

Short Communication

Antimicrobial Characteristics of Silver Aerosol Nanoparticles against *Bacillus subtilis* Bioaerosols

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ABSTRACT

Silver aerosol nanoparticles generated by an atomizer were investigated as an antimicrobial agent against *B. subtilis* bioaerosols, a model for Gram-positive bacteria. The bioaerosols and silver aerosol nanoparticles were mixed in a test duct for 15 s and sampled on nutrient agar plates by an impactor. Peak concentrations of the silver aerosol nanoparticles at the mobility diameter of 80 nm were controlled from 1.5×10^5 to 1.0×10^6 particles/cm³, depending on the concentration of silver hydrosol nanoparticles. The maximal antimicrobial efficiency of silver aerosol nanoparticles was 76% under selected experimental conditions.

Key words: bioaerosols; nanoparticles; air pollution control processes; indoor air quality; disinfection

INTRODUCTION

BIOAEROSOLS ARE AIRBORNE PARTICLES of biological origins, including viruses, bacteria, fungi, and all varieties of living materials. They are known as etiological agents of many diseases such as anthrax, SARS (severe acute respiratory syndrome), asthma, and so on. Bioaerosols of indoor

air accumulate on the filters of heating, ventilating and air-conditioning (HVAC) systems in large quantities, and are able to multiply there under certain conditions, especially if high amounts of moisture on the filter are present (Schleibinger and Rüden, 1999). Numerous engineering solutions are commercially available, and others are under development for removal of bioaerosols such as air filtration,

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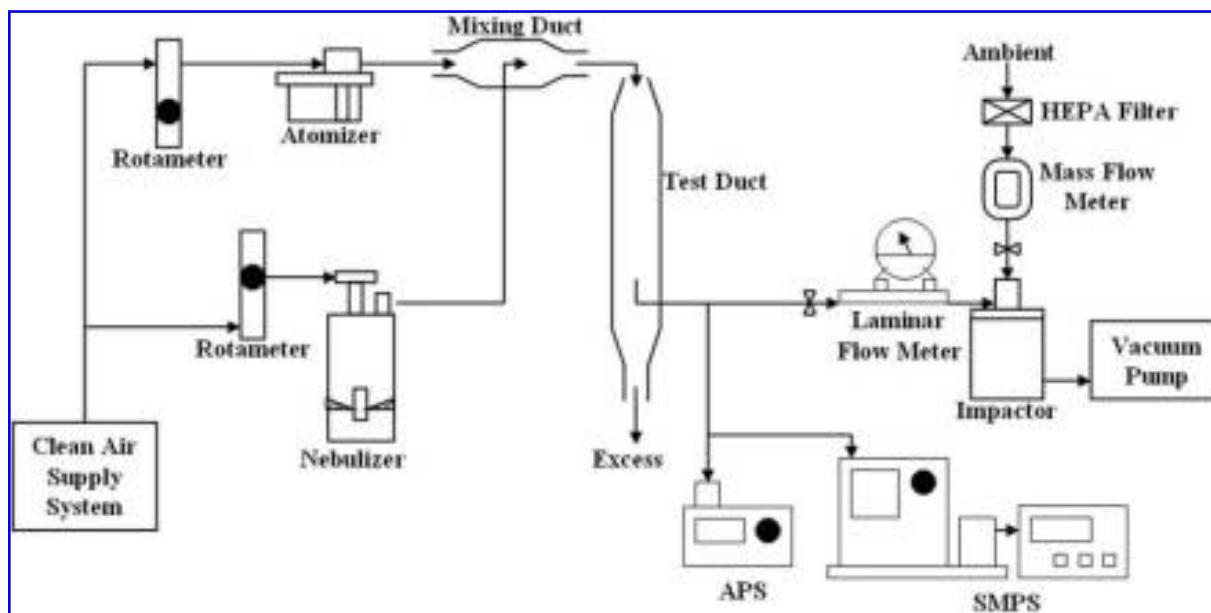


Figure 1. Experimental setup.

