}$ ) <sup>a</sup>	8	28	35
mean crystal size of silver (nm) <sup>b</sup>	12.13	13.17	15.52

<sup>a</sup> Results of the ICP analysis. <sup>b</sup> Results of the XRD analysis and Scherrer equation,  $d = 0.9 \lambda / \beta \cos \theta$ , where  $\lambda$  is the wavelength of the X-ray source (Cu K $\alpha$  = 0.154 nm) and  $\beta$  is the full-width at half-maximum (fwhm) of the X-ray diffraction peak at the diffraction angle  $\theta$  (=19.05).

TABLE 2. Adsorptive Properties of the Silver-Deposited ACF Filters<sup>a</sup>

	TSSA ( $\text{m}^2/\text{g}$ )	MSSA ( $\text{m}^2/\text{g}$ )	TPV ( $\text{cm}^3/\text{g}$ )	MPV ( $\text{cm}^3/\text{g}$ )	APD ( $\text{\AA}$ )
ACF/Ag-00	1598	1583	0.91	0.86	17.7
ACF/Ag-10	1441	1362	0.60	0.55	16.6
ACF/Ag-20	1383	1347	0.58	0.55	17.0
ACF/Ag-30	1341	1280	0.55	0.51	16.5

<sup>a</sup> TSSA (total specific surface area), MSSA (micropore specific surface area), TPV (total pore volume), MPV (micropore volume), APD (average pore diameter).

efficiency and pressure drop can be increased and decreased, respectively.

The results shown in Figures 3 and 4 indicate that the amounts of silver particles were insufficient to cause the variations in the pressure drop or filtration efficiency. The masses of silver particles deposited on the ACF filters were measured using a microbalance (Ohaus, U.S.): 2.2, 7.6,

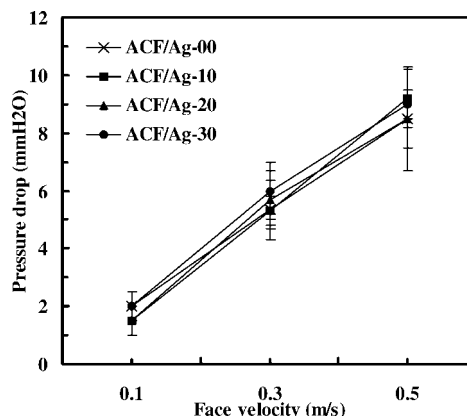


FIGURE 3. Pressure drop of the ACF filters (mean  $\pm$  SD).

and 9.5 mg/m<sup>2</sup> for ACF/Ag-10, ACF/Ag-20, and ACF/Ag-30, respectively. According to Thomas et al. (16), significant changes in the pressure drop of fibrous filters caused by particle deposition occur on the order of grams per square meter of particle deposition.

The antimicrobial effects of the silver-deposited ACF filters were characterized using the disk-diffusion method, and the results are represented by inhibition zone diameters in Table 3. For both bacteria, the diameters of inhibition zones increased with increasing deposition time. For the silver-deposited ACF filters, the bacteria did not grow within or around the filters, while the bacteria did grow on the pristine ACF filter. The test filters with silver particles were considered to release the active forms of silver, for instance, silver ions, which impart antimicrobial activity under humid environment (15). The greater numbers of silver particles on the ACF filter, the greater the amount of silver ions would be expected to diffuse further from the filter because of the larger silver concentration gradient.



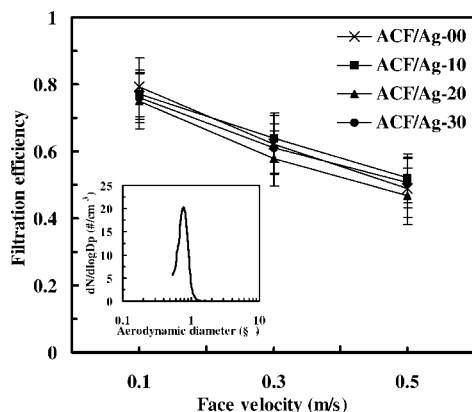


FIGURE 4. Filtration efficiency of the ACF filters (mean  $\pm$  SD).

