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Technical note

In situ real-time measurement of physical characteristics of airborne bacterial particles

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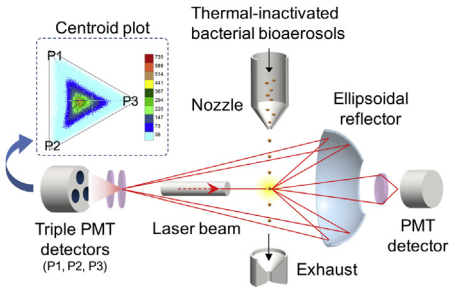
GRAPHICAL ABSTRACT



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ABSTRACT

Bioaerosols, including aerosolized bacteria, viruses, and fungi, are associated with public health and environmental problems. One promising control method to reduce the harmful effects of bioaerosols is thermal inactivation via a continuous-flow high-temperature short-time (HTST) system. However, variations in bioaerosol physical characteristics – for example, the particle size and shape – during the continuous-flow inactivation process can change the transport properties in the air, which can affect particle deposition in the human respiratory system or the filtration efficiency of ventilation systems. Real-time particle monitoring techniques are a desirable alternative to the time-consuming process of microscopic analysis that is conventionally used in sampling and particle characterization. Here, we report *in situ* real-time optical scattering measurements of the physical characteristics of airborne bacteria particles following an HTST process in a continuous-flow system. Our results demonstrate that the aerodynamic diameter of bacterial aerosols decreases when exposed to a high-temperature environment, and that the shape of the bacterial cells is significantly altered. These variations in physical characteristics using optical scattering measurements were found to be in agreement with the results of scanning electron microscopy analysis.

1. Introduction

The National Human Activity Pattern Survey reported that adults spend an average of 87% of their time inside buildings, including houses, offices, and schools, and about 6% of their time in vehicles (Klepeis et al., 2001). Thus, the air quality of the indoor

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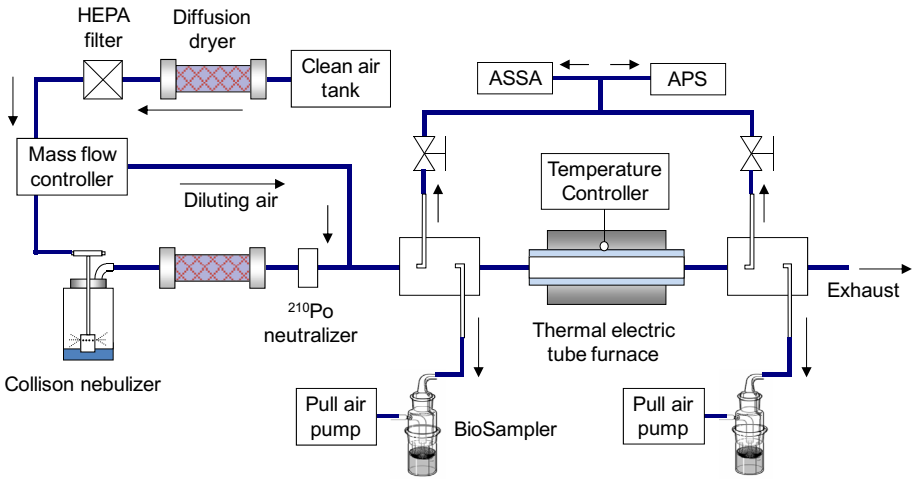

Fig. 1. Experimental apparatus.

environment is significant for human health. Indoor pollution has been ranked by the U.S. Environmental Protection Agency Science Advisory Board and Centers for Disease Control as a high environmental risk (Roberts and Dickey, 1995). Airborne particles of biological origin, termed bioaerosols, account for the majority of ambient contaminants, and are related to disease and public health problems (Burge, 1990). They can be hazardous as pathogenic living materials, can act as allergens or irritants (Cox and Wathes, 1995), and some microorganisms produce toxic metabolites, including endotoxins and mycotoxins.

