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Personal Electrostatic Bioaerosol Sampler (PEBS) with High Sampling Flow Rate

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Abstract

Exposure to airborne biological agents, especially to pathogenic or allergenic microorganisms, is known to cause a wide range of health disorders in occupational and general populations. In order to improve exposure assessment of potentially affected populations, in this work, we explored a concept of a new personal bioaerosol sampling device that features high physical and biological performance when collecting airborne biological agents. The end result of this exploratory project is a developed working prototype of a personal electrostatic bioaerosol sampler (PEBS) for determining *personal* exposures to airborne microorganisms. The PEBS prototype is a self-contained device, i.e., there are no external pumps, tubings, and power supplies; the device is battery-powered and can operate for up to 4 hours.

PEBS is an open channel collector consisting of a novel wire-to-wire particle charger and a collection section housing a double-sided and removable metal collection plate and two quarter-cylinder ground electrodes. The airborne microorganisms are drawn into the device, imparted an electrostatic charge and then deposited on the collection plate by the action of electrostatic forces. The captured particles are easily eluted using water or other fluids.

The sampler's internal geometry and optimum charging and collection voltages were optimized by a combination of computer simulation and iterative design modifications. When PEBS was tested with polystyrene latex particles ranging from 0.026 μm to 3.1 μm in diameter and at 10 L/min collection flow rate, its collection efficiency was approximately 70–80% at charging and collection voltages of +5.5 kV and -7 kV, respectively. Due to the novel charger design, PEBS produced very low ozone concentrations (< 10 ppb).

In further testing, PEBS was challenged with airborne *Bacillus atrophaeus* bacterial cells and *Penicillium chrysogenum* fungal spores when sampling at flowrates of 10 L/min and 20 L/min and sampling times of 10, 60, and 240 min. The collected samples were analyzed using microscopy, adenosine triphosphate (ATP)-based bioluminescence, flow cytometry (Live/Dead test), and culture techniques. PEBS's physical and biological performance was compared against that of an established bioaerosol sampler (BioSampler, SKC Inc., Eighty Four, PA). PEBS achieved physical collection efficiency > 80% at 10 L/min flow rate, and its physical performance in terms of measured bioaerosol concentration was better than that of BioSampler. In addition, the fraction of live microorganisms recovered by PEBS was not different from that of BioSampler. Compared to BioSampler, PEBS measured similar or higher concentrations of culturable bacteria, but lower concentrations of culturable spores. The airborne ATP concentration measured by PEBS was significantly higher than that measured by BioSampler.

In the preliminary field testing of the complete sampler prototype, PEBS was tested outdoors when taking 4 hrs air samples alongside BioSampler and Button Aerosol Sampler (both SKC Inc.). The concentrations of culturable organisms, as well as the viable fraction of the microorganisms determined by PEBS, were not different from that of the other two samplers.

Overall, the developed PEBS sampler prototype is a viable and efficient technology to determine *personal* exposures to airborne microorganisms using multiple sample analysis techniques. Future studies will apply this technology for exposure assessment in various occupational and residential environments.

Significant Findings

The overall goal of this research was to improve our ability to measure exposures to airborne microbiological agents by exploring a concept of a new personal bioaerosol sampling device that features high physical and biological performance when collecting airborne biological agents.

The overall most significant result from this exploratory project is a developed working prototype of a personal electrostatic bioaerosol sampler (PEBS) for determining *personal* exposures to airborne microorganisms. The PEBS prototype is a self-contained and battery-powered device that can operate for up to 4 hours.

PEBS is an open channel collector consisting of a novel wire-to-wire particle charger and a collection section housing a double-sided and removable metal collection plate and two quarter-cylinder ground electrodes. The charger consists of a tungsten wire (25.4 mm long and 0.076 mm in diameter) connected to high voltage and positioned in the center of the charging section (a cylinder 50.8 mm long and 25.4 mm in diameter); a ring of stainless steel wire 0.381 mm in diameter surrounds the hot electrode at its midpoint and is grounded.

The unique charger design resulted in very low ozone emissions (<10 ppb), which is a critical step for applying electrostatics-based collectors for bioaerosol sampling.

PEBS features high physical and biological collection efficiencies. When the sampler was tested with polystyrene latex particles ranging from 0.026 μm to 3.1 μm in diameter and at 10 L/min collection flow rate, its collection efficiency was approximately 70–80% at charging and collection voltages of +5.25 kV and -7 kV, respectively. When PEBS was challenged with airborne *Bacillus atrophaeus* bacterial cells and *Penicillium chrysogenum* fungal spores at a sampling flow rate of 10 L/min and sampling times of 10, 60, and 240 min, its physical collection efficiency was ~80%.

Samples collected by PEBS were successfully analyzed using microscopy, adenosine triphosphate (ATP)-based bioluminescence, flow cytometry (Live/Dead test), and culture techniques. This shows that PEBS is conducive for sample analysis by a variety of methods thus yielding a comprehensive picture of bioaerosol presence.

PEBS's physical and biological performance in laboratory testing was compared against that of BioSampler (SKC Inc., Eighty Four, PA). PEBS measured higher bioaerosol concentrations compared to BioSampler. A fraction of live microorganisms recovered by PEBS was not different from that of BioSampler. PEBS measured similar or higher concentrations of culturable bacteria. The airborne ATP concentration measured by PEBS was significantly higher than that measured by BioSampler. The ability of an electrostatics-based collector such as PEBS to successfully recover culturable microorganisms is a significant technological achievement.

PEBS was tested outdoors to take 4 hr samples alongside the BioSampler and Button Aerosol Sampler (both SKC Inc.). The concentrations of culturable organisms, as well as the viable fraction of the microorganisms determined by PEBS, were not different from that of the other two samplers.

Overall, the developed PEBS sampler prototype is a viable and efficient technology to determine *personal* exposures to airborne microorganisms using multiple sample analysis techniques.

Translation of Findings

Exposure to airborne biological agents is known to result in a high number of respiratory infection episodes and other negative health outcomes and carries a heavy price tag in medical care cost and loss of income. The main objective of this exploratory research was to improve our ability to measure exposures to airborne microorganisms, especially personal exposures by exploring a concept of a new *personal* bioaerosol sampling device that features high physical and biological performance when collecting airborne biological agents. The researchers were successful in designing, developing and then testing the new sampler prototype: personal electrostatic bioaerosol sampler (PEBS). This newly developed prototype is a self-contained device, i.e., it does not require for external pumps, sampling lines, and power supplies; everything, including an air mover, is contained within the sampler's body, and the device can operate on batteries for up to 4 hours when sampling airborne microorganisms. Upon further development and refinement, this device will be introduced into various occupational environments to measure *personal* exposures to airborne microorganisms. Our ability to measure personal exposures to bioaerosols in residential, occupational and other environmental settings for extended periods of time with a single device will improve our understanding of such exposures and will allow developing effective control and prevention measures. Ultimately this will lead to improved worker health protection against respiratory risks. The highlights of this work and the features of the new sampler have and will be disseminated through peer-reviewed publications and presentations and conferences and workshops.

Outcomes/Impact

The main output of this exploratory project is a developed working prototype of a personal electrostatic bioaerosol sampler (PEBS) for determining personal exposures to airborne microorganisms. The biggest advantage of this newly developed technology is that unlike existing technologies for bioaerosol collection, PEBS prototype is a self-contained device, i.e., it does not require external pumps, sampling lines, and power supplies; everything, including an air mover, is contained within the sampler's body, and the device can operate on batteries for up to 4 hours when sampling airborne microorganisms.

This new technology will allow measuring personal exposures to airborne microorganisms in various occupational and residential environments and will contribute to our better understanding of the linkage between bioaerosol exposures and health effects and risks. While the health effect-causing potential of bioaerosol exposures was recognized a long time ago, dose-response relationships between bioaerosol exposure and respiratory effects have not been established, and exposure threshold values have not yet been defined. It is hoped that the development and advancement of new technologies, such as PEBS, to measure airborne biological organisms will help bridge this gap.

The results described in this report show good physical and biological performances of PEBS prototype in laboratory and field tests where it was compared against two established bioaerosol samplers. Further development of this technology will include its miniaturization, and making the sampler "market-ready," including making it lighter and more user-friendly. Before its wide-scale introduction, the sampler will have to be broadly tested in various occupational environments. Overall, the device and its performance show great promise. Intermediate and end outcomes of this project will depend on the adaptation of this technology by health and safety professionals. The end outcome will be our better understating of exposures to airborne microorganisms in occupational environments.

Scientific Report

1. Background

1.1 Introduction

This grant application responded to PAR-12-252: NIOSH Exploratory and/or Developmental Grant Program (R21). This proposal responded to several NORA's Priority Research Agendas: National Agriculture, Forestry, and Fishing; National Services; National Transportation, Warehousing, and Utilities. These Agendas stress the difficulty to quantify environmental exposures of American workers, including *exposures to bioaerosols* and vector-borne agents and call for the development and piloting of advanced technologies to detect and measure such exposures. This research answered that call by developing and testing a novel personal sampler prototype that has potential to improve assessment of exposure to occupational agents and help prevent and reduce work-related respiratory infectious diseases. The proposal also responded to several cross-sector programs, e.g., Exposure Assessment and Respiratory Diseases. The developed sampler prototype could also be adapted to collect nanoparticles in occupational environments thus eventually responding to cross-sector Nanotechnology program and its call to develop and field-test practical methods to accurately measure airborne nanomaterials in the workplace.

Exposure to airborne biological agents, especially to pathogenic or allergenic microorganisms, may cause a wide range of respiratory and other health disorders in occupational and general populations (Douwes et al., 2003) costing billions of dollars in medical care and loss of income (Cox and Wathes, 1995). Various illnesses and infections due to bioaerosol exposures have been reported in numerous industries (Asefa et al., 2009; Duquenne et al., 2013; Eduard et al., 2012; Kennedy et al., 1999; Madsen et al., 2012; Napoli et al., 2012; Persoons et al., 2010; Schachter et al., 1984) and occupational and residential indoor air environments (Fung and Hughson, 2008; Grimsley et al., 2012).

A number of stationary and portable bioaerosol samplers have been developed and used to assess exposures to bioaerosols (Mandal and Brandl, 2011). Several existing personal samplers were adapted for bioaerosol sampling needs, such as Button Aerosol Sampler or IOM cassette used with regular or gelatin filters (Aizenberg, 2000; Chang and Hung, 2012; Wu et al., 2010; Yao and Mainelis, 2007). The use of size-selective polyurethane foams has been explored to sample thoracic and respirable bioaerosol fractions (Haatainen et al., 2009; Kenny et al., 1999). Several new personal bioaerosol sampler concepts, such as using the submerged porous medium (Agranovski et al., 2002), rotating cup (Gorner et al., 2006) and microcentrifuge-tube (Lindsley et al., 2006; Macher et al., 2008; Su et al., 2012) have been proposed.

These and other samplers represent an advancement in the field of personal bioaerosol sampling, but a number of issues remain: the need for a separate and cumbersome sampling pump, high power consumption, inability to operate for extended periods of time (in most cases) and low sampling flow rates. Filter samplers can operate for several hours but only at low flow rates and require external pumps. Plus, sample extraction from filters inevitably leads to losses (Dabisch et al., 2012) and reduced accuracy of exposure assessment, while liquid samplers have been shown to have high latent internal losses (Han and Mainelis, 2012). The elimination of these shortcomings in personal sampling technology sounds like a very tall order; however, the goal of this research was to develop a personal bioaerosol sampler that has the potential to accomplish exactly that: be a self-contained and battery operated (no external pump), capable of collecting airborne biological agents for extended periods of time with virtually lossless

sample transfer into liquid. We strongly believe that such a sampler will enhance our ability to measure personal exposures to biological particles in various occupational environments, especially at their low concentrations and for extended periods of time.

1.2 Innovation

Many stationary and portable bioaerosol samplers are available. However, advances in *personal sampling of bioaerosols* are needed (Eduard et al., 2012), including improved sensitivity and elimination of cumbersome external sampling pumps. Our work was focused on designing and developing a novel personal electrostatic bioaerosol sampler (PEBS) that would feature: 1) high collection efficiency at sampling flow rates that are much higher than those of currently available personal bioaerosol samplers; 2) ability to operate for extended periods of time; and 3) low weight and operation by battery (no external pump needed). These characteristics would allow measuring exposures even to low microorganism concentrations – a feature lacking in current personal bioaerosol samplers – thus substantially improving our ability to identify exposure risks and protect affected populations. In the developed sampler prototype, the particles are drawn into an open channel sampler, electrically charged and deposited onto a plate covered by a superhydrophobic (non-wettable) substance. One of the major innovations and advancements in sampler design is our new concept of particle charger that produces sufficient amount of ions to charge the incoming particles without substantial production of ozone. The details of this design are provided in the technical part of the report.

After sampling, the collection plate is removed and the collected particles washed-off with a desired amount and type of liquid to be analyzed by one or more techniques, including microscopy, molecular tools, and others. The main innovation of this personal sampler is a combination of novel charger design, electrostatic collection method and removable superhydrophobic collection surface in an open channel collector. Due to the low pressure drop of the open channel design and low electrical current requirement, power for both the air mover and the electrostatic collector are provided by a built-in battery. Low power consumption and small size will make this sampler easy to wear and highly applicable for occupational and environmental studies and field deployments. Its potential to sample for several hours will bring us closer to determining dose-response relationships due to exposure to bioaerosols. In addition, the ability to wash-off particles collected on the superhydrophobic surface ensures almost a lossless transfer of particles into liquid for their analysis by various methods, including molecular tools. Such design avoids potential losses associated with liquid and filter samplers (Dabisch et al., 2012; Han and Mainelis, 2012; Schmechel et al., 2003) thus ensuring a more accurate exposure assessment.

In summary, the research described here designed and developed a novel personal electrostatic bioaerosol sampler (PEBS) that is capable of efficiently collecting bioaerosols at high sampling flow rates for extended periods of time – advantageous over existing personal samplers. PEBS is a self-contained and battery-operated device. Its high sampling flow rate and ability to operate for several hours allow more accurate assessment of personal exposures to even low microorganism concentrations – a feature lacking in current personal bioaerosol samplers - and will substantially improve our ability to identify exposure risks and protect affected populations. These features will make the new sampler highly applicable for various occupational and environmental studies.

2. Objectives and specific aims

The main goal of this exploratory research was to improve our ability to measure personal exposures to airborne microorganisms, especially to their low concentrations, by developing and evaluating a novel and self-contained personal bioaerosol sampler. Exposure to airborne biological agents, especially to pathogenic or allergenic microorganisms, has been shown to cause a wide range of respiratory and other health disorders in occupational and general populations. While there are many stationary and portable samplers that collect biological particles, rather few samplers are available to assess *personal exposures to bioaerosols,* and all of them require cumbersome personal pumps. This exploratory research proposed to design and develop a novel personal electrostatic bioaerosol sampler (PEBS), where bioaerosols are drawn into an open channel collector, electrically charged and deposited onto a removable plate covered with a superhydrophobic (non-wettable) substance. Once the sampling is completed, the plate is removed, the collected particles washed-off with a desired amount and type of liquid and analyzed by multiple techniques, including microscopy, molecular tools, and others. This personal bioaerosol sampler was to have the following features: 1) high bioaerosol collection efficiency at sampling flow rates that are higher than those of currently available personal bioaerosol samplers; 2) ability to operate for extended periods of time; and 3) be lightweight and battery-operated (no external pump needed). These characteristics will allow more accurate monitoring of personal exposures to even low microorganism concentrations – a feature lacking in current personal bioaerosol samplers – thus improving our ability to identify the exposure risks and protect affected populations. Low power consumption and small size will make this sampler easy to wear and highly applicable for occupational and environmental studies and field deployments. Thus, our main underlying hypotheses were that: (i) specific personal sampler design in combination with electrostatic collection method and superhydrophobic collection surface would allow achieving collection efficiency of approximately 80% at high sampling flow rates, (ii) the proposed personal sampler will allow assessment of personal exposures to even low airborne microorganism concentrations and for extended exposure durations.

