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Inactivation of Airborne *E. coli* and *B. subtilis* Bioaerosols Utilizing Thermal Energy

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Abstract Airborne microorganisms, which are currently termed bioaerosols, have received attention owing to the harmful effects they have on human health. As the concern over airborne microorganisms grows, there also grows an urgent need to study and develop efficient methods for controlling them. In this study, thermal energy using a thermal tube was tested as a control method, mainly against airborne *E. coli*. For a comparison, *B. subtilis* var. *niger* spores were utilized in the experimentation. It was found that the widely known inactivation conditions for microorganisms were not adequate against airborne microorganisms. The experimental results demonstrated the need for extensive studies that should investigate adequate and economic conditions to control against airborne bacteria. In this study, thermal energy exposed by the thermal tube demonstrated an inactivation performance for controlling *E. coli* bioaerosols.

Key words: Airborne bacteria, bioaerosols, *Escherichia coli*, *Bacillus subtilis* var. *niger*, thermal energy, culturability

Airborne microorganisms, currently termed bioaerosols, have been receiving scientific attention owing to their high potential for harmful effects on human health. There are a number of various bioaerosols such as airborne bacteria, viruses, and fungal spores in the air [8]. Airborne microorganisms can flow freely with air movements and spread widely in a short period of time. Consequently, the skin and respiratory system of humans are highly vulnerable to exposure. Pathogenic airborne microorganisms can cause serious contagious diseases; this concern was clearly justified after the 2001 bioterror incident in the USA, where anthrax bioaerosols were utilized to which hundreds of people were exposed (Sternberg, S., Anthrax alert still echoes, one year later. *USA TODAY*, Oct. 10. 2002).

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Thus far, the general public has taken proper precautions against pathogens in terms of surface and water pathogens; however, precautions against airborne pathogenic microorganisms remain inadequate. In 2004, the Korean government recognized the seriousness of harmful bioaerosols and included airborne microorganisms as a parameter in the national indoor pollutant standards (Ministry of Environment. 2006. Law for indoor air quality control in the public facilities. http://me.go.kr). The parameter was the concentration of total airborne bacteria in the air, which is one of the major parameters for bioaerosols. Because of their health effects and spurred by a governmental desire for action, there is now an urgent need regarding the study and development of control methods against airborne microorganisms.

Once bacteria become airborne, in addition to biological properties, they also possess the physical characteristics of airborne particles called aerosols. Aerosols have unique properties such as thermophoresis, electrophoresis, and diffusion. They are also subject to fluid flow [4]. Therefore, aerosol properties and fluid flow should be considered in a study that centers on control methodology against bioaerosols.

Bioaerosols can cause health problems via several mechanisms, such as hypersensitivities (allergic reactions), toxic reactions, through interactions with other air pollutants, and by causing infections. When living microorganisms invade the human body, they can grow inside the body and cause diseases. This mechanism, known as an infection, is representative of the mechanisms by which bioaerosols cause harmful health effects in people. A critical condition for an infection is that bioaerosols must be alive, meaning that they must be viable. The viability of bioaerosols is one of the most important parameters to consider when attempting control of them.

Several methods have been suggested for controlling the viability of bioaerosols. Given that ultraviolet (UV) irradiation has been known to have a germicidal effect, several studies have examined how UV irradiation affects the viability of bioaerosols. One study utilized *BCG*

mycobacteria and *Serratia marcescens* to test bacterial inactivation by UV irradiation [22], whereas another compared the UV germicidal irradiation with the air ventilation effect [19]. Although UV germicidal irradiation can be easily applied by simply installing and turning on a UV lamp, it is possible that the UV light may have adverse effects, such as skin inflammation, on surrounding humans.

Electric ion emission has also been studied as a means of controlling bioaerosols. Air ion emission can remove indoor aerosol particles, and by increasing the efficiency of the filter, it can enhance respiratory protection devices against bioaerosols [13–15]. Additionally, although air electric ions decrease the viability of airborne bacteria [6], this defense has some shortcomings; for instance, the production of the ions produces ozone, a pollutant; it also causes electric charges to accumulate on surrounding surfaces.