Concern regarding the harmful effects of bioaerosols has increased the demand for efficient methods to control them. Several methods, including ultraviolet irradiation (Lin and Li, 2002; Walker and Ko, 2007), electric ion emission (Grinshpun et al., 2005), heat treatment (Grinshpun et al., 2010a; Jung et al., 2009b), and the use of silver nanoparticles (Ji et al., 2007; Rangari et al., 2010), have been reported to control bioaerosols. Dry heat treatment using a high-temperature short-time (HTST) process can effectively inactivate fungal, bacterial and viral bioaerosols in a continuous-flow system (Grinshpun et al., 2010a, 2010b, 2010c; Jung et al., 2009a, 2009b, 2010; Lee and Lee, 2006). The HTST technique has important applications in biodefense and counterterrorism, in which biological warfare agents can be exceptionally resistant to various stresses, including “dry” and “moist” heat (Setlow, 1995). HTST also has applications in air-quality and sterilization systems (Grinshpun et al., 2010b).

The viability of bacterial cells is the most commonly used evaluation method in environmental bioaerosol control studies. The physical characteristics (*e.g.*, particle size and shape) following the inactivation process of bioaerosols have been regarded as less important than the biological characteristics. However, variations in the physical characteristics may affect their transport characteristics in air, which are strongly related to the aerodynamic particle diameter (Hinds, 1989). In particular, the aerodynamic particle size can significantly affect particle deposition in the human respiratory system and the filtration efficiency of a ventilation system, as well as the performance of collection devices such as cyclone separators and impactors (Friedlander, 2000).

In most previous studies, microscopic analysis has been used to analyze the physical characteristics of airborne microorganisms. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images are useful for examining particles smaller than the limit of resolution of optical microscopes. However, the preparation of microorganism samples is time-consuming and, in addition, the specimen preparation process for SEM analysis includes dehydration and immobilization with various chemicals, which may change the cell morphology. Environmental scanning electron microscopy (ESEM) can be used to reduce artifacts created by the preparation process; however, real-time particle monitoring would more be useful for continuous and rapid bioaerosol measurement. Particle light-scattering techniques are commonly used in process control and monitoring applications.

Here, we report the change in the size and shape of bacterial aerosols following HTST thermal treatment using real-time optical scattering measurements.

2. Materials and methods

The experimental system is shown in Fig. 1. There were four major components: a system for generating airborne bacterial particles, an aerosol measurement system, a sampling system for various analyses of the bioaerosols, and a heating system.

2.1. Bacterial aerosol generation and continuous thermal exposure

Escherichia coli and *Bacillus subtilis* were used as test bioaerosols. Stock cultures of *E. coli* (Gram-negative, ATCC No. 8739) and *B. subtilis* (Gram-positive, Korean Agricultural Culture Collection [KACC] No. 10111) were obtained from the KACC (Suwon, South Korea). *E. coli* is sensitive to adverse environmental conditions (Huang and Juneja, 2001; Jung et al., 2009a; Lee et al., 2010; Palaniappan et al., 1992). Airborne *E. coli* is found in indoor and outdoor environments, and pathogenic *E. coli* O157:H7 can be spread in an airborne manner (Varma et al., 2003). *B. subtilis* is widely occurring and is more resistant to harsh environmental conditions (Agranovski et al., 2003a, 2003b; Burton et al., 2005; Dhayal et al., 2006; Hamilton and Sale, 1967; Jung et al., 2009a; Lee et al., 2010; Palaniappan et al., 1992; Yao and Mainelis, 2006, 2007). *E. coli* cultures were grown in tryptic soy broth (TSB; Becton Dickinson, Franklin Lakes, NJ, USA) at 37 °C for 18 h. *B. subtilis* cultures were grown in nutrient broth (NB; Becton Dickinson) at 30 °C for 24 h. The bacteria were harvested through centrifugation at 5000 *g* for 10 min. The pellets were washed three times with sterilized deionized distilled water, which was also used to dilute the cells and obtain a bacterial culture with an optical density of 0.89–0.91 at 600 nm.

A 20-mL aliquot of each sample was taken and placed in a six-jet collison nebulizer (BGI Inc., Waltham, MA, USA). The cell