TABLE 3. Diameters of the Inhibition Zones of the Silver-Deposited ACF Filters (Mean  $\pm$  SD)

materials	diameters of inhibition zones (mm)	
	<i>E. coli</i>	<i>B. subtilis</i>
ACF/Ag-00	0	0
ACF/Ag-10	10.85 $\pm$ 0.20	11.95 $\pm$ 0.21
ACF/Ag-20	11.25 $\pm$ 0.21	13.7 $\pm$ 0.85
ACF/Ag-30	11.8 $\pm$ 0.28	14.55 $\pm$ 0.64

The diameters of the inhibition zones of *B. subtilis* were larger than those of *E. coli*. Therefore, silver had better antimicrobial effects against *B. subtilis*, Gram-positive bacteria, than against *E. coli*, Gram-negative, as discussed in previous studies (17, 18). The lower sensitivities of Gram-negative bacterial strains could probably be explained by the biochemical and physiological characteristics of those bacteria. It is well-known that the outer membrane of Gram-negative bacteria is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective resistibility barrier (8, 19–21).

Figures 5 and 6 show the temporal variations in the colony ratio ( $C/C_0$ ) of *E. coli* and *B. subtilis* on the tested ACF filters over a 240 min period, respectively. On the pristine ACF, the colony ratio of both bacteria increased with time, implying multiplication of bacteria on the pristine ACF filters. As the contact time or amount of silver increased, the colony ratio decreased, as shown in both figures. While *E. coli* was completely inhibited for all silver-containing ACF filters after 60 min, the colony ratio of *B. subtilis* decreased more rapidly because of the lower resistivity of *B. subtilis* against silver, as discussed in the disk-diffusion experiments.

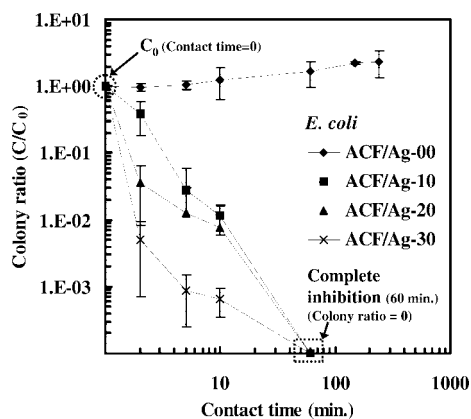


FIGURE 5. Antimicrobial effects of the test filters on *E. coli* (mean  $\pm$  SD).

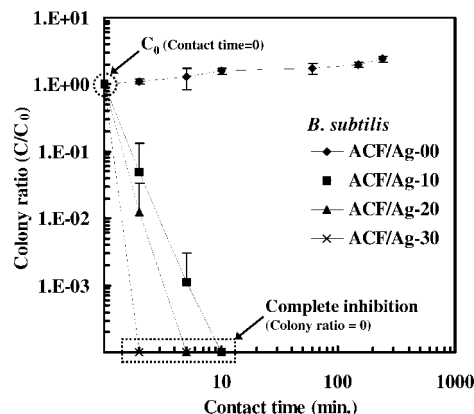


FIGURE 6. Antimicrobial effects of the test filters on *B. subtilis* (mean  $\pm$  SD).

In conclusion, silver-deposited ACF filters were successfully prepared using an electroless deposition method, and their characteristics were investigated. Silver-deposited ACF filters show antimicrobial activities and were effective in the control of bioaerosols by inhibition of bacterial survival on the ACF filters, which eventually prevented secondary contamination of ACF filters by breeding of bacteria. Even though the electroless silver deposition did not influence the physical properties of the ACF filters such as the pressure drop and filtration efficiency, the adsorptive efficacy was decreased by silver deposition. Therefore, the silver content needs to be optimized according to the species and the concentrations of background bioaerosols in the intended area of application of the silver-deposited ACF filters.

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