Thermal energy has long been considered as a suitable method for controlling microorganisms [16, 20, 21]. The high temperature environment kills microorganisms by causing the denaturation of critical enzymes [17]. Two types of heat are generally used; moist heat and dry heat. Moist heat utilizes steam under pressure, whereas dry heat involves high temperature exposure without additional moisture. Several types of heat treatments are currently used for killing microorganisms. The treatments include incineration, Tyndallization, pasteurization, and autoclaving [17]. However, most of these technologies were originally targeted to kill microorganisms in food or water. For instance, it is known that *E. coli* can be inactivated by exposure to moist heat at 121°C for approximately 15 min (Health Canada. 2001. Material safety data sheet - infectious substances, http://www.msdsonline.com/Support/Resources/Health_Canada/HC000066.pdf). This method is adequate for *E. coli* in food or in water; however, it may not be adequate for controlling airborne *E. coli*, because the environment of airborne microorganisms is obviously different from the conditions of food or water. Therefore, it is necessary to find adequate and practical conditions for controlling airborne bacteria.

Thus far, there have been several investigations into using thermal energy against airborne bacteria. Some of these studies have been targeted at airborne spores widely used as simulants for biological warfare agents [1, 5, 18], and others focused on several vegetative cells [2, 3, 24]. However, in these studies, the novel techniques for aerosols (such as measuring aerosol particle size distributions and concentrations) necessary to confirm and elaborate the experimental processes were not utilized. Recently, the potential of usage of thermal energy against airborne *E. coli* [12] was proposed by the authors; however, the detailed experimentation methodology, although including various conditions, was insufficient, and an analysis and comparison with other microorganisms was absent in that study.

Here, thermal inactivation against airborne *E. coli* is looked at in more detail. *E. coli* is a popular microorganism in bioaerosol studies [10, 11]. In addition, airborne *E. coli* has been found in occupational air environments [26], and a recent study implicated the airborne spread of *E. coli* O157:H7 during an outbreak investigation [25]. The main focus in this study was *E. coli*, and for a comparison, *B. subtilis* spores were also tested. As *B. subtilis* spores have been known to be very resistant to high temperatures, it was felt necessary to confirm this fact in the present experimental system.

To examine the effect of thermal energy on airborne bacteria, a thermal tube system was developed in which bioaerosols were exposed to a high temperature environment. The tube system was designed as a possible control device for an indoor environment, and as a component of an experimental aerosol system. Techniques for quantitatively generating and measuring aerosol particles were used.

MATERIALS AND METHODS

E. coli and *B. subtilis* var. *niger* spores (BG spore) were used as test microorganisms in this study. *E. coli* is a typical Gram-negative bacterium that is 0.6 μm to 0.8 μm wide and 1.3 μm to 1.4 μm long. It was purchased from the Korean Culture Center of Microorganisms (KCCM 70089). The *E. coli* was grown in Luria-Bertani (LB) growth medium (Luria-Bertani broth 25 g and agar 15 g per one liter of deionized water) for 14 to 18 h at 37°C. In this study, the main focus was airborne *E. coli*, but a confirmation of the strong resistance of the *B. subtilis* spores was also sought.

The Gram-positive *B. subtilis* var. *niger* is rod-shaped and can form a special structure, termed an endospore, which is very resistant to environmental stressors. The spore consists of several concentric structures; a plasma membrane, a germ cell wall, a cortex, a coat, and an exosporium; and its size can range from 0.5 μm to 0.8 μm in width and from 1.3 μm to 1.8 μm in length. The strain has been used for several decades as a test organism in biodefence studies, as it is a feasible representative of a resistant Gram-positive bacteria bioaerosol. The sample utilized in the present study was obtained from the Korean Collection for Type Cultures (KCTC 1022). To induce sporulation, the sample was inoculated in a special NB growth medium (nutrient broth 25 g, agar 15 g, $MnSO_4$ 10 mg, and $MgSO_4$ 500 mg per one liter of deionized water), incubated for 7 days at 37°C, and then kept at 4°C for 1 month (Urzica, A. A. C. 2004. High hydrostatic pressure inactivation of *B. subtilis var. niger.* Spores: the influence of the pressure build-up rate on the inactivation. Dissertaion. Ruprecht Karls Universität, Heidelberg, Germany) [7, 23]. To prevent contamination by vegetative cells, the sample was heated at 80°C for 30 min.