ultraviolet germicidal irradiation (UVGI), negative air ionization, electrostatic precipitation, photocatalytic oxidation, air ozonation, and so on (Griffiths *et al.*, 2005). Direct application of functional materials in aerosol state has been suggested as a novel method for air quality control. For instance, carbon aerosol particles were applied to adsorb VOCs (Byeon *et al.*, 2006). Traditionally, silver is known as antimicrobial agent. Sondi and Salopek-Sondi (2004) and Cho *et al.* (2005) investigated the antimicrobial effect of silver nano colloid particles against *Escherichia coli* (Gram-negative bacteria) and *Saccharomyces aureus* (Gram-positive bacteria). Other researchers have been trying to develop new materials containing silver and verify their antimicrobial characteristics against various kinds of bacteria (Holt and Bard, 2005; Wang *et al.*, 2006; Yoon *et al.*, 2008). In this study, *Bacillus subtilis* bioaerosols, a model for Gram-positive bacteria in the aerosol state, were selected to investigate the efficacy of silver nanoparticles generated by an atomizer as an antimicrobial agent.

MATERIALS AND METHODS

Suspension of *B. subtilis* (ATCC 6633) was prepared by culturing again 0.1 mL of an overnight culture inoculated in 15 mL of nutrient broth for 18 h at 30°C. Nutrient broth was made by dissolving 5 g of peptone and 3 g of meat extract in 1,000 mL of deionized water and then by sterilizing the broth with an autoclave. The suspension was diluted with sterile deionized water to obtain a base suspension with a bacterial density of 10^4 cells/mL. A suspension of silver

nano particles (SARPU, ABC Nanotech Co. Ltd., Korea) was purchased. The average diameter of the silver nanoparticles was informed as 40 nm, and these nanoparticles were suspended in water at the concentration of 10 wt%. The suspension of silver nanoparticles was diluted with deionized water to obtain suspensions of various concentrations, 0.3, 0.1, and 0.03 wt%.

Figure 1 shows the experimental setup. *B. subtilis* bioaerosols were dispersed into air by a nebulizer (1 jet, BGI Inc.) at a flow rate of 2 L/min. To generate the silver aero-

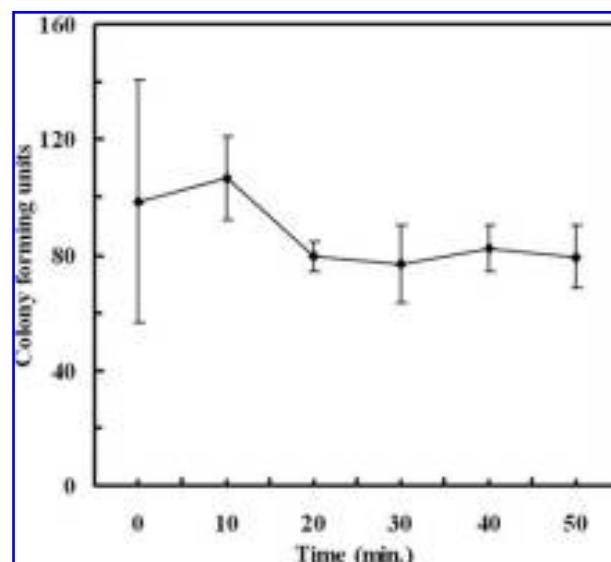


Figure 2. Stability of bioaerosol generation and sampling.

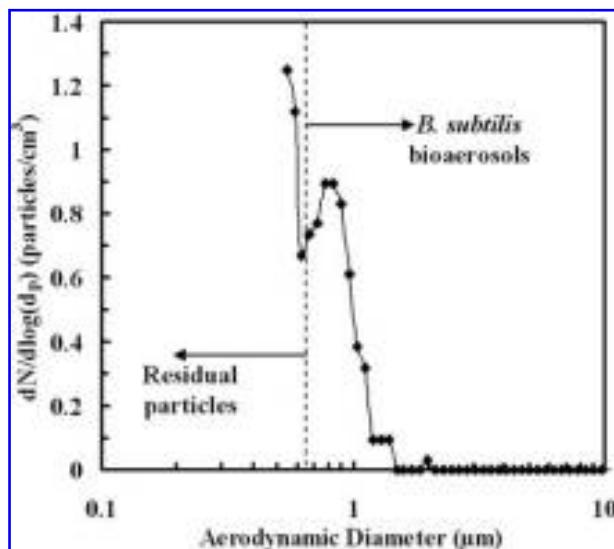


Figure 3. Size distribution of *B. subtilis* bioaerosols.

sol nanoparticles, water-based colloidal silver nanoparticles at the concentrations of 0.3, 0.1 and 0.03 wt% were atomized by an atomizer (model 9302, TSI Inc.).