The goal of this research was achieved through the following Specific Aims:

I. Design and manufacturing of a personal electrostatic bioaerosol sampler (PEBS) with high sampling flow rate. The researchers used their expertise in working with electrostatic samplers to design a prototype and then test its performance at different geometries and operational parameters (flow rate and charging/collection voltage). Once a satisfactory design was achieved, the prototype sampler was manufactured using 3D printing.

II. Laboratory evaluation of PEBS when collecting non-biological particles. The developed prototype was challenged with polystyrene latex particles of bioaerosol-relevant sizes (0.5 – 5 μm) to determine its collection efficiency. The testing was performed at sampling times ranging from 10 minutes to 4 hours. Based on the results, the sampler's geometry and operational parameters were adjusted.

III. Laboratory evaluation of PEBS when collecting bacteria and fungi. Using the parameters determined above the sampler was challenged with bacterial and fungal species. The biological efficiency and physical collection efficiency was determined for sampling times ranging from 10 minutes to 4 hours.
    a) Measurement of sampler's performance using culturable and total microorganism counting methods
    b) Measurement of sampler's performance using ATP-bioluminescence and flow cytometry Live/dead methods (ADDED)

IV. Laboratory evaluation of PEBS against other bioaerosol samplers. The prototype sampler was compared against one liquid-based sampler. The test and reference samplers were concurrently challenged with bacterial and fungal species, and the determined bioaerosol concentrations were compared. Sampling times ranged from 10 minutes to 4 hours.
    a) Measurement of sampler's performance using culturable and total microorganism counting methods
    b) Measurement of sampler's performance using ATP-bioluminescence and flow cytometry Live/dead methods (ADDED)

V. Preliminary sampler prototype testing in the field. The new sampler was compared with two reference samplers in a field environment for its ability to determine culturable and total microorganism concentrations and operate as a self-contained unit. In addition, the samples were analyzed using ATP-bioluminescence and flow cytometry Live/dead methods (ADDED)

The Results section presented below describes the main findings and developments achieved as part of this research project.

3. *Specific Aim I: Design and manufacturing of a personal electrostatic bioaerosol sampler (PEBS) with high sampling flow rate;*

Specific Aim II: Laboratory evaluation of PEBS when collecting non-biological particles

The design and manufacturing of the sampler PEBS and analysis of its performance when collecting non-biological particles respond to both Specific Aims I and II, and thus they are described together in the following sections.

3.1 General design principles

A number of factors affect ESP design and performance, including particle terminal drift velocity which is determined by the operational voltage(s) and particle electrical mobility, sampler's geometrical parameters, and volumetric air flow rate. The performance of traditional wire-to-plate ESPs could be typically described by Deutsch-Andersen equation (Nóbrega et al., 2001) or its modified version (Lin et al., 2012). However, in most cases, this equation serves only as guidance because the actual collection efficiency is considerably affected by air-ion mixing, non-ideal collection patterns and particle re-entrainment (Yang et al., 2009).

Since our goal was to achieve good collection efficiency while maintaining low ozone production, we departed from the traditional wire-to-plate design and used the wire-to-wire approach as described below. Because of the new design and our previous experience in ESP design (Han et al., 2015a; Han and Mainelis, 2008), it seemed more prudent to apply general ESP design principles and develop the sampler by iteration: optimize one design parameter to achieve a collection efficiency of 70% or better while others remain fixed. When deciding on these parameters we were cognizant that our goal is to design a personal sampler, i.e., the sampler had to be compact. Also, for improved user experience, the collection plate had to fit easily into a standard 50-mL disposable and sterile centrifuge tube for a convenient way to *remove*, handle and store the collected particles.

Ozone production is an inescapable consideration when designing an ESP, especially one to be used as a personal bioaerosol sampler. The ozone is typically produced during particle charging which is needed to impart sufficient electrostatic charge on the incoming particles so that they could be collected by an electrostatic process. The charging of particles is achieved either via diffusion or field-charging mechanism (Liu and Yeh, 1968). For the latter, wire-plate (Xiangrong et al., 2002) or wire-cylinder (Niewulis et al., 2014) designs are most commonly used. While the field-charging mechanism is an efficient charging process, a number of issues related to its application for bioaerosols still remain: ozone emission during charging process (Chang et al., 1991), charger degradation over time (Koutsoubis and MacGregor, 2000), and difficulty in charging smaller particles (Tsai et al., 2010). Among these concerns, ozone emission is probably the biggest issue not only due to its effect on the collected microorganisms (Kammer, 2005) but also because ozone is an irritant to the lungs (Kleinman, 2000). While the current U.S. National Ambient Air Quality Standard for ozone is 70 ppb (50CFR65292), there are currently limited regulations governing ozone emissions from personal-use devices. For example, the Food and Drug Administration (FDA) standard limits ozone output of indoor medical devices to 50 ppb (21CFR801.415); this level is stricter than 100 ppb standard for 8-hour exposures (not

emissions) in occupational environments set by Occupational Safety and Health Administration (54CFR23332). Thus, at the very least, a personal sampling device should satisfy FDA requirements and, even better, have much lower ozone emissions. Overall, ozone emissions depend on the sampling flow rate, operational voltage and its polarity, current level, electric field strength, relative humidity, and size and material of a charging electrode (Boelter and Davidson, 1997; Castle et al., 1969; Goheen et al., 1984; Kulkarni et al., 2002; Plank et al., 2014).
To minimize ozone production, we undertook the following steps: the sampler was designed as a two-stage system (separate charging and collecting sections) for better control of charging process, application of novel wire-to-wire charger design, use of positive corona discharge to minimize ozone production (Chen and Davidson, 2003), use of lowest possible corona current, and elimination of any sharp edges within the sampler to minimize strong, local electrical fields. The text below describes these iteration steps in detail.

3.2 Design features of Personal Electrostatic Bioaerosol Sampler (PEBS) with a Wire-to-Wire charger

The PEBS is comprised of a static “air blender,” a wire-to-wire charger and a collection chamber (Fig. 3.1). The entire PEBS has a shape of a cylinder of 2.54 cm (1 inch) in diameter, ~14 cm (5.5 inches) in length, and is made of a static dissipative material (homopolymer acetal, or Delrin; Professional Plastics Inc., Fullerton, NY). The static blender, which is positioned at the sampler’s inlet, has been designed to improve mixing of the incoming aerosol particles with the produced ions. The blender has the shape of a disk 2.54 cm (1 inch) in diameter and 0.56 cm (0.22 inches) in height; the blender has 6 blades in the inner circle (1.45 cm in diameter) and 15 blades in the outer circle and was printed using 3D printing technology.

To achieve high collection efficiency with low ozone production, the sampler features a novel wire-to-wire charger, where a tungsten wire 2.54 cm (1 inch) in length and 0.076 mm (0.003 inches) in diameter (W91, Scientific Instrument Inc., Ringoes, NJ) is positioned at a distance of 1.27 cm (0.5 inches) downstream of the inlet and in the center of the charging chamber (i.e., 1-inch diameter cylinder); it is connected to DC high voltage. A ring of stainless steel wire 0.381 mm (0.015 inches) in diameter is installed on the inside of the cylinder at the middle point and at 90-degree angle to tungsten the wire and is grounded. The tungsten wire is supported by ceramic mini posts of 1.575 mm (0.062 inches) outer diameter and 0.787 mm (0.031 inches) inner diameter which provide insulation for a conduit to the wire. Since the tungsten wire is connected to the positive voltage and the stainless steel wire electrode is grounded, this wire-to-wire configuration creates sufficient ions which charge the incoming particles while producing low ozone emissions during the charging process. Positive charging is the preferred approach for biological particles as the production of ozone can be up to one order of magnitude lower than the production of ozone in the negative corona (Chen and Davidson 2003).

While many studies have examined how the ozone production is affected by various ESP design parameters (e.g., current level, ionizer wire diameter, wire material, and electric field strength) (Awad and Castle 1975; Castle et al. 1969; Plank et al. 2014; Nashimoto 1988; Ohkubo et al. 1990; Viner et al. 1992), we did not see studies on the physical dimensions of the ground electrode and ozone production. In our design, the area of the ground electrode is minimized, and it should lead to a lower deposition of particles and ions on the ground electrode in the charging section. This, in turn, should result in a lower corona current and, since the ozone production is proportional to the current level (Castle et al. 1969; Viner et al. 1992), lower ozone production.

The collection section consists of two grounded stainless steel plates having the shapes of the quarter cylinder and a stainless steel collection plate, which is connected to collection voltage. The collection plate divides the collection section into two half-cylinder collection chambers. The collection plate automatically connects to the collection voltage once it is slid into the grooves in the inner wall of the chamber with an electric connection. After completing the sampling, the collection plate is removed from the collector for sample elution and analysis. Because the design of the collection section is symmetrical and the particles are collected on both sides of the plate, particles from each side could be eluted separately thus allowing to have two identical samples (Fig. 3.1). The two samples could be analyzed separately for different purposes or by different techniques (e.g., microscopy, fluorometry, etc.) or they could be combined if needed. The collection plate is 3.81 cm (1.5 inches) long, 2.54 cm (1 inch) wide, and 0.16 cm (1/16 inches) thick. Each quarter-cylinder grounded electrode has a length of 3.81 cm (1.5 inches), the circumference of 1.99 cm (π×1/4 inches), and thickness of 0.04 cm (1/64 inches); they are inserted into grooves in the middle of the chamber. The outlet of the collector connects to an air mover with adjustable flow rate.

3.3 Experimental setup for testing PEBS with PSL particles in laboratory

The test system is shown in Fig. 3.2, and it consisted of a flow controller, a particle generator, an air-particle mixing element, a flow straightener, a test chamber, and a particle monitor. The system was housed in a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN).
A six-jet Collison nebulizer (Mesa Laboratories Inc., Butler, NJ) with a glass jar was used to aerosolize test particles from a liquid suspension at a flow rate (Q_A) of 5 L/min (pressure of 12 psi), and the aerosolized particles were combined with a dry air flow, Q_d (5 L/min). The dry air and aerosolized particle stream were combined ($Q_d + Q_A$ = 10 L/min) and passed through a 2-mCi Po-210 charge neutralizer (Amstat Industries Inc., Glenview, IL) to reduce aerosolization-imparted particle charges to Boltzmann charge equilibrium. A HEPA-filtered dilution air flow, Q_D (60 L/min), provided by an in-house compressor was used to dilute the particle stream; it was controlled by a pressure regulator and monitored by a mass flowmeter (TSI Inc., Shoreview, MN). The electrically neutralized particles then passed through two mixing boxes connected by a U-type duct connector to improve the uniformity of particle distribution across the flow cross-section (Han et al., 2005). A well-mixed flow stream then entered a raised test duct 15.2 cm (6 inches) in diameter and a 61 cm (24 inches) in length, as shown in Fig. 3.2. A flow straightener (honeycomb) was placed at the exit of the second elbow to eliminate large-scale turbulence and flow swirl generated by the mixing boxes and the 90-degree elbows. A raised test duct allowed the PEBS collector to be perpendicularly oriented relative to the air stream. This arrangement simulated a real-world sampling situation, where a person would wear a sampler in a vertical orientation in the upper part of their chest. The sampler was positioned six duct diameters downstream of the exit of the flow straightener in order to provide a uniform cross-sectional profile of test particles.

The PEBS was tested with six different aerodynamic diameters (0.026, 0.1, 0.2, 0.5, 1.0, and 3.1 μm) of green fluorescent polystyrene latex (PSL) particles (Duke Scientific Corp., Palo Alto, CA). The airborne concentration of fluorescent PSL particles was approximately 10^3 ~ 10^4/Liter. The coefficient of variation (*COV*) of 0.5 μm PSL concentration across the test duct was about 2.7% at the measurement location. The *COV* was measured over five equally distributed sampling points in the cross-sectional area of the duct in triplicate.

In our tests, the PEBS was operated at a sampling flow rate (Q_S) of 10, 20, and 30 L/min provided by a vacuum pump. The collector was tested at charging voltages ranging from +5 kV

to +8 kV, and collection voltages ranging from -3 kV to -7 kV. The sampling time varied from 2 to 240 minutes. At this stage of the project, the stainless steel collection electrode was not coated with any materials. The ozone concentration was measured using a UV photometric ozone monitor (Model 202, 2B Technologies Inc., Boulder, CO) downstream of the PEBS (Fig. 3.2).

3.4 Determination of PEBS collection efficiency

The collection efficiency of the PEBS was determined by comparing particle number concentration downstream of the collector with its charging/collection voltages ON and OFF either using a Grimm optical particles counter (OPC) (model 1.108, Grimm Technologies Inc., Douglasville, GA) or a P-Trak (UPC 8525, TSI Inc., Shoreview, MN) connected to an isokinetic probe (Apex Instruments Inc., Fuquay-Varina, NC). The Grimm was used for larger particles (0.5, 1, and 3.1 μm PSL), while P-Trak was used for smaller particles (0.026, 0.1, and 0.2 μm PSL). The use of direct reading instruments allowed performing a high number of experiments, which was important when the sampler underwent multiple adjustments in the initial phase of its development. When determining the collection efficiency by comparing the particle concentration downstream of the sampler with its voltage ON and OFF, one has to keep in mind that this efficiency $\eta_{EFF,}$ is a sum of the collection efficiency of the charging section ($\eta_{EFF,\ CHARGER,}$ i.e., losses in the charging section) and the subsequent collection of particles in the collection section ($\eta_{EFF,\ COLLECTOR}$). The collection efficiency of the charging section, i.e., losses:

$$\eta_{EFF,\ CHARGER} = 1 - \frac{C_{CHARGER-ON}}{C_{OFF}}, \qquad [3.1]$$

and the collection efficiency of the collection section:

$$\eta_{EFF,\ COLLECTOR} = \frac{C_{CHARGER-ON} - C_{CHARGER\&COLLECTOR-ON}}{C_{OFF}}, \qquad [3.2]$$

where $C_{CHARGER\text{-}ON}$ is particle number concentration with charger voltage ON and collector voltage OFF; $C_{CHARGER\&COLLECTOR\text{-}ON}$ is particle number concentration when both charger and collector voltages are ON; C_{OFF} is particle number concentration with both charger and collector voltages OFF.

This metric does not take into account particle losses inside the PEBS. However, our separate investigation of particles deposited inside the sampler on other sampler components showed that those losses were <1% for 1 μm PSL particles when the sampler operated at 10 L/min. The losses inside the PEBS due to its other components (e.g., static blender or walls) were minimal: e.g., transmission efficiencies of approximately 93% through the static blender were observed when testing with 1 μm PSL particles at the highest investigated flowrate of 30 L/min. Thus, to simplify the measurement procedures, the C_{OFF} was used as a reference value for calculating the sampler's performances in the development stage.

While the collection estimation method described above allowed for the quick development of the sampler, it does not represent the actual collection efficiency, only its surrogate. To determine the actual collection efficiency, one has to compare the concentration of airborne test particles determined by the sampler, which is based on particles deposited on the collection plate and the sampling flow rate, and particle concentration upstream of the sampler determined by the reference method. Thus, the actual collection efficiency, $\eta_{ACTUAL,\ COLLECTOR}$ was determined by comparing the mass concentration of particles deposited on the PEBS collection electrode and removed by 5 mL of ethyl acetate with the mass of PSL particles isokinetically

sampled onto a reference filter (25 mm cellulose membrane, Pall Inc., East Hills, NY) positioned upstream of the sampler and operated at flow rate 2.2 L/min. PSL particle concentration in each sample was determined by measuring its fluorescence intensity using a digital filter fluorometer (Turner Quantech model FM109515, Barnstead/Thermolyne Corp., Dubuque, IA) as described previously (Han et al., 2015a; Han and Mainelis, 2008). The actual collection efficiency, $\eta_{ACTUAL,\ COLLECTOR}$, was determined as follows:

$$\eta_{ACTUAL\ ,\ COLLECTOR} = \frac{C_{collector}}{C_{reference\ \ filter}}, \qquad [3.3]$$

where $C_{collector}$ and $C_{reference\ filter}$ are the concentrations of airborne PSL determined based the amount of PSL captured by the collection plate and the reference filter respectively. The calculations of these concentrations take into account the sampling flow rates, sample elution volumes and volumes of sample aliquots used to measure fluorescence intensities (Han and Mainelis, 2008). Because sample analysis by fluorescence microscopy is more time-consuming that the use of direct reading instruments described above, it was used only in the final stages of the sampler development. One of the figures presented below compared the efficiencies determined by the two methods.