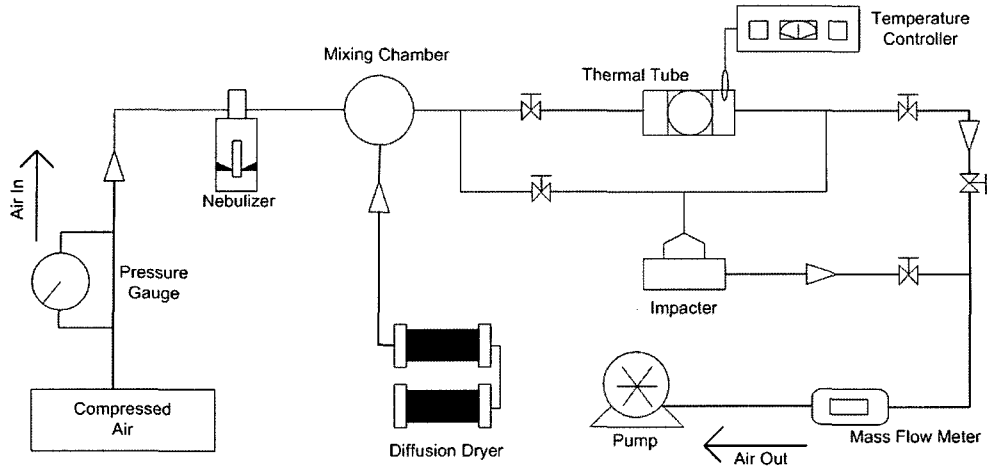

Fig. 1. The experimental setup for testing the effect of thermal energy on bioaerosols.

To aerosolize the *E. coli* and *B. subtilis* var. *niger* spores in a nebulization process, a liquid suspension of microorganisms [6] was created. The nebulization method has been used in several studies [6, 10, 11], and bioaerosols generated from the nebulization process can be regarded as a surrogate for environmental bioaerosols that were generated from wavy liquid sources, such as lakes, rivers, and so on. Fifty ml of deionized water was used to dilute 1 ml of the liquid suspension of either *E. coli* or *B. subtilis* var. *niger* spores. The diluted suspension of bacteria was then poured into a nebulizer. For this process, a Microbiological Research Establishment type 6-Jet Collison Nebulizer (BGI Collison Nebulizer, Porton, U.K.) was utilized. This nebulizer sprayed the solution into a number of tiny droplets that contained bacteria. The sprayed droplets flowed into the experimental system via the flowing gas.

Fig. 1 shows the experimental setup. The generated bioaerosols, which contained moisture, were mixed with dry, clean air that passed through a HEPA filter and a diffusion dryer. The moisture of the droplets was vaporized by mixing with the air, and the bacteria, which were contained inside the droplets, became bioaerosols in the experimental system. The diluted bioaerosols entered the thermal tube and were exposed to thermal energy while passing through the thermal tube. The relative humidity at the inlet of the thermal tube was approximately 40± 10%.

The thermal tube was designed and manufactured specifically for this study. This thermal tube, which consists of a conical inlet and outlet with a cylindrical middle zone, was 210 mm in length, and the inner diameter of its middle zone was 80 mm. The flow rate through the thermal tube was 28.3 l per min. There was an electric heating wire inside the thermal tube, which was connected to an electric current controller. The temperature inside the thermal tube was automatically fixed to a specific target value by the electric controller. The electric controller, which monitored the temperature via a thermocouple at the inner center of the thermal tube, adjusted the electric current until the temperature reached the target value. The *E. coli* and *B. subtilis* var. *niger* spores, which were generated by the nebulization process, were exposed to maximum temperatures of 140°C and 180°C in this thermal tube, respectively. The estimated exposure time through the heating control zone of the thermal tube was approximately 0.3± 0.2 sec.

To investigate the transport loss through the thermal tube, a Particle Size Distribution Analyzer (TSI 3603) was used to measure the aerosol particle size distribution of the bioaerosols at both the inlet and outlet of the thermal tube.