Bioaerosols and silver aerosol nanoparticles were introduced into the test duct (i.d. = 80 mm) through a dilutor (i.d. = 42 mm), which were connected with a flexible tube having a much smaller diameter (i.d. = 7 mm). This geometric reduction and expansion at the connection tube is effective for aerosol mixing, because the tube acts like an orifice (Ji *et al.*, 2004).

Size distributions of the bioaerosols and silver aerosol nanoparticles were measured using APS (Aerodynamic Particle Sizer; model 3321, TSI Inc., Shoreview, MN) and SMPS (Scanning Mobility Particle Sizer; model 3936, TSI Inc.), respectively. APS measures the aerodynamic diameter of particles by the time of flight between two laser beams. SMPS classifies the particles as electrical mobility and measures the number of the classified particles by growing the size through the evaporation and condensation processes. The measurable ranges of APS and SMPS are 0.5–20 μm and 14.1–710 nm, respectively.

The bioaerosols and silver aerosol nanoparticles were mixed in a test duct for about 15 s and sampled on nutrient agar plates by an impactor (TE-10-800, Tisch Environmental Inc., Cleves, OH). The cutoff size of the impactor was 500 nm in aerodynamic diameter. For 2 min, 3 L/min was sampled from the test duct and 25.3 L/min was filled up from ambient through the HEPA filter because the operating flow rate of the impactor was 28.3 L/min. In the sampling process, the bioaerosols (with or without silver nanoparticles on their surfaces) larger than 500 nm impacted on the nutrient agar plate, whereas the silver aerosol nanoparticles smaller than 500 nm passed the plate. The

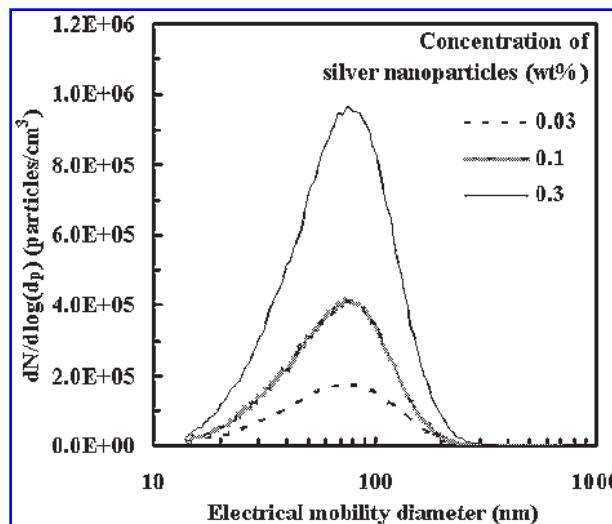


Figure 4. Size distributions of silver aerosol nanoparticles.

aerosols sampled on the nutrient agar plates were incubated for 24 h at 30°C. Through incubation, it was expected that the bioaerosols without silver nanoparticles on their surfaces would form colonies so that visual inspection was possible, while the bioaerosols aggregated with silver nanoparticles would not form colonies because of the antimicrobial effect of the silver nanoparticles.

Prior to the antimicrobial test, the stability of bioaerosol generation was tested by sampling bioaerosol particles with a time interval of 10 min and the results are shown in Fig. 2. The number of bacterial colonies was stabilized after 20 min of nebulization. All the experiments in this paper were