In addition to collection efficiency, the sampler's concentration rate, R_C (min^{-1}), was calculated using the sampler's operational parameters as follows (Han et al., 2010; Han and Mainelis, 2008; Han et al., 2015b):

$$R_C = \frac{Q_S}{V_W} \times \eta_{EFF\ ,COLLECTOR} \qquad [3.4]$$

where Qs (L/min) is the sampling flow rate, V_W (L) is the volume of the sample elution liquid and $\eta_{EFF,\ COLLECTOR}$ is the collection efficiency based on Eq. 3.2.

3.5 Results and discussion

Fig. 3.3 shows the collection efficiencies of the charger (i.e., losses) and the collector of the PEBS as a function of charging voltage when sampling 1 μm PSL particles at different flow rates (10, 20, and 30 L/min) at a fixed collection voltage of -7 kV. The charging voltage was varied from +5 to +6 kV at 10 L/min sampling flow rate, from +6 to +7 kV at 20 L/min, and from +7 to +8 kV at 30 L/min sampling flow rate. As the charging voltage increased at each sampling flow rate, the collection efficiencies of the charger and collector increased to 5.5–25.2% and 25.1–72.6% at 10 L/min, respectively; 5.3–14.6% and 46.2–60.9% at 20 L/min; 10.4–17.2% and 33.3–43.8% at 30 L/min. Overall, as could be expected, when the sampling flow rate increased from 10 to 30 L/min (Fig. 3.3), the average collection efficiency in the charging and collection sections decreased at all settings of the charging voltage. The decrease was observed because with increasing sampling flow rates particles spent less time in the collection chamber and had a lower chance of being collected. Fig. 3.3 also shows ozone concentrations emitted during PEBS operation. In the data presented here, the background concentration is already subtracted. During each test, the temperature in the test chamber stayed in the range of 21–25°C and the relative humidity ranged from 26 to 41%. Ozone emission concentration increased with increasing charging voltage (i.e., field strength): from 2.7 to 17.7 ppb when voltage was increased from +5 to +6 kV at 10 L/min, from 7.4 to 18.8 ppb when voltage was increased from +6 to +7 kV at 20 L/min, and from 9.0 to 17.7 ppb when voltage was increased from +7 to +8 kV at 30 L/min. As could be expected, the ozone concentration decreased with increasing flow rate because the same amount of ozone was diluted in a larger air volume: 17.7 ± 1.4 ppb (10 L/min) versus 7.4 ± 0.1 ppb (20 L/min) at +6 kV charging voltage and 18.8 ± 1.2 ppb (20 L/min) versus

9.0 ± 0.4 ppb (30 L/min) at +7 kV charging voltage. The ozone concentration is not inversely proportional to the flow rate due to its production affected by atmospheric conditions (e.g., temperature, relative humidity) (Chen and Davidson, 2002).

Based on the one-way ANOVA, for 10 L/min sampling flow rate, the collector's efficiency at charging voltage of +5 kV was statistically significantly lower than that at other voltages ($p < 0.05$); at 20 L/min collection flow rate, the collector's efficiency was not statistically significantly different ($p > 0.05$) within +6–6.5 kV range of the charging voltage; at 30 L/min sampling flow rate, the collection efficiency was not statistically different ($p > 0.05$) within +7.25–8 kV range; except for charging voltage of +7 kV. As a result, we selected the +5.5 kV charging voltage at 10 L/min sampling flow rate for further experiments because it yielded a relatively good collection efficiency (~ 72%) and a relatively low ozone concentration (< 10 ppb). Thus, remaining experiments at 10 L/min sampling flow rate were carried out using this charging voltage of +5.5 kV. For sampling at 20 and 30 L/min flow rates, we selected +6.5 kV and +7.5 kV charging voltages, respectively. These values were used in experiments presented later (for Fig. 3.8).

Once the ionizer's charging voltage of -5.5 kV was selected, we determined the optimal collection voltage by testing the collection section efficiency, $\eta_{EFF,\ COLLECTOR,}$ when the collection voltage was varied from -3 to -7 kV at 10 L/min sampling flow rate (Fig. 3.4). The collection section efficiency was not statistically different for voltages from -4 to -7 kV ($p > 0.05$); all collection efficiencies at these voltages were higher than the collection efficiency at -3 kV collection voltage. For the collection voltages of -4 kV and higher, the average efficiency in the collection section was 79.6 ± 3.7%, and the average ozone emission concentration was 6.3 ± 0.7 ppb. As shown here, the ozone emissions were independent of the collection voltage. However, additional ozone emissions in the collection section could still occur due to stray discharges (i.e., strong electrostatic fields due to sharp edges of the collection plate or ground plate). This was prevented by smoothing and rounding edges of electrodes in the collection section. Since the collection voltage of -7 kV resulted in the lowest coefficient of variation (*COV* = 0.009) of the collection efficiency and similar ozone production compared to other voltages above -4 kV, it was selected for further experiments.

In the next step, the wire-to-wire charger performance was optimized by varying and selecting the wire diameter (i.e., 0.076, 0.203, 0.381, and 0.813 mm) of the ground electrode. The results are presented in Fig. 3.5. Since the wire diameter of the ground affects the strength of the electrostatic field, it affects ion emission and, in turn, the collection efficiency of both the charging and collection sections as well ozone emission. When +5.5/-7.0 kV for charging/collection was used and the wire diameter was increased from 0.076 mm (0.003 inches) to 0.813 mm (0.032 inches), the collection efficiency on the charging and collection sections increased from 8.7 ± 2.6% to 16.0 ± 3.1% and from 4.4 ± 3.5% to 76.5 ± 0.7%, respectively. At the same time, with increasing wire diameter, ozone emissions increased as well even though the value of the charging voltage remained fixed. As mentioned in our general design principles, it could be attributed to increasing ion current level in the charging section (Castle et al., 1969; Viner et al., 1992). For the investigated wire diameters, the collection efficiency of the collection section was not statistically significantly different ($p > 0.05$), except for dw = 0.076 mm (0.003 inches) where the efficiency was ~5%. Thus, for further experiments, we chose wire of 0.381 mm (0.015 inches) in diameter because it resulted in a lower ozone concentration than 0.813 mm (0.032 inches) wire and a lower *COV* than 0.381 mm (0.015 inches) wire.

In the last step of PEBS optimization, we varied the length of the collection plate from 2.54 cm (1 inch) to 5.08 cm (2 inches), which resulted in the collection surface area, A_s, of 6.45, 8.06, 9.68, 11.29, and 12.90 cm^2. The results are shown in Fig. 3.6. The surface collection area did not substantially affect the collection efficiency once it reached 8.06 cm^2 (1.25 $inches^2$). The collection section efficiencies for the surface areas of 8.06 cm^2 and larger were not statistically different. They were all statistically significantly higher than the collection efficiency at A_s = 6.45 cm^2 (1.00 $inch^2$) ($p<0.05$). For further experiments, we chose the 9.68 cm^2 (1.50 $inch^2$) collection surface area because it had the same length (1.5 inches) as the ground electrodes and collection efficiency for this surface area had lowest *COV* (1.1%) among the investigated options.

Thus, based on the results presented above, the PEBS, a two stage bioaerosol collector, had the following parameters: 1) the charging electrode is a tungsten wire 0.0762 mm (0.003 inches) in diameter and 2.54 cm (1 inch) in length, 2) the ground electrode in the charging section is a ring-type stainless steel wire 0.381 mm (0.015 inches) in diameter and 7.98 cm (3.14 inches) in length, 3) the collection electrode is a stainless steel plate (width: 2.54 cm (1 inch) × length: 3.81 cm (1.5 inches)) resulting in 9.68 cm^2 (1.50 $inch^2$) of surface area on one side, and 4) the ground electrode in the collection section is a quarter-cylindrical stainless steel plate with 7.61 cm^2 (1.18 $inch^2$) of surface area. The optimized charging voltages for PEBS were +5.5 kV at 10 L/min sampling flow rate, +6.5 kV at 20 L/min, +7.5 kV at 30 L/min, while -7.0 kV would be used for the collector.

Fig. 3.7 presents a performance of the PEBS with those design and operational parameters as described in the previous section when collecting PSL of 0.026, 0.1, 0.2, 0.5, 1.0 and 3.1 μm in aerodynamic diameter (d_a). The average overall collection efficiency for the collection plate was 73.4 ± 4.9% over the entire range of tested particles and the average ozone emission concentration was 7.4 ± 1.4 ppb. The highest collection efficiency of 78.3 ± 1.3% was observed for 1 μm particles, while the average efficiency for smaller particles (0.5, 0.2, and 0.1 μm) was slightly lower. Because the number of charges acquired by particles is proportional to particle diameter squared (Hinds, 1999), this decrease in efficiency could be expected as the particles get smaller. The average collection efficiency for the smallest 0.026 μm particles has increased (to 77.2 ± 3.0%) compared to 0.1-0.5 μm particles, most likely due to their greater diffusion (Hinds, 1999) and high electrical mobility. The average collection efficiency for 3 μm PSL particles is also slightly lower than that for 1 μm particles. Our analysis of the particle deposition pattern inside the PEBS showed the increase of losses inside the charging section of the PEBS for 3.1 μm particles. However, the efficiency analysis by ANOVA showed that the collection section efficiency was not statistically significantly affected by the particle size.

Fig. 3.8 presents the performance of PEBS for three different sampling flow rates. An optimal charging voltage was used for each flow rate: +5.5 kV for 10 L/min, +6.5 kV for 20 L/min, and +7.5 kV for 30 L/min. The collection voltage was fixed at -7 kV for all three sampling flow rates. Because the charging voltage increased for increasing sampling flow rate, the ozone emission concentration increased from approximately 4.8 ppb at +5.5 kV and 10 L/min, to 11.9 ppb at +6.5 kV and 20 L/min, and then to and 16.0 ppb at +7.5 kV and 30 L/min. The collection efficiency decreased with increasing sampling flow rate: 78.3 ± 1.3% at 10 L/min, 57.6 ± 3.0% at 20 L/min, and 40.2 ± 0.9% at 30 L/min (Fig. 3.8). This could be expected because the particles spent less time in charging and collection sections. The increase in ozone concentration was caused by the increase in charging voltage.

Fig. 3.8 also shows sampler's concentration rates, R_C, based on the presented collection section efficiency, the 1 mL of elution liquid, and sampling flow rates of 10–30 L/min. Depending

on the sampling flow rate, the concentration rates ranged from 7.8×10^3/min to 1.2×10^4/min for 1 μm PSL particles. If the volume of collection fluid could be decreased to 0.1 mL, then the concentration rate would increase by a factor of 10 exceeding values of 10^5/min. These calculations assume that particles are eluted from both sides of the collection plate.

In the latest part of the development, the collection efficiency of PEBS was determined when collecting 1 μm PSL particles for 10, 60, and 240 min at 10 L/min and the results are presented in Fig. 3.9. Here we show the collection section efficiency $\eta_{EFF,\ COLLECTOR}$, determined using Grimm OPC and the actual collection efficiency, $\eta_{ACTUAL,\ COLLECTOR}$, determined by comparing a number of particles deposited on the actual collection surface with that collected on the reference filter. Before setting out this test, it is important to mention that the two approaches to determine collection efficiency (Eq. 3.2 and Eq. 3.3) were compared and found to be within 0.6% (1 μm PSL particles at 10 L/min), 3.5% (1 μm at 30 L/min), and 9.2% (3 μm at 10 L/min). Concentrations of airborne PSL particles were ~10^6–10^7/m^3. As could be seen, the PEBS performance indicators determined by the two methods are very close: 82.4 ± 1.8% versus 81.7 ± 2.6%, 77.4 ± 5.4% versus 76.6 ± 2.5%, and 73.3 ± 7.2% versus 73.6 ± 4.1%, for 10, 60, and 240 min sampling time, respectively. The average values for the two parameters are approximately the same (78% versus 77%) and not different statistically. For all three sampling times, the average ozone emission concentration was 8.6 ± 0.6 ppb. When the sampling time increased from 10 to 240 min, the collection efficiency decreased by approximately 10% on an absolute scale. However, the decrease was not statistically different. Also, for all sampling times, the actual collection efficiency was not statistically different from the collection section efficiency determined by OPC ($p > 0.05$).

3.6 Figures for Chapter 3

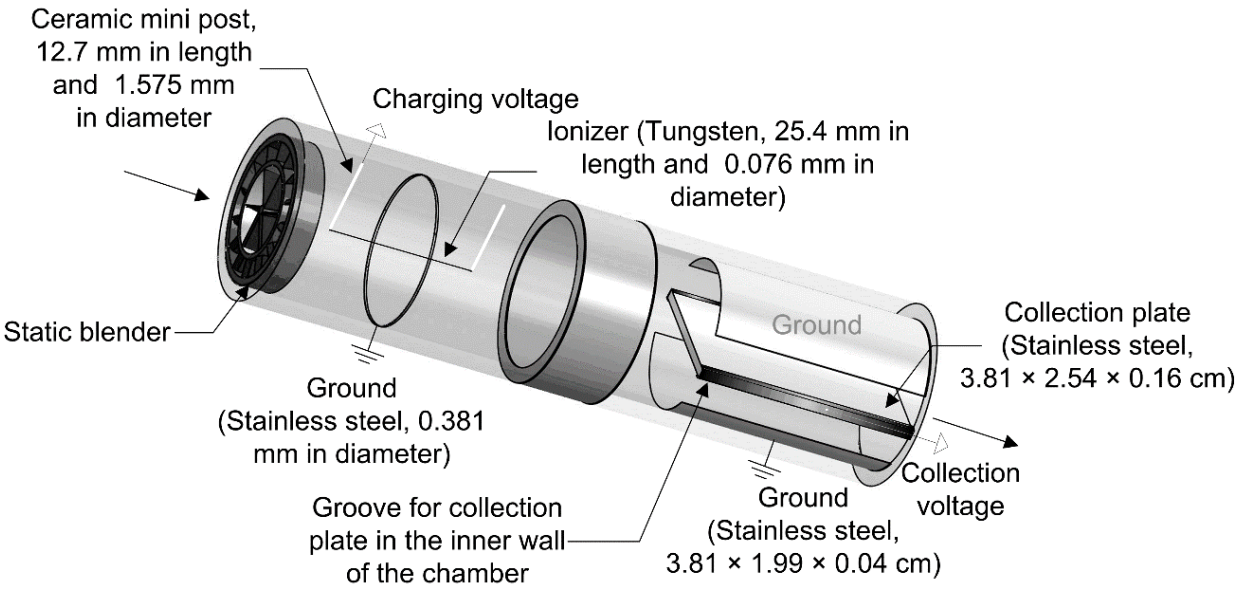


Fig. 3.1. Schematic diagram of the Personal Electrostatic Bioaerosol Sampler (PEBS) with a wire-to-wire charger. The sampler incorporates a novel particle charger with a 25.4 mm (1 inch) long tungsten wire 0.076 mm (0.003 inches) in diameter positioned in the center of the charging chamber (a cylinder 25.4 mm or 1 inch in diameter) and connected to high voltage; a ring of stainless steel wire 0.381 mm (0.015 inches) in diameter) is surrounding the hot electrode at its midpoint and is grounded.