In this study, the culturability was measured as a parameter for the viability of airborne bacteria. An Andersen sampler was used to measure the culturability of the bioaerosols. In this sampler, nozzles were used to accelerate the bioaerosols, which were impacted onto an agar plate. Bioaerosols that were deposited on the plate were incubated for 18 h, and the colonies that formed on the plate were enumerated. The colony-forming units (CFUs) of bioaerosols per sampled volume of air (CFU/m^3) were enumerated at the inlet and the outlet of the thermal tube as a parameter that represented the viability of the bioaerosols.

RESULTS AND DISCUSSION

Figs. 2 and 3 show the particle size distribution of the *E. coli* and *B. subtilis* var. *niger* spore bioaerosols, respectively. The particle size distribution was measured at the inlet and the outlet of the thermal tube under different temperature conditions. The particle concentration was normalized in terms of the highest concentration measured

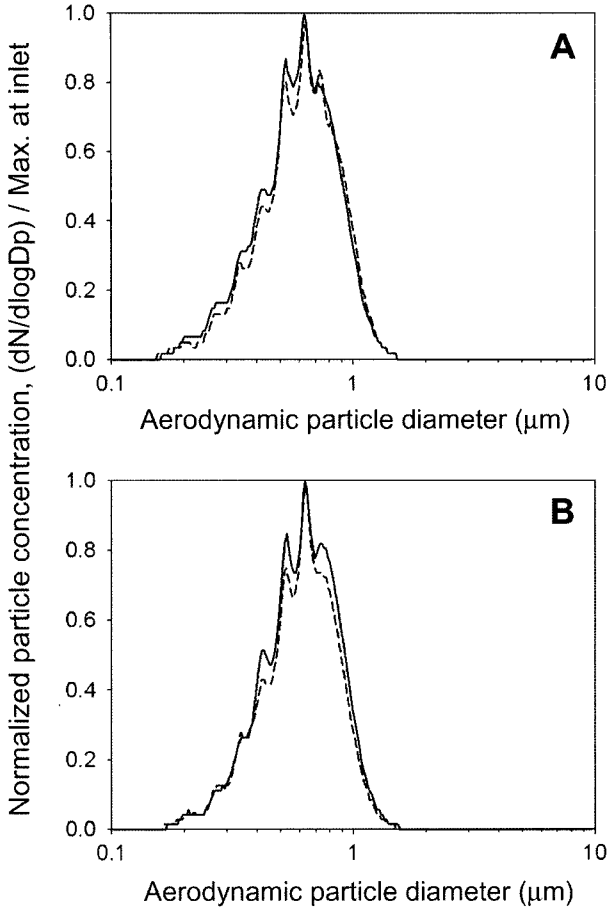

Fig. 2. A comparison of size distributions of aerosolized *E. coli* particles at the inlet (straight line) and the outlet (dashed line) of the thermal tube.
The particle concentration was normalized in terms of the highest particle number concentration at the inlet. The temperature of the heating filters was 27°C (**A**) and 140°C (**B**), respectively.