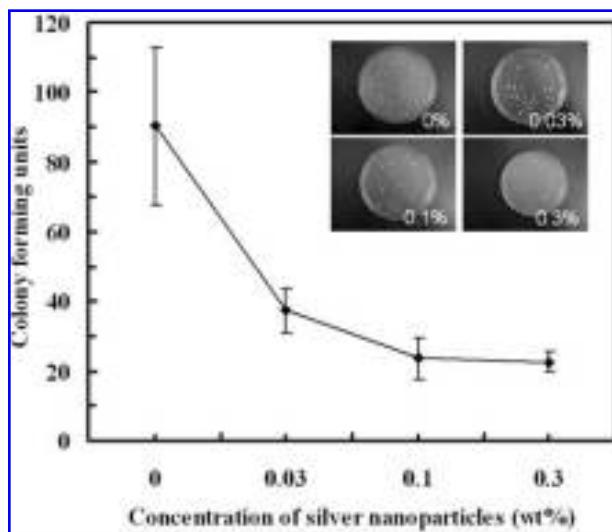


Figure 5. Antimicrobial results of silver aerosol nanoparticles (Mean \pm SD).

carried out once the bioaerosol generation was at steady state, with and without the generation of silver nanoparticles. This initial fluctuation of bioaerosol generation within 20 min also occurred in the work by Stewart *et al.* (1995). Each experiment was repeated three times with fresh bacterial suspensions for generation of bioaerosols.

RESULTS AND DISCUSSION

Figures 3 and 4 show the size distributions of *B. subtilis* bioaerosols and silver aerosol nanoparticles, respectively. The peak (aerodynamic) diameter of *B. subtilis* bioaerosol was approximately 0.8 μm and this diameter was in good agreement with the result of a previous study (Lee *et al.*, 2002). In the generation of bioaerosols, a considerable amount of residual particles smaller than 0.6 μm was generated with bioaerosols. These small particles may have originated as fragments of cells and other components associated with bacteria culture (Qian *et al.*, 1995; Terzieva *et al.*, 1996).

The peak (electrical mobility) diameter of the dispersed silver aerosol nanoparticles was about 80 nm, even though the mean size of commercial silver nanoparticles was informed as 40 nm. It is believed that the 80 nm silver aerosol nanoparticles would be aggregates of several 40 nm silver particles.

The electrical mobility diameter could be converted to the aerodynamic diameter by equation (1) (Sioutas *et al.*, 1999).

$$d_{\text{SMPS}} = \left[\frac{\kappa C_{\text{APS}}}{\rho_p C_{\text{SMPS}}} \right]^{1/2} d_{\text{APS}} \quad (1)$$

C is the Cunningham correction factor, κ is the dynamic shape factor (=1 for a spherical droplet particle), d_{APS} is the aerodynamic diameter, d_{SMPS} is the electrical mobility diameter, and ρ_p is the particle density (=10 g/cm³ for silver). The converted peak (aerodynamic) diameter of the 80 nm silver aerosol nanoparticle was approximately 370 nm. Therefore, the silver aerosol nanoparticles could pass the nutrient agar plate without being filtered in the impaction plate.

The peak concentration of silver aerosol nanoparticles increased from 1.5×10^5 to 1.0×10^6 particles/cm³ as the concentration of the water-based colloidal silver nanoparticles increased from 0.03 to 0.3 wt%.

Figure 5 shows the number of *B. subtilis* bacterial colonies as a function of the concentration of silver suspensions. The curve shows a trend in which the number of colonies decreases with the increasing concentration of silver nanoparticles. On average, 90 colonies were formed on the agar plates without silver nanoparticles, whereas 37 and 22 colonies were formed when the bioaerosols were mixed with silver aerosol nanoparticles dispersed from the silver suspensions of 0.03 and 0.3 wt%, respectively. As a result, the maximal antimicrobial efficiency ($\eta = 1 - C_{\text{w/o Ag}}/C_{\text{w/o Ag}}$)

of airborne silver nanoparticles was 76% under the selected experimental conditions.

CONCLUSION

The applicability of silver nanoparticles in aerosol state as antimicrobial agent against *B. subtilis* bioaerosols was investigated. Bioaerosols and silver aerosol nanoparticles were dispersed into air and sampled by an impactor after mixing them for 15 s in a test duct. The antimicrobial efficiency was evaluated by colony counting after incubation of the samples for 24 h at 30°C. Silver aerosol nanoparticles were found to have the maximal antimicrobial efficiency of 76% under selected experimental conditions. Therefore, silver nanoparticles can be applied to improve the indoor air quality by dispersing them in the front of filters in air handling units (AHU) of HVAC systems.

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