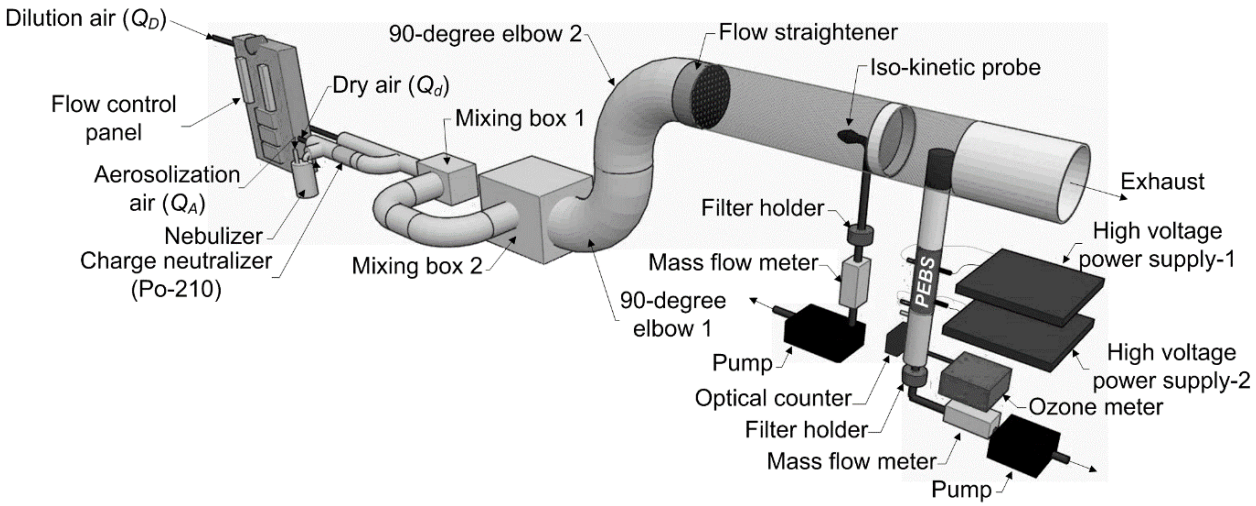


Fig. 3.2. Schematic diagram of the experimental setup.

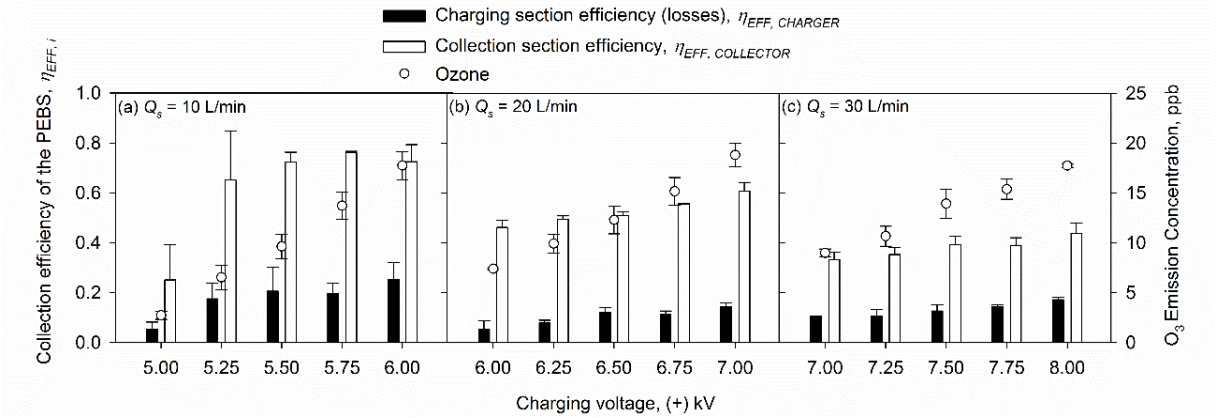


Fig. 3.3. Collection efficiencies of the charging section (i.e., losses) and the collection section of the PEBS as a function of charging voltage when sampling 1 µm PSL particles at different flow rates (10, 20, and 30 L/min) at a fixed collection voltage of -7 kV. The charging voltage was varied from +5 to +6 kV at 10 L/min sampling flow rate, from +6 to +7 kV at 20 L/min, and from +7 to +8 kV at 30 L/min sampling flow rate. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviation. In these experiments, a tungsten wire 25.4 mm (1 inch) long and 0.076 mm (0.003 inches) in diameter was used in the charger. The tungsten wire at its midpoint was surrounded by a grounded ring of stainless steel wire 0.381 mm (0.015 inches) in diameter. In the collection section, the collection electrode was a dual-sided stainless steel plate 3.81 × 2.54 × 0.16 cm (1.5 × 1.0 × 1/16 inches). The plate was positioned in the middle of the collection chamber. The ground electrodes were two conductive half-cylinder with dimensions 3.81 × 1.99 × 0.04 cm (1.5 × 0.78 × 1/64 inches).

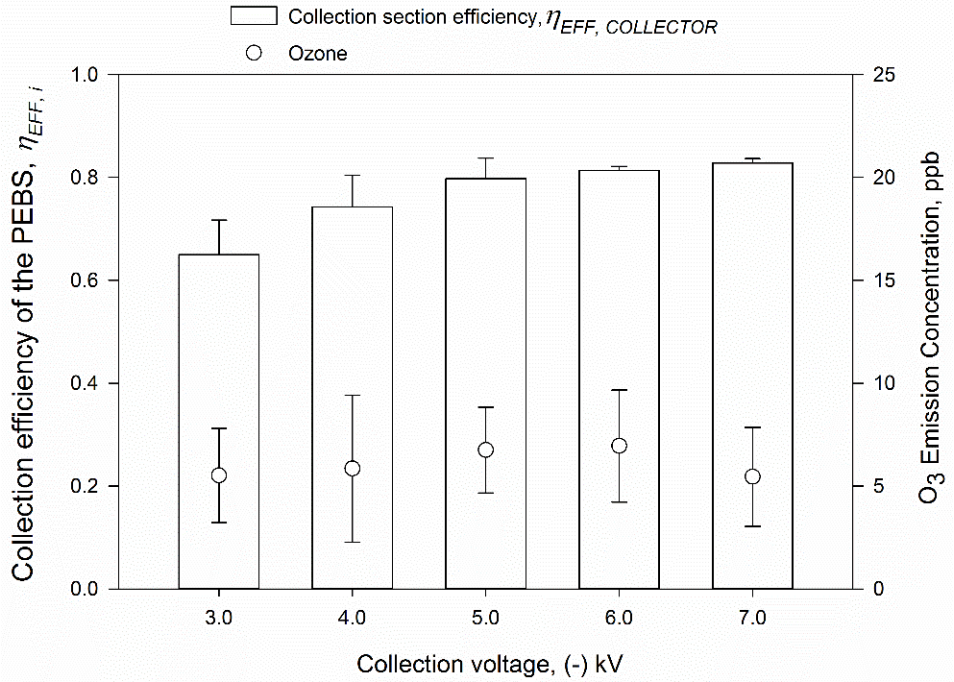


Fig. 3.4. The collection efficiency of the PEBS collection section as a function of collection voltage (varied from -3 to -7 kV) when collecting 1 μm PSL particles at 10 L/min flow rate and the fixed charging voltage of +5.5 kV. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviations. In these experiments, a tungsten wire 25.4 mm (1 inch) long and 0.076 mm (0.003 inches) in diameter was used in the charger. The tungsten wire at its midpoint was surrounded by a grounded ring of stainless steel wire 0.381 mm (0.015 inches) in diameter. In the collection section, the collection electrode was a dual-sided stainless steel plate 3.81 × 2.54 × 0.16 cm (1.5 × 1.0 × 1/16 inches). The plate was positioned in the middle of the collection chamber. The ground electrodes were two conductive half-cylinder with dimensions 3.81 × 1.99 × 0.04 cm (1.5 × 0.78 × 1/64 inches).

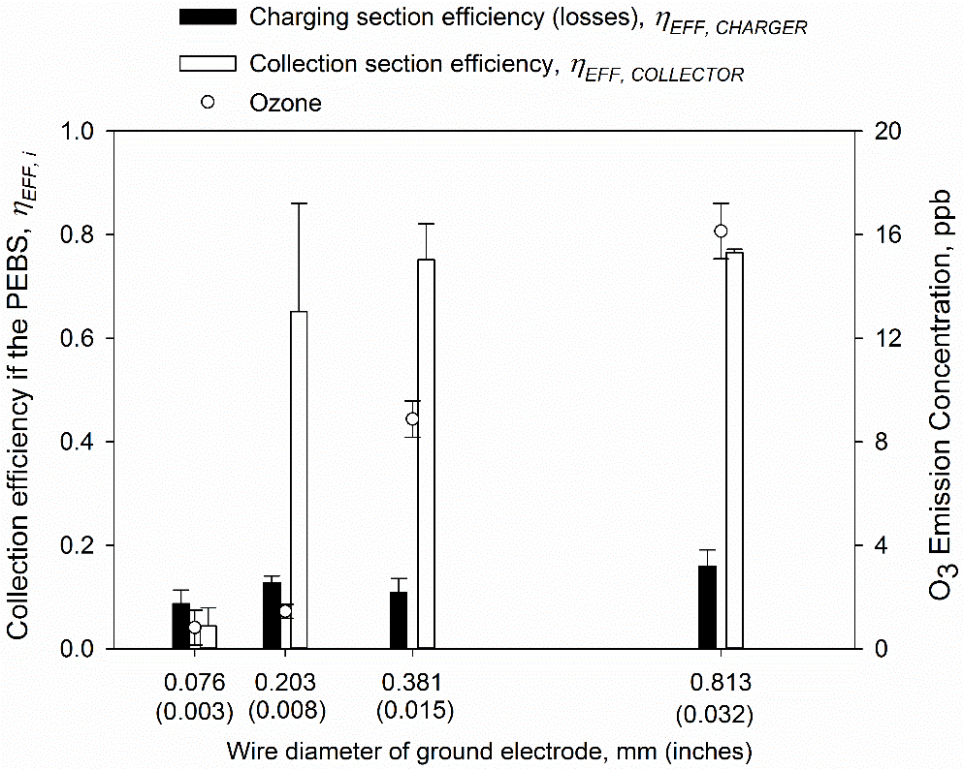


Fig. 3.5. The collection efficiency of the PEBS as a function of the wire diameter of the ground electrode in the charger. The experiments were performed with 1 μm PSL particles at a 10 L/min sampling flow rate and +5.5 kV/-7 kV charging/collection voltage. The efficiency was determined by measuring particle concentration downstream of PEBS with its voltage ON and OFF. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviations.

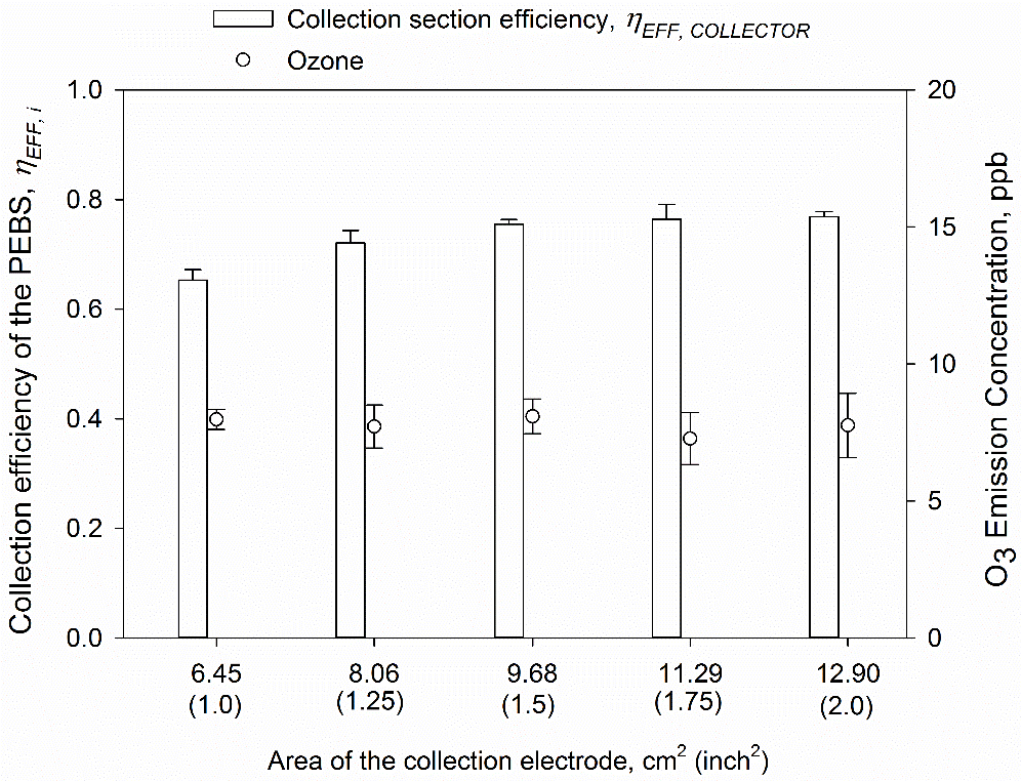


Fig. 3.6. The collection efficiency of the PEBS as a function of the collection electrode area. The experiments were performed with 1 μm PSL particles at a 10 L/min sampling flow rate and +5.5 kV/-7 kV charging/collection voltage. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviations.

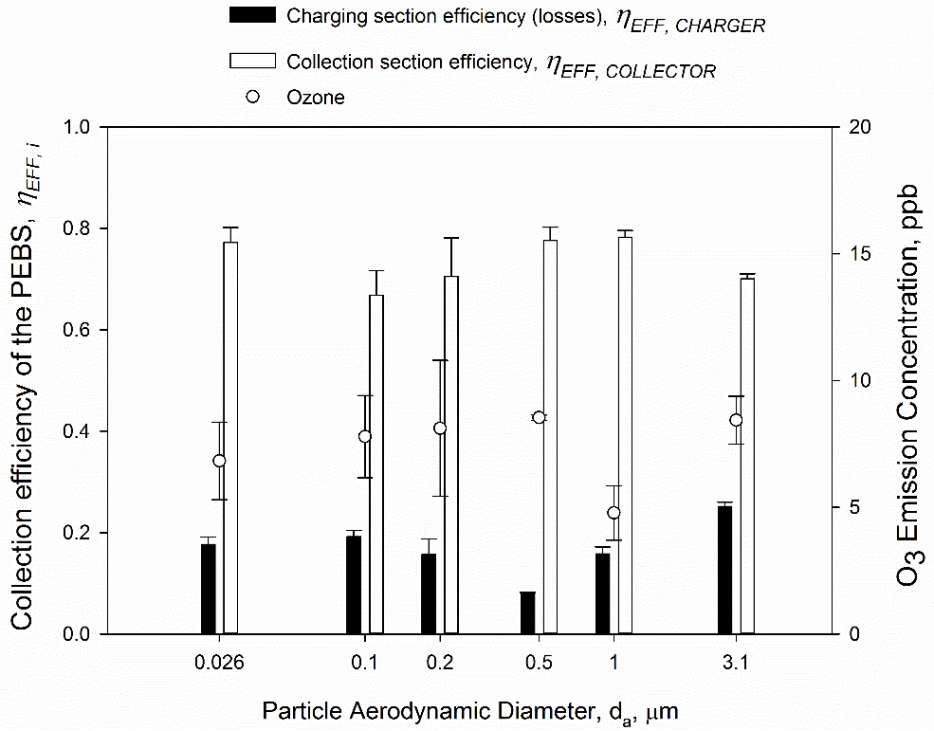


Fig. 3.7. The collection efficiency of the PEBS as a function of PSL particle size (ranging from 0.026 to 3.1 μm). The experiments were performed with 1 μm PSL particles at a 10 L/min sampling flow rate and +5.5 kV/-7 kV charging/collection voltage. The efficiency was determined by measuring particle concentration downstream of PEBS with its voltage ON and OFF by a GRIMM OPC and P-Trak CPS. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviations.