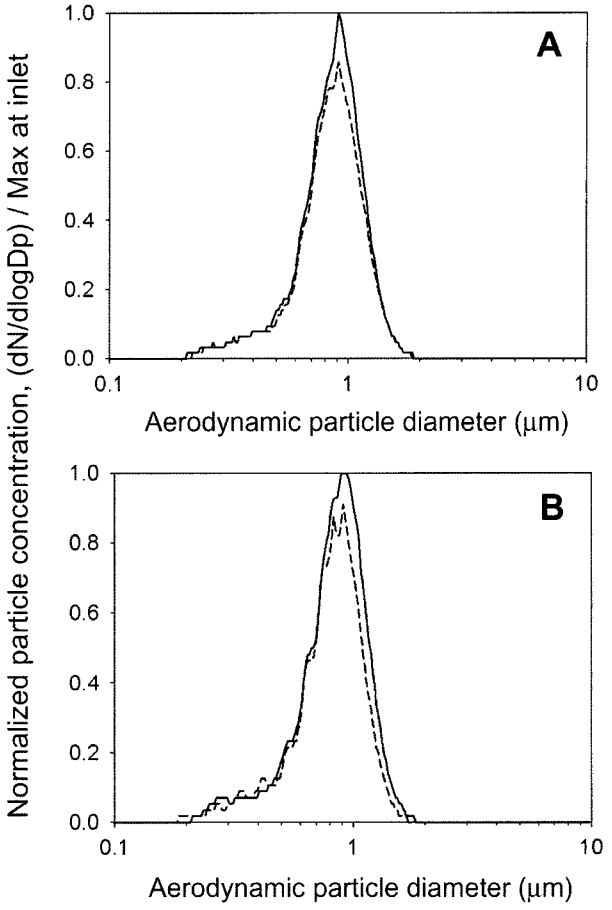

Fig. 3. A comparison of size distributions of aerosolized *B. subtilis* var. *niger* spore particles at the inlet (straight line) and the outlet (dashed line) of the thermal tube.
The particle concentration was normalized in terms of the highest particle number concentration at the inlet. The temperature of the heating filters was 27°C (**A**) and 180°C (**B**), respectively.

at the inlet. From this figure, it was found that the bioaerosols kept the shape of their particle size distribution while they passed through the thermal tube. The transport loss was small and insignificant compared with the level of concentration. The shapes of the particle size distributions at the inlet and the outlet were similar, regardless of temperature. Furthermore, the similarity of the particle size distributions can be seen in Tables 1 and 2, which show the geometric mean diameters and standard deviations of the particle size distributions. From Figs. 2 and 3, it was found that the airborne bacteria retained their physical properties as aerosols while passing through the thermal tube. This indicates that the developed thermal tube can influence airborne bacteria without causing a physical particle loss. The airborne bacteria had different particle sizes compared with the one that was microscopically measured. The Particle Size Distribution Analyzer (TSI 3603) utilized in this study detected the aerodynamic diameter of airborne bacteria; in other words, this device measured microorganisms as physical aerosols. Thus, these airborne microorganisms had the diameters of aerosols, as shown in Figs. 2 and 3. The total concentration of the airborne *E. coli* bacteria ranged from approximately 10^4 CFU/m^3 to 2×10^4 CFU/m^3, and the airborne *B. subtilis* spores ranged from approximately 4×10^3 CFU/m^3 to 2×10^4 CFU/m^3.

Fig. 4 shows the culturability loss of *E. coli* bioaerosols through the thermal tube. This loss is expressed as follows:

Table 1. Average geometric mean diameter and standard deviation of aerosolized *E. coli* particles.

	Geometric mean diameter (μm)	Standard deviation
27°C inlet	0.578	1.47
27°C outlet	0.595	1.46
140°C inlet	0.596	1.44
140°C outlet	0.588	1.44

Table 2. Average geometric mean diameter and standard deviation of aerosolized particles of *B. subtilis* var. *niger* spores.

	Geometric mean diameter (μm)	Standard deviation
27°C inlet	0.828	1.40
27°C outlet	0.825	1.41
140°C inlet	0.806	1.42
140°C outlet	0.771	1.43

$$\text{Culturability loss} = \frac{CFU_{inlet} - CFU_{outlet}}{CFU_{inlet}} \quad (1)$$

Most *E. coli* bioaerosols did not lose their culturability when they passed through the thermal tube at room temperature. This result supports two findings. First, this small loss of culturability of airborne *E. coli* at room temperature was in harmony with the estimation in Fig. 2, specifically that there would be a small transport loss through the thermal tube. Second, at the same time, airborne *E. coli* maintained its culturability while passing through the thermal tube at room temperature.

As the temperature increased, the culturability of *E. coli* bioaerosols quickly decreased, and the culturability loss curve began to resemble an S-shape curve. The rate of culturability loss was high at nearly 80°C, which represented approximately a 30% culturability loss for an increment of 20°C. More than 99% of the *E. coli* bioaerosols lost their culturability at 140°C; thus, the aerosol particles measured at the outlet at 140°C represented non-living *E. coli* bioaerosols. Thus far, it has been understood that *E. coli* could be inactivated by dry heat at 160–170°C for approximately 1 h (Health Canada. 2001. Material safety data sheet - infectious substances, http://www.msdsonline.com/Support/Resources/Health_Canada/HC000066.pdf); however, in this study, airborne *E. coli* bacteria were inactivated by exposure to a dry heat condition using the thermal tube at 140°C for about 0.3 sec. This finding supports the contention stated in the Introduction that the inactivation conditions for microorganisms in food or in water are different from conditions for airborne microorganisms.

