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Liquid impinger BioSampler's performance for size-resolved viable bioaerosol particles

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ABSTRACT

This study aimed at studying the collection efficiencies of the widely used SKC BioSampler when sampling size-resolved biological viable aerosol particles using an Ultraviolet Aerodynamic Particle Sizer (UV-APS) unit. The test bioaerosols include aerosolized bacterial species and those from indoor air. For optimal performance, different flow rates (Q_{sampl}) (5, 12.5 and 20 L/min) and different volumes of collection liquids (V_{cl}) (5, 10 and 20 mL) were tested for bioaerosol particles from 0.5 to 10 μm. In addition, the DNA stain method-the LIVE/DEAD BacLight dye and culturing were also utilized to confirm the viable bioaerosol collection efficiencies of the BioSampler.

Experimental data showed that when sampling aerosolized *Bacillus subtilis* at the flow rate of 12.5 L/min, the collection efficiency of the BioSampler decreased from 82.7% (at V_{cl} = 20 mL) to 24.8% (at V_{cl} = 5 mL). The results from the BacLight dye and culturing performed for the samples collected by the BioSampler achieved similar conclusions. When sampling indoor air particles, the overall collection efficiencies of all viable biological particles were 95.3%, 87.7% and 65.5%, respectively, for V_{cl} = 20 mL, 10 mL and 5 mL at the flow rate of 12.5 L/min. When Q_{sampl} was 20 L/min, the BioSampler performed better with V_{cl} = 5 mL than other volumes. The results from this work suggest that the sampling flow rate plays a dominant role in the overall physical collection efficiency while the collection volume is crucial in preserving the viability of bioaerosol particles. Different from general practice, the operating parameters for the BioSampler should thus be adjusted respectively for target viral, bacterial and fungal aerosol particles.

1. Introduction

Bioaerosols can have an important impact on air quality, climate and health (Xu & Yao, 2011; Wei et al., 2015). These airborne biological particles could be bacteria, fungi, virus, pollens and other biological derivatives (Xu et al., 2011). In general, these materials can remain airborne for a sustained period without being removed. Exposure to these biological materials could cause numerous adverse health effects including respiratory disorders, allergic diseases and infectious diseases (Ostro, Lipsett, Mann, Braxton-Owens & White, 2001; Fabian et al., 2008; Xu et al., 2011). To protect people from their exposure, we need first to identify their presence and then quantify them.

Frequently, Andersen-type impactors are used to study airborne culturable bacterial and fungal species. However, the samplers can only detect those culturable ones which on the other hand only represent a very small fraction, i.e., generally less than 5%. In

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addition, use of Andersen-type impactors would result in significant underestimates of culturable microbial aerosol loads due to the high impaction stress, dessication and embedding problem (Zhen et al., 2009). To overcome these limitations, electrostatic sampler is being increasingly used (Yao & Mainelis, 2006a; Han & Mainelis, 2008; Tan, Shen, Yao & Zhu, 2011). It was shown that use of electrostatic sampling could obtain up to 9 times higher culturable bacterial concentration compared to Andersen sampler-BioStage (Yao & Mainelis, 2006a). Despite of these achievements, these culture-based methods fail to detect those viable but non-culturable (VBNC) species in the air. A large fraction of those VBNC cells are missed and not analyzed as a result of the culture-based limitations.