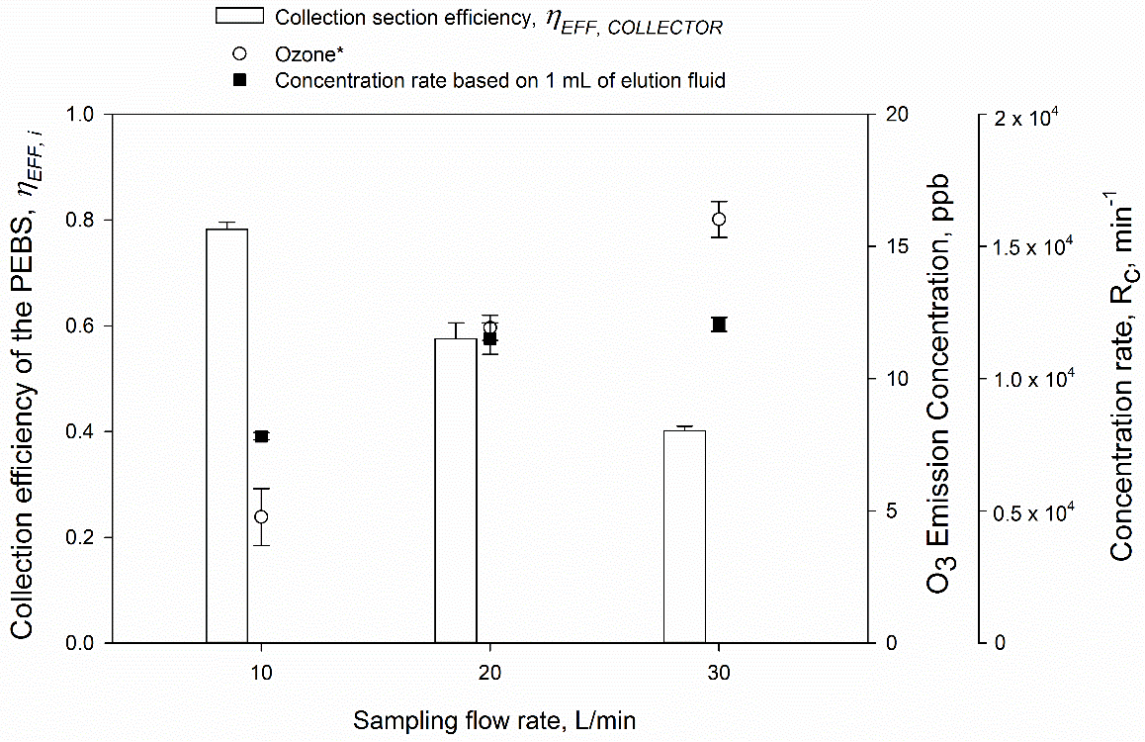


Fig. 3.8. The collection efficiency of the PEBS as a function of sampling flow rate (10, 20, and 30 L/min). The experiments were performed with 1 μm PSL particles and 10 min sampling time. The charging voltage was different for each flow rate: +5.5 kV (10 L/min), +6.5 kV (20 L/min), and +7.5 kV (30 L/min), but the collection voltage was fixed at -7 kV. The collection efficiency was determined by measuring particle concentration downstream of PEBS with its voltage ON and OFF by GRIMM. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. The third y-axis shows PEBS's concentration rate determined using Eq. 3.4. Each data point is an average of least three repeats, and the error bars represent standard deviations.

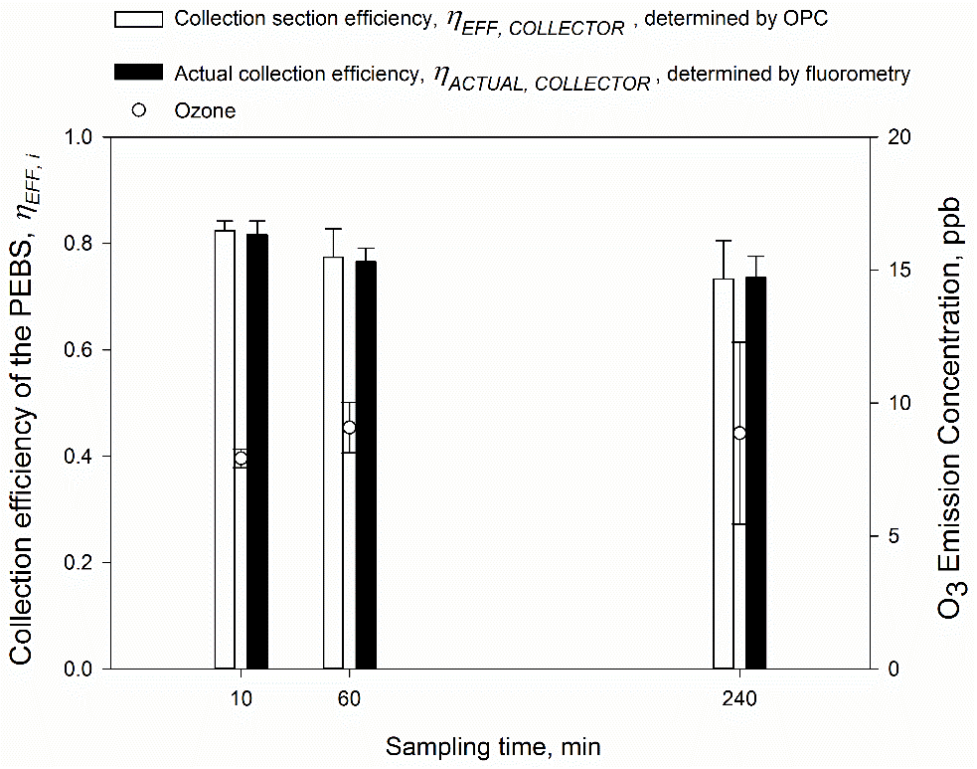


Fig. 3.9. The performance of PEBS determined by two different metrics as a function of sampling time (10, 60, and 240 min). The experiments were performed with 1 μm PSL particles at a 10 L/min sampling flow rate and +5.5 kV/-7 kV charging/collection voltages. The concentrations of test particles were ~10^3–10^4/L. The second y-axis shows ozone emission concentrations by the PEBS with ozone background concentrations removed. Each data point is an average of least three repeats, and the error bars represent standard deviations.

4. Specific Aim III: Laboratory evaluation of PEBS when collecting bacteria and fungi; Specific Aim IV: Laboratory evaluation of PEBS against another bioaerosol sampler

In this chapter, we describe laboratory testing of PEBS when collecting bacteria and fungi as well as its comparison against another bioaerosol sampler.

4.1 Design features of PEBS

As described above, PEBS is a two-stage electrostatic precipitator comprised of a static air blender, a wire-to-wire charger, a transition section, and a collection chamber (Fig. 4.1). The entire PEBS has a shape of a cylinder of 2.54 cm (1 inch) in diameter and ~14 cm (5.5 inches) in length. The static blender positioned at the sampler's inlet improves mixing of the incoming aerosol particles with the produced ions; the wire-to-wire charger configuration creates efficient field charging without a significant loss of the incoming particles and also results in low ozone emissions (less than 10 ppb); the collection chamber consists of two stainless steel quarter-cylinder grounded electrodes and a removable dual-sided collection plate made of stainless steel and connected to the collection voltage.

4.2 Test particles and their preparation

The physical and biological performance of PEBS in the laboratory was determined using two microorganisms: gram-positive *Bacillus atrophaeus* bacterial cells (ATCC 49337, American Type Culture Collection, MD) and *Penicillium chrysogenum* fungal spores (ATCC 10135). Both microorganisms have been widely used in bioaerosol studies as typical test particles (Hill et al., 1999; Johnson et al., 1994; Nadkarni et al., 2002). The preparation of microorganisms used in this study is described in previous publications (Han et al., 2015c; Han et al., 2017). Briefly, *Bacillus atrophaeus* cells were inoculated in Nutrient Broth (Becton, Dickinson and Co., Sparks, MD) and incubated for 18 hours at 30°C prior to experiments (Han et al., 2015c); *P. chrysogenum* spores were streaked on Malt Extract Agar (Becton, Dickinson and Co.) and incubated for seven days at room temperature prior to experiments (Han et al., 2011). The spores were harvested by adding a few ml of sterile Milli-Q water (EMD Millipore Corp., Billerica, MA) to a plate and then gently scraping the surface of mycelium with a scraper (Yao and Mainelis, 2006). All cells were washed four times by repeated centrifugation for five minutes at 7000 rpm (BR-4 centrifuge, Jouan, DEC Inc., Lorton, VA) and then resuspended in 20 mL sterile Milli-Q water (Han et al., 2017). The final liquid suspension was prepared by mixing the final bacterial pellet with phosphate-buffered saline (PBS); the harvested and washed fungal spores were suspended in sterile DI water. Target airborne microorganism concentrations of ~10^7 (cells or spores)/m^3 were obtained as determined by a Grimm optical particles counter (OPC) (model 1.108, Grimm Technologies Inc., Douglasville, GA, USA). Fresh liquid suspension of 10 mL of each species was prepared for each test, and it was aerosolized using a three-jet Collison nebulizer with a polycarbonate jar and operated at a flow rate of 5 L/min (pressure of 12 psi). Polycarbonate jar instead of glass jar was used to minimize potential damage to the microorganisms during aerosolization (Zhen et al., 2013, 2014).

4.3 Experimental setup for testing PEBS in laboratory

The test system is shown in Fig. 4.2, and it consisted of a flow controller, a particle generator, an air-particle mixing element, a flow straightener, a test chamber, and a particle monitor (Han et al., 2017). The system was housed in a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN). The test system used for this study was based on the setup described in our previous publication (Han et al., 2017), and it had only a minor modification of the test section downstream of the test chamber (Fig. 4.2): two more reference samplers (reference filter and BioSampler) were installed in the test section. The aerosolized particles were combined with a dry air flow, Q_d (5 L/min) (Han et al., 2015c; Zhen et al., 2013). The flow stream passed through a 2-mCi Po-210 charge neutralizer (Amstat Industries Inc., Glenview, IL) to reduce aerosolization-imparted particle charges to Boltzmann charge equilibrium. A HEPA-filtered dilution air flow, Q_D (60 L/min), provided by an in-house compressor was used to dilute the particle stream. A well-mixed flow stream passed through a flow straightener (honeycomb) (Han

et al., 2005). The sampler was positioned six duct diameters downstream of the exit of the flow straightener in order to provide a uniform cross-sectional particle profile (Han et al., 2017). The raised test duct allowed to accommodate a perpendicularly oriented PEBS collector and an empty tube of the same diameter positioned side by side. The latter was used to convey air to the reference filter (25 mm PTFE membrane, Pall Inc., East Hills, NY, USA) when measuring physical collection efficiency of PEBS. The number of test particles captured on the reference filter and an after-filter positioned downstream of non-operating PEBS agreed within 5.3 ± 1.1% when tested with *B. atrophaeus* bacteria and 10 min sampling. The samples here were analyzed using ATP-bioluminescence as described below. Another reference sampler, a BioSampler with 5 mL collection fluid cup, was used when investigating PEBS's biological performance, and it was positioned ½ duct diameters downstream of the PEBS.

PEBS was operated at sampling flow rates (Q_S) of 10 and 20 L/min provided by a vacuum pump, at sampling times of 10, 60, and 240 minutes, and at +5.25 kV/ –7 kV charging/collection voltages. The corresponding electrostatic field strength at –7 kV collection voltage is 5.5 kV/cm, which corresponds to our earlier data showing that an electrostatic field of 5 kV/cm does not affect microorganism culturability (Yao et al., 2005).

The stainless steel collection plate of PEBS was coated with a superhydrophobic substance (HIREC-1450, NTT Corporation Inc., Japan) to ensure easy and efficient removal of collected particles. The coating substance and its application procedure were described previously (Han and Mainelis, 2008; Han et al., 2011; Han et al., 2015c; Han et al., 2017). Briefly, the substance was applied on the electrode twice within a few minutes to achieve a uniform coating, and then the electrode was left to dry at 60°C for about 1 h (Han et al., 2015a). After completing the sampling, the collection plate was removed from the collector and transferred into a 15 mL autoclaved jar; the reference filter was transferred into a centrifuge tube (50 mL); 5 mL of sterile deionized water was added to the jar and the centrifuge tube. The particles collected on the reference filter were eluted into 5 mL of sterile DI water using a previously described procedure (Han et al., 2015c; Wang et al., 2001). The collection liquid that remained in BioSampler after sampling was transferred into a centrifuge tube and measured, then its volume was reconstituted to 5 mL by adding PBS (for bacterial cells) or sterile deionized water (for fungal spores). Each 5 mL sample from PEBS, BioSampler, and reference filter was equally subdivided into five microcentrifuge vials in equal volumes (1 mL) for subsequent analysis by acridine orange epifluorescence microscopy (AOEM) or direct microscopic counting, flow cytometry, adenosine triphosphate (ATP)-based bioluminescence, flow cytometry (Live/Dead test), and culture-based methods. The ozone concentration produced by PEBS was measured using a UV photometric ozone monitor (Model 202, 2B Technologies Inc., Boulder, CO) downstream of PEBS (Fig. 4.2). For 60 and 240 min sampling, the collection fluid of BioSampler was refilled to 5 mL every 15 minutes.

4.4 Methods used to determine physical collection efficiency, viability, and culturability

(a) Optical particle counter. Particle number concentrations downstream PEBS with its charging/collection voltages ON and OFF were determined using a Grimm optical particle counter).

(b) Microscopy. The collected bacterial cells were counted using epifluorescence microscopy. Here, a 1 mL sample was serially diluted in 10-fold dilutions with sterilized water to achieve a comfortably countable concentration. Each microscope slide was prepared by filtering 1 mL aliquot of a selected dilution through a 25 mm black polycarbonate filter (0.22 μm pores size,

Fisher Scientific, Suwannee, GA) and then staining it with 1 mL of 0.1 μg/mL Acridine Orange solution (Becton Dickinson Microbiology Systems, Sparks, MD) for 15-30 min (Han et al., 2015c). After washing the filter with 3 mL of sterilized water and air-drying, the filter was mounted on the glass slide, and at least 20 microscope fields were counted twice using the 100× oil-immersion objective. The concentration of cells per 1 mL aliquot, $C_{sample,bacteria}$, was calculated as follows:

$$C_{sample,bacteria} = n \times X \times D_{10-fold},\ \#/mL \qquad [4.1]$$

Here, n is the average cell count in each microscope view field, X is the number of fields (X = 6125) for the entire 25mm filter, and $D_{10-fold}$ is the dilution factor.
The collected fungal spores were counted using direct light microscopy and a hemocytometer counting chamber (Hausser Scientific Company, Horsham, PA) (Freimoser et al., 1999). Here, 0.01 mL of the 1 mL fungal spore sample aliquot was transferred to the counting chamber and then a coverslip was affixed to the hemocytometer. To determine the spore concentration per ml, the average number of spores in either just the large center square or four corner squares plus a center square of the hemocytometer chamber was counted depending on the spore concentration in the sample. The number of fungal spores per 1 mL, $C_{sample,fungi}$, was calculated as:

$C_{sample,fungi} = n \times 50000,\ \#/mL$ (when spores in the center square of the hemocytometer were counted)
$C_{sample,fungi} = n \times 10000,\ \#/mL$ (when spores in the four corner squares plus the center square of the hemocytometer were counted) [4.2]

Here, n is the total counted spore number.

Based on the counted number of microorganisms, the airborne number concentration of microorganisms, $C_{N,i}$ (N/m^3), was determined as follows:

$$C_{N,i} = \frac{C_{sample,i} \times V_s}{Q \times t} \qquad [4.3]$$

Where V_s is the entire sample volume in mL; Q is the sampling flow rate; t is the sampling time in min; subscript i refers to either the bacterial cells or fungal spores.

(c) ATP-based bioluminescence. The bioluminescence intensity of a sample is proportional to its ATP contents; i.e., the concentration of viable biological particles in a sample (Eydal and Pedersen, 2007; Han et al., 2015b). When applying this method to our samples, we followed procedures developed in our previous studies (Han et al., 2015b; Seshadri et al., 2009). From each 1 ml sample, triplicate 100 μL aliquots were transferred into 1.5 mL centrifuge tubes; a 100 μL aliquot was combined with an equal volume of BacTiter-Glo reagent (Pro-mega Crop., Madison, WI). The contents were briefly vortexed for ~5 sec and then left at room temperature for 1 min. The luminescence intensity of the resulting aliquot was measured by a luminometer (model 20/20n, Turner Biosystems Inc., Sunnyvale, CA) and recorded as relative luminescence units (RLU).