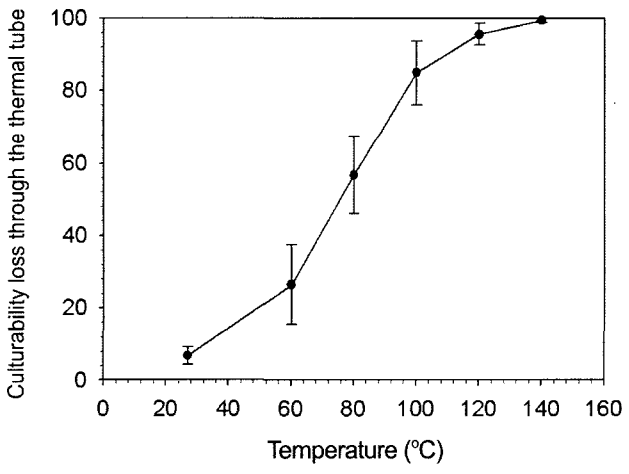

Fig. 4. The culturability loss of aerosolized *E. coli* at elevated temperatures.
A minimum of three measurements were taken for each datum point.

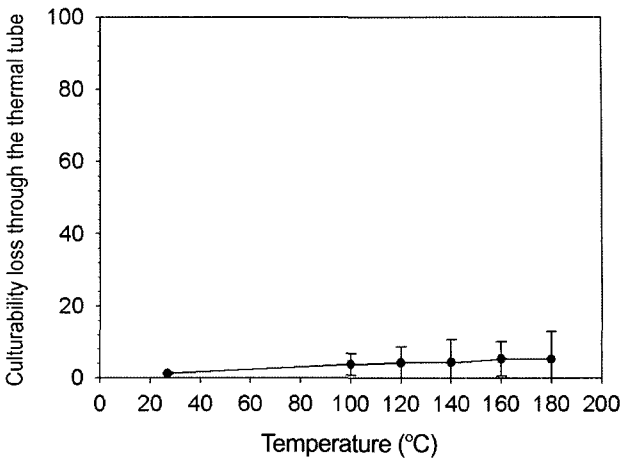

Fig. 5. The culturability loss of aerosolized *B. subtilis* var. *niger* spore at elevated temperatures.
A minimum of three measurements were taken for each datum point.

Fig. 5 shows the culturability loss of *B. subtilis* var. *niger* spore bioaerosols, most of which did not lose their culturability even after being exposed to a temperature of 180°C. The loss of culturability was found to be approximately 5%. A previous study demonstrated that aerosolized *Bacillus subtilis* var. *niger* (*B. subtilis* var. *niger*) spores could be killed in 0.02 sec when exposed to temperatures above 260°C [18]. It is difficult to directly compare the current study and this study, however, as the authors of the referenced study did not use the aerosol techniques of measuring particle size distributions; additionally, the exposure time in the referenced study was different from the present study. In the present study, it can be confirmed that the *B. subtilis* var. *niger* spore bioaerosols were resistant to harsh conditions, such as being exposed to 180°C for about 0.3 sec.

Overall, in this study, a thermal tube that inactivated airborne *E. coli* was developed, and *B. subtilis* var. *niger* spores were used for a comparison. To quantify the effect of the heat stress on the culturabilities of the *E. coli* and *B. subtilis* spore bioaerosols, an Andersen viable impactor was used at both the inlet and outlet of the thermal tube in order to measure the number of CFUs per sampled volume (CFU/m^3). More than 99% of the *E. coli* bioaerosols lost their culturability at 140°C, although the *B. subtilis* spores showed no significant culturability loss attributable to thermal energy, even at 180°C. Thus, the thermal tube was found to be very effective for controlling *E. coli* vegetative cell bioaerosols.

This type of inactivation information against airborne microorganisms is useful as the need for developing extensive control methods for airborne bacteria increases.

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