Over the years, many liquid-impingers have been developed, including the first one that was reported in *Science* journal in 1908 (Winslow, 1908). The air samples collected into liquids by the impinger can be further analyzed for all microbial species including those VBNC as well as their derivatives by a variety of available methods such as qPCR, ELISA and nano-biosensor. In modern days, a typical example of impingers commonly used is the BioSampler (SKC, Inc.), an improvement to the AGI-30 impinger through combined impaction and centrifugal motion (Willeke, Lin & Grinshpun, 1998; Lin et al., 2000). It is designed to mimic human nose, and has many advantages: relatively stable for a long time with a non-evaporating liquids; less particle bounce; less particle reaerosolization. Regardless of target aerosol particles, the BioSampler was generally operated at a standard sampling flow rate of $Q_{sampl} = 12.5$ L/min for sampling airborne particles and biologically inert airborne particles in the literature. The sampler can efficiently transfer aerosols into hydrosols and then the available molecular techniques or biochemistry methods can be utilized for adequate bioaerosol sample analysis (Xu et al., 2011). The BioSampler has been widely used since Willeke et al. invented it in 1998. There are approximately more than 200 papers involving the BioSampler. Generally, studies regarding the BioSampler can be grouped into the following: air sampling (Hader, Wright & Petters, 2014; Tseng et al., 2014; Zhen, Han, Fennell & Mainelis, 2014), evaluating the collection efficiencies (Dybwad, Skogan & Blatny, 2014; Zhen, Han, Fennell & Mainelis, 2013; Chang & Wang, 2015), developing new aerosol-to-hydrosol samplers (Wang et al., 2013), or combining it with other instruments for sample analysis (Han, Wren, DuBois, Therkorn & Mainelis, 2015; Wang, Shafer, Schauer & Sioutas, 2015). However, the BioSampler's viable biological aerosol collection efficiency and the influences of its operating parameters such as collection volume as well as its impact on the biological cell viability are not fully characterized and significantly lacking for size-resolved bioaerosol particles in the literature.

In this work, we aimed to evaluate the size-resolved biological aerosol collection efficiency of the SKC BioSampler using an Ultraviolet Aerodynamic Particle Sizer (UV-APS) unit and culturing. For this, the operating parameters such as collection volumes and sampling flow rates that influenced the sampling performance were studied. Besides, the DNA stain-the LIVE/DEAD BacLight dye was also employed in this work to study the sampling stress impact on the viability of biological aerosol cells. The results from this work can enhance the understanding of the capability of the commonly used liquid sampler, and are useful for future development of robust bioaerosol sampler.

2. Materials and methods

2.1. Test sampler and monitoring instrument

In this study, we examined the biological collection efficiencies of the BioSampler (SKC, Inc.) using a real-time fluorescence-based monitoring instrument Ultraviolet Aerodynamic Particle Sizer (UV-APS, model 3312A, TSI Inc., MN, USA). In this study, we monitored the biological aerosol collection efficiencies of the BioSampler with different flow rates ($Q_{sampl} = 5$, 12.5 and 20 L/min) and different volumes of collection liquids ($V_{cl} = 5$, 10 and 20 mL). Here, the UV-APS measures the intrinsic fluorescence (emitted by NADH, NADPH, and riboflavin) level in viable bioaerosol particles in the 0.37–20 μm range upon the UV irradiation at a wavelength of 355 nm.

2.2. Test bacteria

The physical and biological aerosol collection efficiencies of the BioSampler were determined using *Bacillus subtilis* vegetative cells. *B. subtilis* are Gram positive bacteria which have an aerodynamic diameter (d_a) of about 1.0 μm. The species is a commonly found environmental microbe and known to be very resistant to various environmental stresses when forming spores (Sneath, 1986; Friis, Davis, Figueira, Paquette & Mucci, 2003). In addition, we selected *B. subtilis* as test biological particles because they are often used as a surrogate for *B. anthracis* because of similarity in physical and biological aspects (Hill et al., 1999).

The cells of *B. subtilis* were obtained from colonies grown agar plates. Bacteria suspensions were prepared following the same procedure described in a previous study (Yao & Mainelis, 2006b). In details, *B. subtilis* first were grown on Tryptic Soy Agar (TSA, Becton, Dickson and Company, Sparks, MD) plates at 30 °C for 18 h. Before the experiments, autoclaved water was added into the agar plate and colonies of *B. subtilis* were gently scraped from the agar surfaces using an inoculation loop. The resulting bacterial suspension was washed three times by pouring them into a 50 mL tube and centrifuged at a vortex rate of 7000 rpm (Eppendorf Centrifuge 5804R, Eppendorf, Hamburg, Germany) for 7 min. After this step, the resulting pellet was re-suspended in autoclaved water and centrifuged. The final pellet of bacteria from the last centrifugation was suspended in the autoclaved water for subsequent aerosolization experiments.