The airborne ATP concentration, $C_{RLU,i}$ (RLU/m^3), was determined as follows:

$$C_{RLU,i} = \frac{\frac{RLU}{V_a} \times V_s}{Q \times t} \qquad [4.4]$$

Where *RLU* is luminescence intensity for the biological particles; V_a is the aliquot volume used for analysis (100 µL); V_s is the entire sample volume in mL; *Q* is the sampling flow rate; *t* is the sampling time in min; subscript *i* refers to either the bacterial cells or fungal spores.

(d) Flow cytometry (Live/Dead test). The quantification of microorganism fractions with different physiological states (live, dead, injured, and unstained) in collected samples was performed using a rapid and reliable method based of the intactness of cell membranes (Jones and Senft, 1985). Prior to the fluorescent dual stain labeling, stock solutions of both cFDA-AM (5-Carboxyfluorescein Diacetate, Acetoxymethyl Ester; Life Technologies, Eugene, Oregon, USA) and PI (Propidium Iodide; Life Technologies, Eugene, Oregon, USA) were prepared using the following procedure. 1.9 mM of cFDA- AM solution was prepared by dissolving 1mg of cFDA- AM powder in 1 ml DMSO (Dimethyl sulfoxide; Life Technologies, Eugene, Oregon, USA) solvent and storing it at –20°C in the dark. 1.0 mM of PI was prepared in distilled water from the supplier's solution of 1mg/ml and stored at 4°C in the dark. Both stock solutions were thawed at room temperature and vortexed prior to analysis. From each 1 mL sample, triplicate 0.3 mL aliquots were transferred into 1.5 mL centrifuge tubes. A final concentration of 50 µM cFDA-AM was added into the tubes, and then the samples were incubated at 37°C for 30 min in the dark followed by addition of 25 µM PI (Jepras et al., 1995; King, 2000). Controls from the same species as the samples were also prepared to identify live and dead cell populations using single stains (single stained controls): the live cell control was washed cell suspensions of untreated cells stained with only cFDA-AM and the dead cell control was prepared by killing cells in a water bath at 100°C for 20 min prior to only PI staining.

Stained samples were immediately put on ice in the dark and used within 1 hr for flow cytometry analysis. Within one hour of staining the samples, flow cytometry analysis was performed using the BD Accuri C6 Flow Cytometer (BD Life Sciences, San Jose, CA). The bacterial samples were first gated using side scatter (SSC) threshold which represents the cell density or granularity (Müller and Nebe-von-Caron, 2010). The forward scatter (FSC) threshold was applied only for pure fungal spore samples (Mesquita et al., 2013). The analysis yielded unstained, live, injured and dead cells that were differentiated by a gated fluorescent plot using channels FL1 (fluorescence 530 nm bandpass filter) vs. FL3 (fluorescence 660 nm bandpass filter) as the emission wavelength for both dyes was at 488 nm; the excitation wavelength of cFDA-AM was at 530/30 (FL1) and PI at > 660 LP (FL3) (Banin et al., 2006; Lee et al., 1986). An unstained sample from initial cell suspension (e.g., before any aerosolization and sampling stress) and single stained positive controls were analyzed first to gate the positions of the unstained, live, and dead cells in the plot. The samples were then run using the same gates, and the percentages of live, injured, dead and unstained each microorganism populations were determined from the plot for each sample.

(e) Culture-based method. From each 1 ml sample, 100 µl aliquots were plated on freshly prepared agar plates immediately after sampling. *B. atrophaeus* was plated on Nutrient Agar (NA; Difco, Becton, Dickinson and Co., Sparks, MD) and then incubated for 24 hours at 30°C (Nakamura, 1989). *P. chrysogenum* was plated on Malt Extract Agar (MEA; Difco, Becton, Dickinson and Co.) and then incubated for 48 - 72 hours at room temperature. New colonies counted every 24 hours (Yao and Mainelis, 2006) and added to the total count. All samples including blanks were performed in triplicates. The resulting airborne CFU concentration, $C_{CFU,i}$ (CFU/m^3) was determined as follows:

$$C_{CFU,i} = \frac{\frac{N_{CFU}}{V_a} \times V_s}{Q \times t \times C_{Initial}} \qquad [4.5]$$

Where N_{CFU} is the average number of counted colony forming units; V_a is the aliquot volume of 0.1 mL; V_s is the entire sample volume in mL; Q is the sampling flow rate; t is the sampling time in min; $C_{Initial}$ is the culturability of the initial suspension prior to aerosolization. The $C_{Initial}$ was determined by the total number of CFUs divided by the total number of cells in 1 mL of the initial suspension before aerosolization with the same procedure as described above.

4.5 Determination of the physical and biological efficiencies

The physical collection efficiency of PEBS was determined using OPC measurements and also by counting the collected particles using microscopy (AOEM for bacteria and direct light microscopy for fungal spores).

When using OPC measurements, the physical collection efficiency, $\eta_{OPC,}$ was determined as:

$$\eta_{OPC} = \frac{C_{CHARGER\_ON} - C_{CHARGER\_ON\ \&\ COLLECTOR\_ON}}{C_{OFF}} \quad [4.6]$$

where $C_{CHARGER\_ON}$ is particle number concentration downstream of PEBS with charger voltage ON and collector voltage OFF; $C_{CHARGER\_ON\ \&\ COLLECTOR\_ON}$ is particle number concentration downstream of PEBS when both charger and collector voltages are ON; C_{OFF} is particle number concentration downstream of PEBS with both charger and collector voltages OFF. This metric is a good approximation of the actual collection efficiency (i.e., number of particles on the collection plate relative to the upstream particle number) because it does not include the collection efficiency or, rather, losses, of the charging section. This metric was described in detail in our earlier study (Han et al., 2017).

The actual physical collection efficiency, $\eta_{Microscopy}$ was determined by comparing the number concentration of particles deposited on the PEBS collection plate and counted using microscopy with the particle concentration on the reference filter as determined by Eq. 4.1:

$$\eta_{Microscopy} = \frac{C_{PEBS}}{C_{reference\ filter}} \quad [4.7]$$

where C_{PEBS} and $C_{reference\ filter}$ are the concentrations of cells captured by PEBS and reference filter, respectively, and determined as number concentration per 1ml of elution liquid (Eq. 4.1).

In addition to the absolute physical collection efficiency of PEBS, we also determined its relative physical efficiency, where a number concentration of airborne particles measured by PEBS, $C_{N,i,PEBS}$, was compared to that measured by BioSampler, $C_{N,i,BioSampler}$, as per Eq. 4.3, and the resulting relative physical efficiency, R_P, was calculated as:

$$R_P = \frac{C_{N,i,PEBS}}{C_{N,i,BioSampler}}\text{ , (subscript } p \text{ for physical performance)} \quad [4.8]$$

Similarly to the relative physical collection efficiency, biological efficiencies of PEBS relative to those of BioSampler were determined for each analysis method that was used: ATP, flow cytometry, and culture.

When using ATP analysis, the resulting airborne ATP concentrations (RLU/m^3) determined by Eq. 4.4 were compared as:

$R_V = \frac{C_{RLU,i,PEBS}}{C_{RLU,i,BioSampler}}$, (subscript *V* for viability) [4.9]

Since the ATP method measures the presence of viable cells, this method could be thought of as a comparison of the ability of the two samplers to measure viable cells.

When using flow cytometry to determine the viable cells (i.e., live cells), the viable cell fractions from both samplers were compared:

$R_{VF} = \frac{F_{Live\ cells,i,PEBS}}{F_{Live\ cells,i,BioSampler}}$, (subscript *VF* for a viable fraction) [4.10]

When using culture analysis method, the resulting airborne culturable microorganism concentrations (CFU/m^3) determined by Eq. 4.5 were compared:

$R_C = \frac{C_{CFU,i,PEBS}}{C_{CFU,i,BioSampler}}$, (subscript *C* for culturability) [4.11]

4.6 Statistical analysis

The physical and biological performances were compared as a function of sampling time, flow rate, microorganism type, and sample analysis method using two- or three-way ANOVA (Sigmaplot 2011, Version 12.3, Systat Software Inc., San Jose, CA). If there was a significant effect of one of the variables, the differences between individual pairs of variables were examined by using the Holm-Sidak method, which takes into account multiple comparisons. The $p < 0.05$ was considered significant at $\alpha = 0.05$.

4.7 Results and discussion

4.7.1 Physical performance

Fig. 4.3 shows the physical collection efficiency of PEBS and emitted ozone concentration as a function of sampling time when sampling *B. atrophaeus* bacterial cells and *P. chrysogenum* fungal spores at 10 L/min and at fixed charger/collection voltages of +5.25 kV/–7 kV. Here, we show the collection efficiency η_{OPC}, determined using the OPC, and the actual collection efficiency, $\eta_{Microscopy}$, determined using microscopy (Fig. 4.3a). Airborne concentrations of both test microorganisms were ~10^7/m^3. As could be seen in Fig. 4.3a, the PEBS's physical collection efficiency determined by the OPC versus microscopy for all sampling times are very similar: for *B. atrophaeus*, 73.6 ± 8.5% versus 79.2 ± 8.4% for 10 min sampling, 80.2 ± 3.5% vs. 86.8 ± 2.4% for 60 min sampling, and 76.2 ± 8.3% vs. 81.9 ± 2.4% for 240 min sampling; for *P. chrysogenum,* the efficiencies were 69.2 ± 7.4% versus 77.3 ± 2.6%, 83.4 ± 4.7% vs. 85.2 ± 8.2%, and 70.7 ± 13.6% vs. 76.7 ± 15.3% for 10 min, 60 min and 240 min sampling, respectively. A three-way ANOVA analysis indicated no statistically significant effect sampling time, analysis method, and species, except for the following pair: 10 min versus 60 min sampling ($p = 0.027$) with 60 min sampling yielding higher values. Fig. 4.3b shows ozone concentrations emitted during PEBS operation, with ozone background concentrations removed. While it slightly decreased with longer sampling times, the change was not statistically significant ($p > 0.05$), and the average ozone emission concentration was 5.6 ± 1.6 ppb. During each test, the temperature in the test chamber stayed in the range of 24-27 ^{o}C, and the relative humidity ranged from 30% to 36%.

As the sampling flow rate increased from 10 to 20 L/min (Fig. 4.4a), the average collection efficiencies decreased for both microorganisms as determined by both methods: from 76.4% to 53.4% for *B. atrophaeus* and from 73.2% to 52.0% for *P. chrysogenum*. This change was statistically significant as per three-way ANOVA ($p < 0.001$) and the difference of the means between the two flow rates was 22% on the absolute scale. The decrease was observed because with increasing sampling flow rates particles spent less time in the collection chamber and had a lower chance of being collected by electrostatic forces. Similar to Fig. 4.3, there was no statistical difference among the sample analysis methods as per three-way ANOVA. Fig. 4.4b also shows ozone concentrations emitted during PEBS operation: 6.4 ± 1.5 ppb at 10 L/min and 8.5 ± 2.6 ppb at 20 L/min sampling flowrates. While the ozone concentration increased slightly, the increase was not statistically significant ($p > 0.05$). During the tests, the temperature in the test chamber stayed in the range of 21-25°C, and the relative humidity ranged from 26 to 41%.

Fig. 4.5 presents airborne concentrations of particles (#/m^3) determined by PEBS relative to those determined by BioSampler, expressed as $C_{N,i,PEBS}/C_{N,i,BioSampler}$ ratio and presented as a function of sampling time (10, 60, and 240 min) for *B. atrophaeus* and *P. chrysogenum*. The average $C_{N,i,PEBS}/C_{N,i,BioSampler}$ ratio is above > 1: 1.60 for *B. atrophaeus* and 1.92 for *P. chrysogenum.* Thus, PEBS determined higher total airborne microorganism concentrations compared to BioSampler. This difference is due to the higher physical collection efficiency of PEBS and also innate losses of particles inside BioSampler when the particles are collected by the device but remain inside during sample elution (Han and Mainelis, 2012). These innate BioSampler losses are of somewhat stochastic nature, and that could explain elevated data uncertainty. The higher physical collection efficiency of PEBS is a positive feature when sampling in low concentration environments. The pairwise comparison of ratios showed that there was a statistically significant effect of sampling time and the two species as per two-way ANOVA ($p < 0.002$) and Holm-Sidak pairwise comparison: the ratio increased significantly with longer sampling time, and the ratio was higher for fungi.

4.7.2 The physiological state of captured cells

The physiological status of microorganisms captured and later maintained by and within a bioaerosol sampler is one of the sampler's key performance parameters because it determines the types of analyses and accuracy of those analyses when analyzing the collected sample.

A) Application of flow cytometry Live/Dead method. The physiological fractions of *B. atrophaeus* (Fig. 4.6a) and *P. chrysogenum* (Fig. 4.6b) microorganisms collected by PEBS and BioSampler for 10, 60, and 240 min and determined by flow cytometry Live/Dead method are shown in Fig. 4.6. The Y-axis in Fig. 4.6 shows fractions of collected microorganisms that are live, injured, dead, and unstained. It could be seen that for each sampling time, the distribution of fractions is similar for both samplers, although the two longer sampling times (60 or 240 min) seem to have a slightly lower fraction of live cells compared to 10 min sampling. Over the entire range of tested sampling times, the average fraction of live *B. atrophaeus* cells captured by PEBS decreased from 61.7 ± 3.4% to 48.2 ± 4.4%; for BioSampler, the fraction of measured live cells decreased from 60.0 ± 5.3% to 49.5 ± 8.5%. For *P. chrysogenum* spores, the average fraction of live spores captured by PEBS was 88.3 ± 2.0% during 10 min sampling and 81.5 ± 7.4% after 240 min sampling; for BioSampler, these ranges were 81.8 ± 5.7% and 91.3 ± 3.7% for 10 min and 240 min sampling, respectively.

For both samplers, the fraction of injured *B. atrophaeus* cells increased from ~8% after 10 min sampling to approximately ~20% after 60 and 240 min sampling. The fraction of dead bacterial cells stayed in 15-20% range for both samplers and all sampling times.

When sampling *P. chrysogenum* spores, the fractions of injured and dead spores did not change much with sampling time or sampler used and were less than 5% (dead spores) and less than 11% (injured spores). For *B. atrophaeus* cells, the fraction of unstained cells was approximately 13% for both samplers and all three sampling times. For *P. chrysogenum* spores, it was approximately 3% independent of sampling time and sampler.

According to three-way ANOVA and Holm–Sidak pair-wise comparison, for both *B. atrophaeus* and *P. chrysogenum,* the type of sampler and the sampling time were not significant factors; however, the fractions of live bacteria and spores were significantly higher than other fractions (injured, dead, and unstained cells) ($p < 0.001$); for both bacteria and fungi, the fraction of dead microorganisms was higher compared to unstained microorganism fraction ($p = 0.035$). For fungi only, the injured fraction was higher than dead or unstained fractions ($p < 0.003$).

B) ATP-based bioluminescence method. This method can quickly determine the presence of total bioaerosol mass in a sample, and the method's output as Relative Luminescence Units (RLU) can be converted into the estimate of bioaerosol presence in the air expressed as RLU/m^3. Due to its convenience, this method has been applied in laboratory investigations of bioaerosol samplers (Seshadri et al., 2009) as well as in the field studies to determine the presence of bioaerosols (Han et al., 2015c; Park et al., 2015). At the same time, existing studies show that the strength of ATP signal depends on bioaerosol species and the collection device (Han et al., 2015b). Since the ATP method is not species specific, a contribution of a particular bioaerosol species prone to give a strong bioluminescence signal could have a substantial and disproportionate effect on the overall bioluminescence signal thus affecting our estimate of bioaerosol presence, which is typically expressed as RLU/m^3.

Thus, the ATP signals produced by PEBS and BioSampler after three different sampling times were converted into airborne ATP concentrations (RLU/m^3) to account for different sampling flowrates, and the ratios of those ATP concentrations ($C_{RLU,i,PEBS}$/ $C_{RLU,i,BioSampler}$) are presented in Fig. 4.7a. ATP molecules needed for the bioluminescence reaction are produced by viable cells (Venkateswaran et al., 2003) and, therefore, the ATP signal could be thought of as the ability of a particular sampler to capture and maintain viable cells. Since the flow cytometry Live/Dead method also measures the fraction of viable cells, the ratios of these fractions between the two collectors (i.e., $F_{Live\ cells,i,PEBS}/F_{Live\ cells,i,BioSampler}$) are presented in Fig. 4.7b for comparison.

As could be seen from Figs. 4.7a and 4.7b, these ratios determined by two different methods are quantitatively and qualitatively different. Surprisingly, for *B. atrophaeus,* the relative viability ratio analyzed by the ATP method increased from 0.8 to 5.3 when the sampling time increased from 10 to 240 min ($p < 0.001$ for overall effect and $p < 0.005$ for all individual pairs); for *P. chrysogenum* fungal spores, the ratio stayed well below unity, but also increased with increasing sampling time: from 0.22 at 10 min sampling to 0.40 at 240 min sampling ($p < 0.001$ for overall sampling time effect and for ratio at 240 min vs. ratios at 10 and 60 min sampling).

Regarding the difference between the microorganisms, we speculate that the aerosolization and sampling process of *B. atrophaeus* cells makes the ATP molecules more readily available for bioluminescence reaction compared to the ATP molecules in fungal spores, which are considered hardier microorganisms membranes, including the release of intracellular material (e.g., ATP), which is then available to be involved in bioluminescence reaction (Zhen et al., 2014).

At the same time, the viability fraction ratios (average ± propagated error) for *B. atrophaeus* and *P. chrysogenum* as determined by flow cytometry were 1.11 ± 0.45 and 0.97 ± 0.18, respectively, (Fig. 4.7b) and did not significantly depend on species and sampling time.

While the output from both flow cytometry and ATP-based bioluminescence depends on the presence of viable cells, it is clear that the output from the two methods is not the same. It is obvious from Fig. 4.7a that the two investigated species react very differently to sampling by the two devices while data from Fig. 4.7b does not suggest that. Also, the effect of sampling time was observed only for analysis by bioluminescence. Since aerosolization and sampling processes are the same for data presented in Fig. 4.7a and 4.7b, the difference is likely due to different microorganism components participating in the analysis and differences in the analysis methodology.

At the same time, the relative viability for *B. atrophaeus* as measured by the ATP bioluminescence increased by almost a factor of 7 while the relative viability for *P. chrysogenum* increased by approximately a factor of 2 when sampling time increased from 10 to 240 min. It would suggest that the damage to bacterial cells and their ATP captured by BioSampler increased with increasing sampling time leading to higher relative viability ratio of microorganisms captured by PEBS. Lower ATP signal from bacterial cells after longer sampling with BioSampler has been observed in earlier studies (Han et al., 2015b).

4.7.3 PEBS performance when measuring culturable microorganisms

Airborne concentrations of culturable *B. atrophaeus* and *P. chrysogenum* determined by PEBS (expressed as CFU/m^3, as per Eq. 4.5) after 10, 60, and 240 min sampling were compared to those determined by BioSampler as per Eq. 4.11 and are presented as relative culturability of PEBS samples in Fig. 4.8. For 10 min sampling, the relative culturability (average ± propagated error) of PEBS for *B. atrophaeus* and *P. chrysogenum* was 1.16 ± 1.60 and 0.67 ± 0.32, respectively. When the sampling time increased from 10 to 60 min and then to 240 min, the relative culturability gradually decreased, and for 240 min sampling, it was 0.79 ± 1.09 and 0.27 ± 0.11, for *B. atrophaeus* and *P. chrysogenum,* respectively. The decrease on the relative scale was approximately 32% for *B. atrophaeus* and 76% for *P. chrysogenum*. However, the effect of time was not significant when all data were analyzed together ($p > 0.05$) and when each species analyzed separately: $p > 0.05$ for bacteria and $p > 0.05$ for fungi.

Overall, the relative culturability averaged over the three sampling times was close to 1 (1.0 ± 2.1) for *B. atrophaeus* and close to 0.5 (0.5 ± 0.4) for *P. chrysogenum* and the difference was significant ($p = 0.002$). Since the relative culturability of PEBS when sampling bacteria is approximately 1, it seems that both devices maintain culturability of bacteria equally well. On the other hand, the observed lower relative culturability of fungal spores is unexpected because fungal spores are generally considered to be robust when it comes to their sampling (Morris et al., 2000). One possible explanation could be that the slightly jagged surface of fungal spores results in strong local electrostatic fields when the spores are deposited on the collection electrode thus resulting in the loss of culturability. This result seems to echo the results presented in Fig. 4.7a, where the lower relative viability of *P. chrysogenum* was observed, and it was independent of sampling time. It is likely that both results are connected. This phenomenon definitely warrants further investigations into the effect of electrostatic fields on the culturability and viability of fungal spores whether that depends on fungal species and their surface structure.

4.8 Figures for Chapter 4

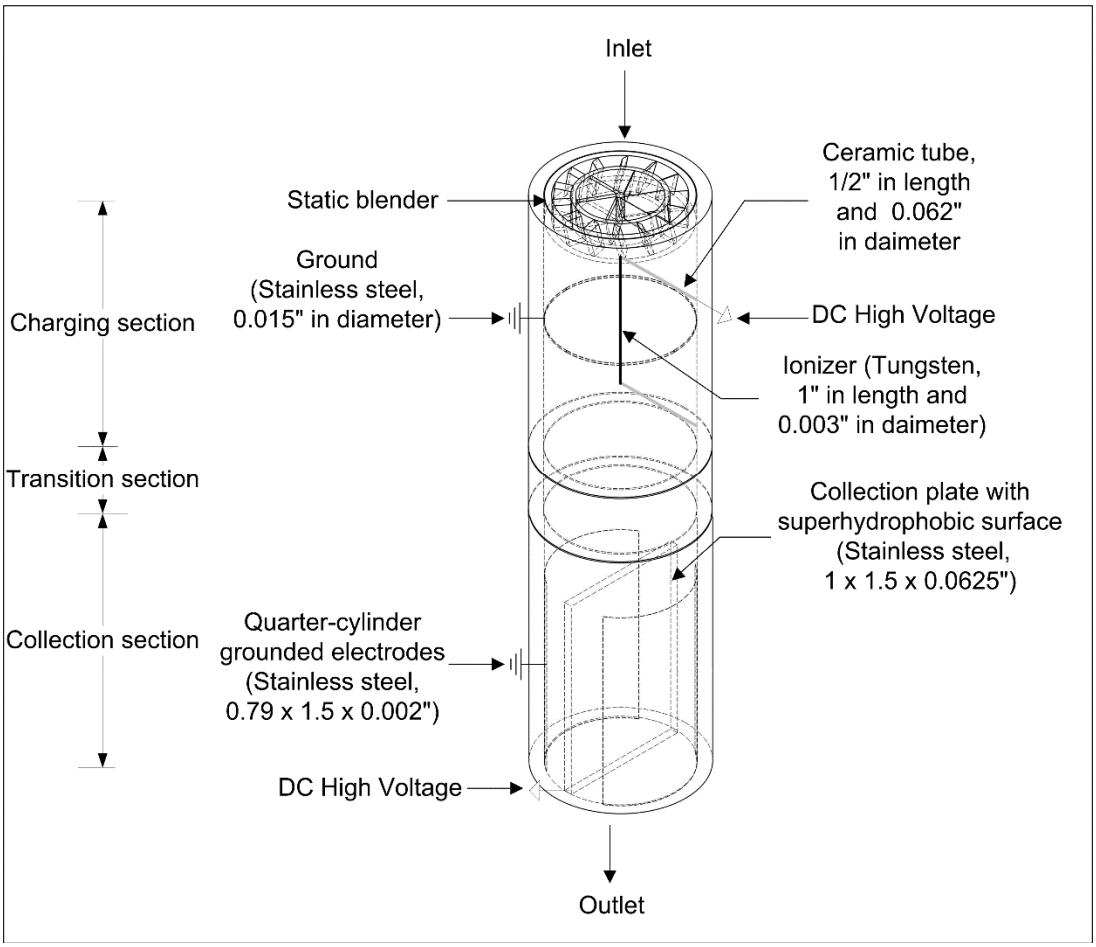


Fig. 4.1. Schematic diagram of the personal electrostatic bioaerosol sampler (PEBS) with a wire-to-wire charger.

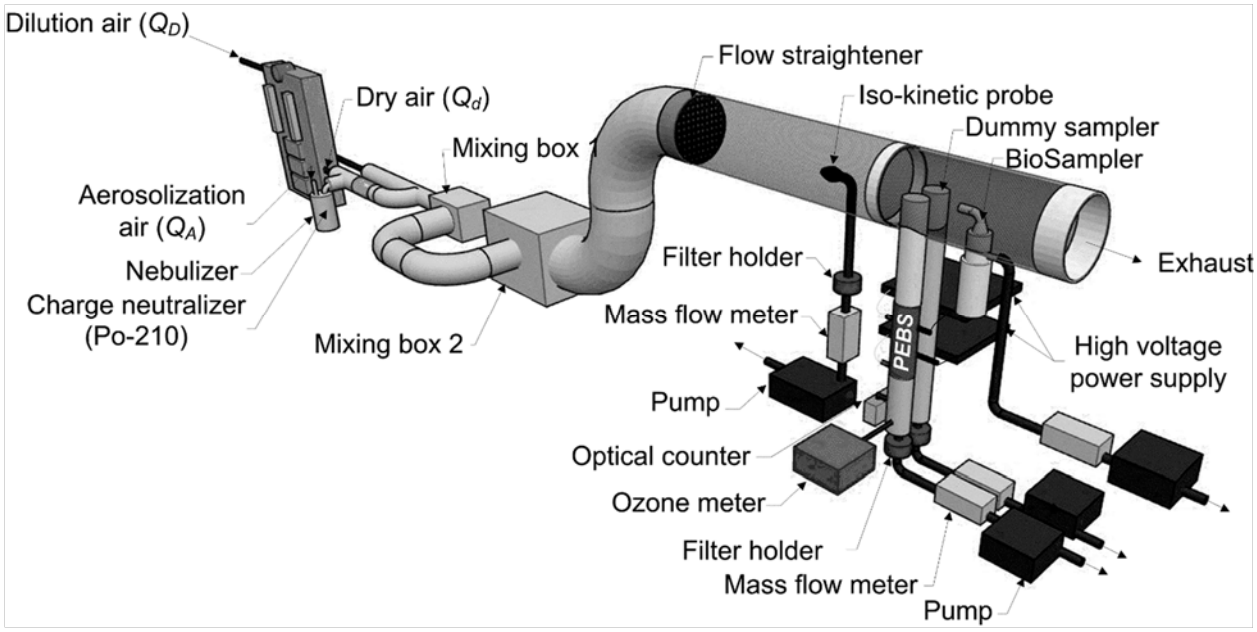


Fig. 4.2. Schematic diagram of the experimental setup.

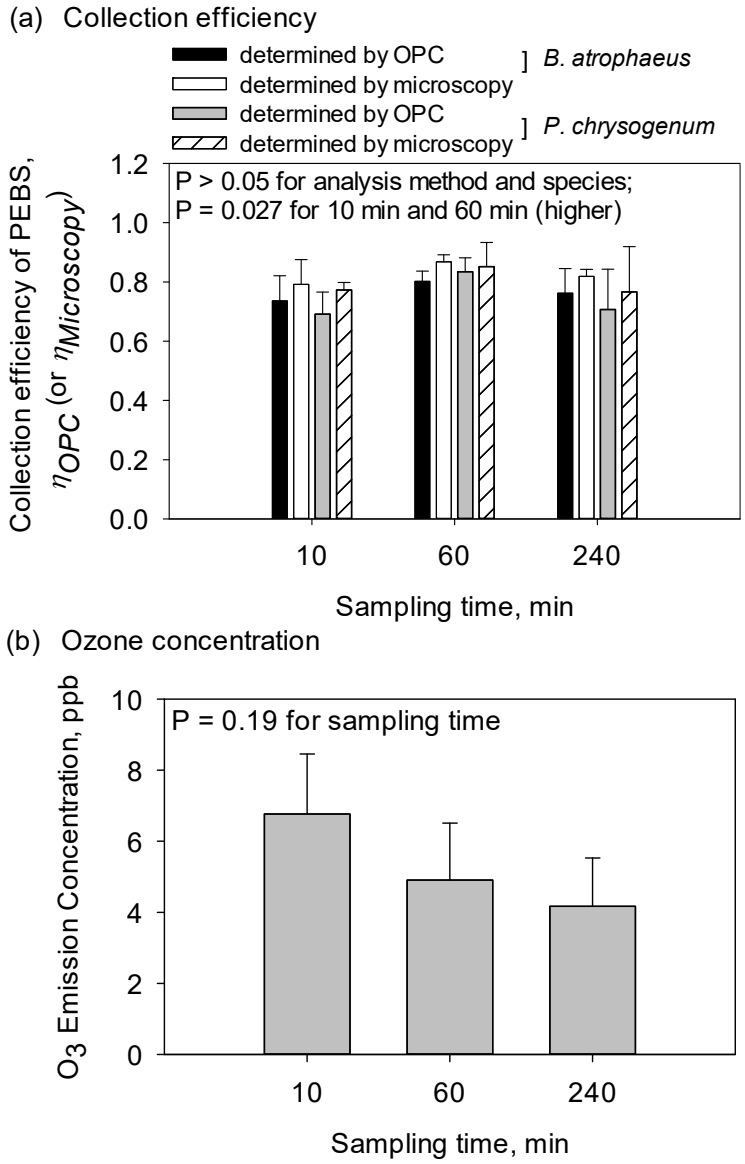


Fig. 4.3. (a) Physical collection efficiency of PEBS determined by direct particle counting and microscopy as a function of sampling time (10, 60, and 240 min) when sampling B. atrophaeus bacteria and P. chrysogenum fungal spores; (b) ozone emission concentrations, with background ozone concentrations removed. The experiments were performed at a 10 L/min sampling flow rate and +5.25 kV/-7 kV charging/collection voltages. The concentrations of test particles were ~104/L. Each data point is an average of least three repeats, and the error bars represent standard deviations.

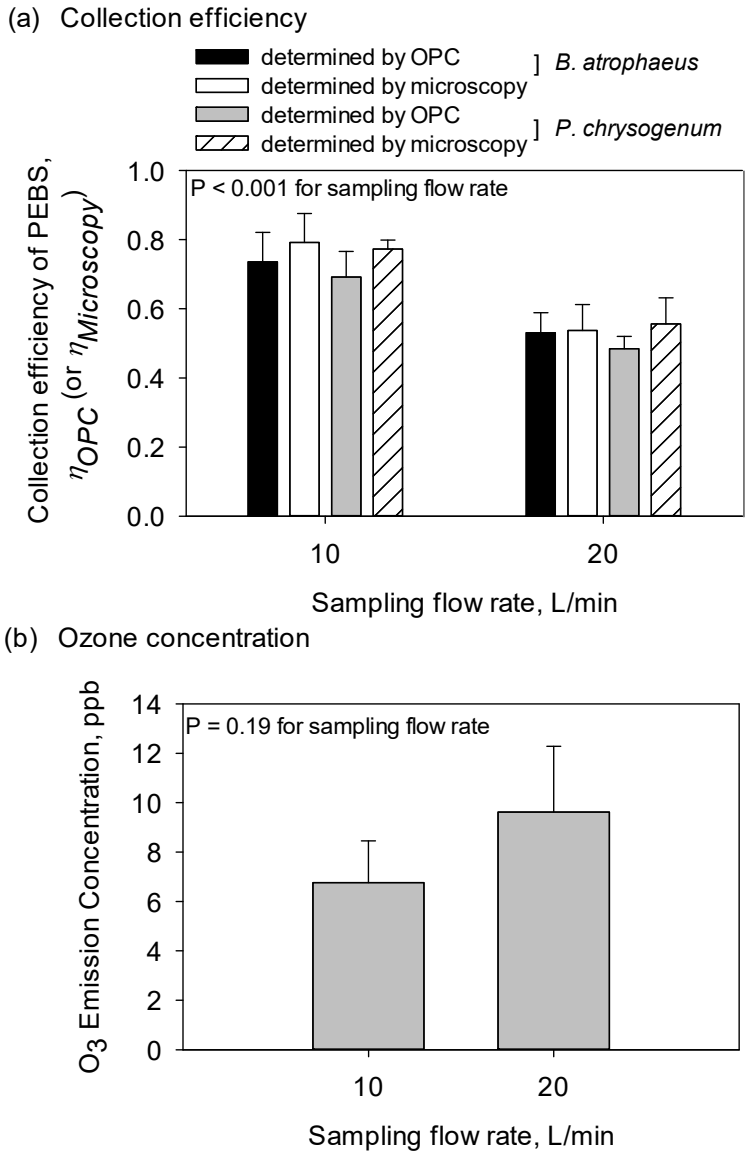


Fig. 4.4. (a) Physical collection efficiency of PEBS determined by direct particle counting and microscopy as a function of sampling flow rate (10 and 20 L/min) when sampling *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores; (b) ozone emission concentrations, with background ozone concentrations removed. The experiments were performed at 10 min sampling time and +5.25 kV/-7 kV charging/collection voltages and a sampling flow rate of 10 L/min. Each data point is an average of least three repeats, and the error bars represent standard deviation.

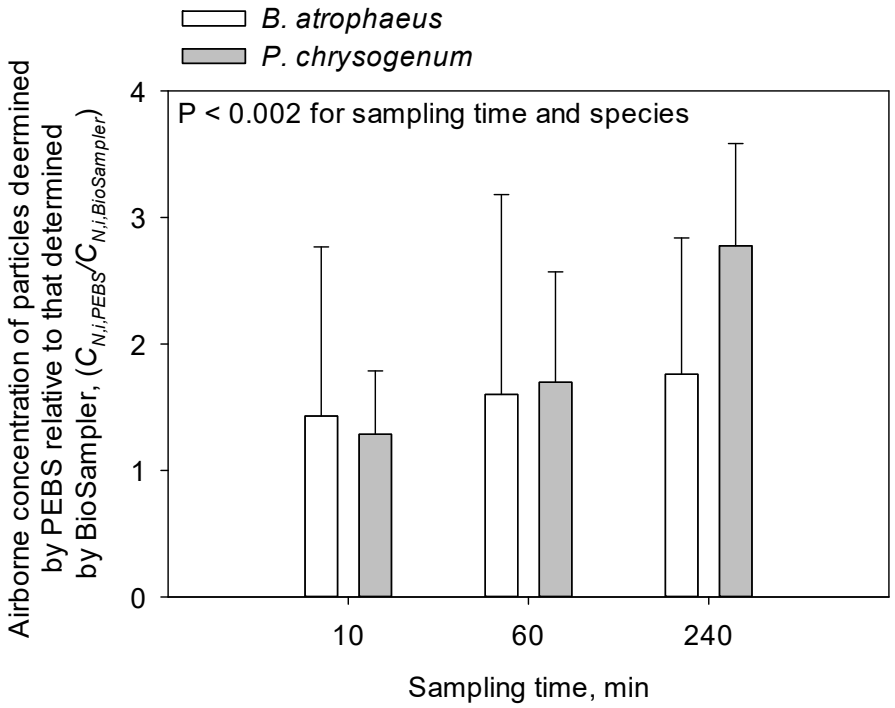


Fig. 4.5. Physical performance of PEBS presented as a ratio of the airborne particle number concentration (N/m^3) determined by PEBS to the number concentration of BioSampler for different sampling times (10, 60, and 240 min) when sampling *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores. PEBS was operated at 10 L/min sampling flow rate and +5.25 kV/-7 kV charging/collection voltages. BioSampler was operated at 12.5 L/min sampling flow rate. Each data point is an average of least three repeats, and the error bars represent a propagated error.

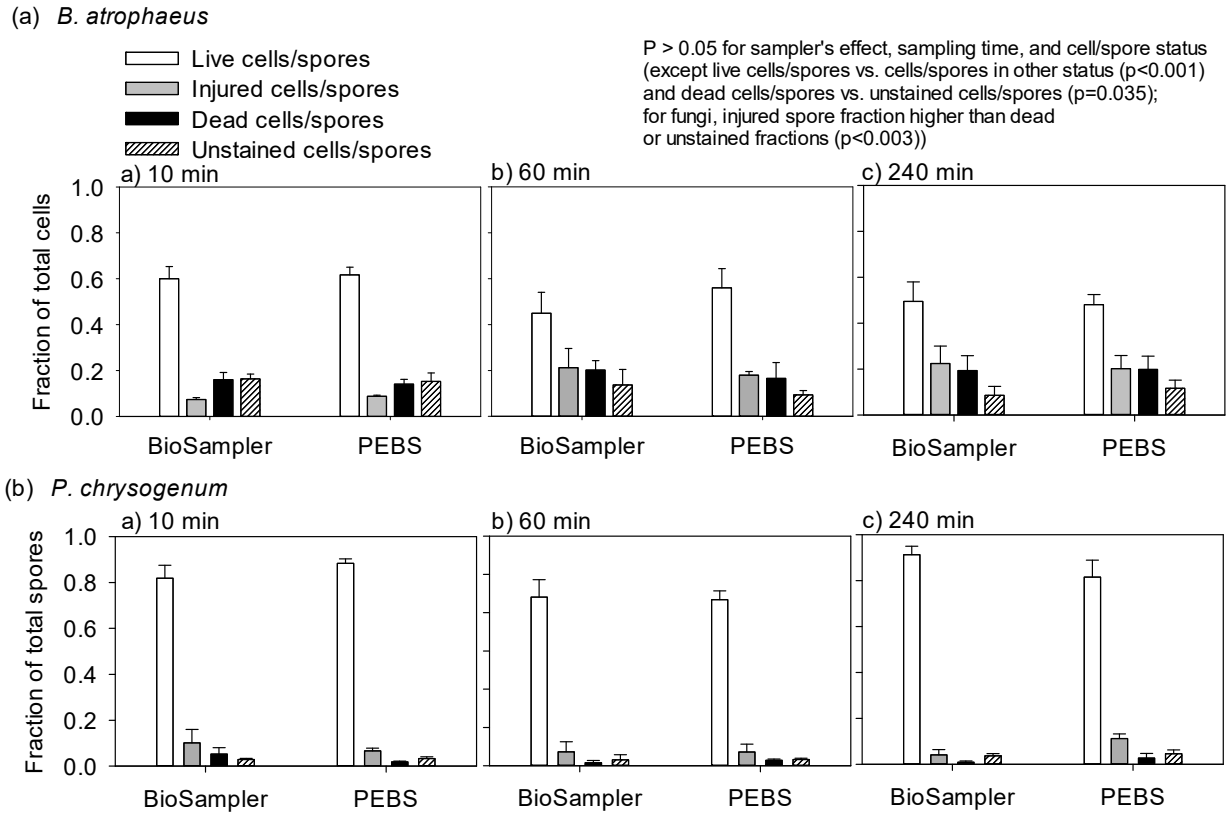
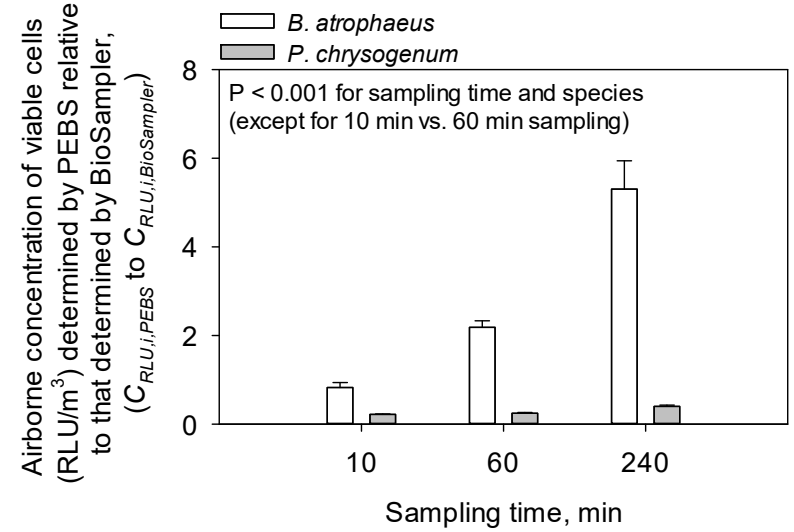


Fig. 4.6. Physiological status of test microorganisms collected by PEBS and BioSampler as a function of sampling time (10, 60, and 240 min). The presented values are fractions of total cells as measured by flow cytometry (Live/Dead) method. The experiments were performed with biological particles, a) B. atrophaeus and b) P. chrysogenum. PEBS was operated at 10 L/min sampling flow rate and +5.25 kV/-7 kV charging/collection voltages. BioSampler was operated at 12.5 L/min sampling flow rate. Each data point is an average of least three repeats, and the error bars represent standard deviation.

(a) analyzed by ATP method



(b) analyzed by flow cytometry method

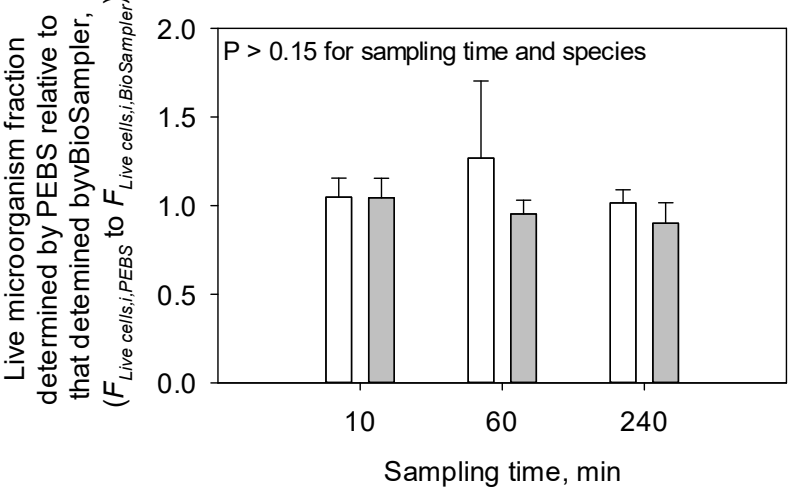


Fig. 4.7. Relative viability of samples collected by PEBS and presented as (a) a ratio of airborne ATP concentrations expressed as RLU/m3 relative to those determined by BioSampler (b) the fraction of live cells measured by flow cytometry relative to that in BioSampler samples. The ratios were determined for three sampling times (10, 60, and 240 min). The experiments were performed with B. atrophaeus bacteria and P. chrysogenum fungal spores. PEBS was operated at 10 L/min sampling flow rate and +5.25 kV/-7 kV charging/collection voltages. BioSampler was operated at 12.5 L/min sampling flow rate. Each data point is an average of least three repeats, and the error bars represent a propagated error.

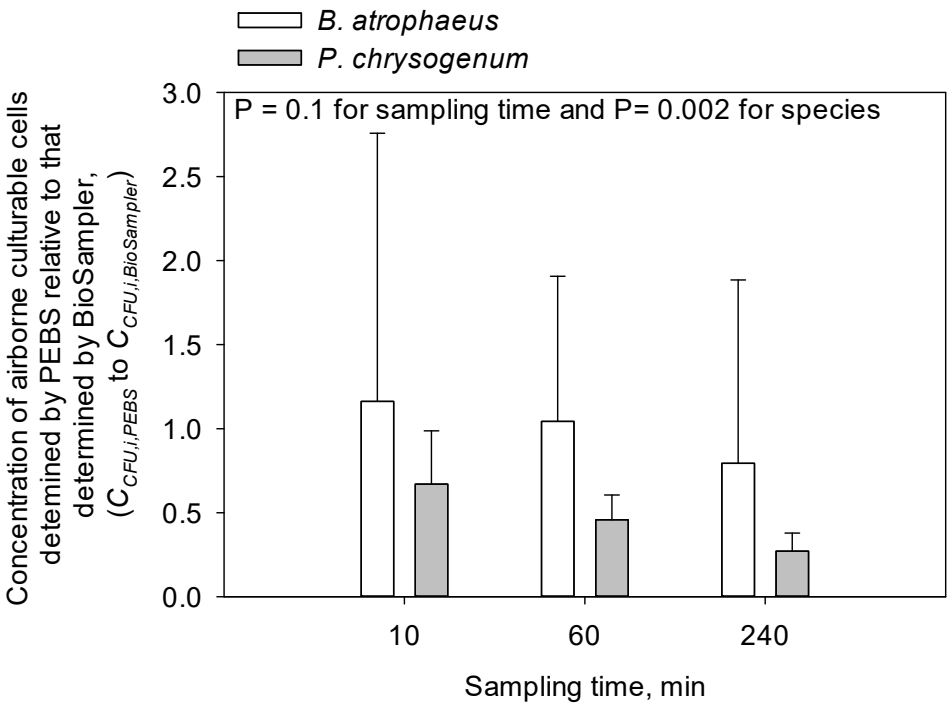


Fig. 4.8. Relative culturability of microorganism samples collected by PEBS: presented as a ratio of airborne CFU concentration (CFU/m3) determined by PEBS to that determined by BioSampler for three sampling times (10, 60, and 240 min). The experiments were performed with biological particles, a) B. atrophaeus and b) P. chrysogenum. The experiments were performed with B. atrophaeus bacteria and P. chrysogenum fungal spores. PEBS was operated at 10 L/min sampling flow rate and +5.25 kV/-7 kV charging/collection voltages. BioSampler was operated at 12.5 L/min sampling flow rate. Each data point is an average of least three repeats, and the error bars represent a propagated error.

5. *Specific Aim V: Field evaluation of the new electrostatic sampler against leading bioaerosol samplers*

5.1 Setup for field evaluation

Following our successful laboratory development of PEBS and its testing with non-biological and biological particles as described above, we built the 1st version of a field-deployable personal electrostatic bioaerosol sampler (FD - PEBS) shown in Fig. 5.1b. This is the first self-contained personal electrostatic bioaerosol sampler with all the necessary components integrated into one unit. FD-PEBS consists of an electrostatic sampler (i.e., PEBS shown in Fig. 5.1a; details of PEBS design were described earlier in this Report) and an integrated control unit (Fig. 5.1). The control unit is divided into three sections to accommodate the charger, collector, and air mover (e.g., a computer fan). All components necessary to operate the FD-EPSS, including miniature DC-to-DC high voltage power converters (Q101-5 and Q80-5, Gigi-Key Electronics Corp., Thief River, MN), potentiometers (www.amazon.com), voltmeters (Amazon), switches (Amazon), and batteries (e.g., 3.6 V & 1200 mAh lithium) are integrated in a control box (width: 45 mm × length: 80 mm × height: 153 mm) which was fabricated by 3D printing. The removable collection plate is easily installed in the collection chamber of PEBS by inserting it through a narrow vertical opening (0.16 cm) behind the cover (Fig. 5.1b). The control box also houses the computer fan (Gdstime Technology Co., China) downstream of the collection chamber of PEBS. The controls mounted on the front of the control box can adjust power for each component (i.e., charger, collector, and fan) turn them on and off as needed. The high voltage power supply is a DC-to-DC converter, which receives an input voltage from two 3.6 V batteries with a regulator. Voltages applied to the charger and collector can be monitored using a separate voltmeter through integrated leads. The power to the air mover (i.e., computer fan) is provided by a separate battery and can be easily adjusted to achieve the desired sampling flow rates (e.g., 10 L/min). The flow rate was verified by measuring the air velocity entering the FD-PEBS through its inlet.

The performance of FD-PEBS was pilot-tested when collecting samples outdoors on Rutgers University Cook campus in New Brunswick, NJ, in summer of 2017. The concentrations of airborne microorganisms collected by FDPEBS and determined by various analysis methods were compared against those measured by Button Aerosol Sampler (SKC Inc., Eighty Four, PA) and BioSampler (SKC Inc.) when sampling bioaerosols for 240 min. The FDPEBS was operated at a flow rate of 10 L/min with the charging/collection voltage of +5.25/-7 kV. The Button sampler was operated with a 0.44 μm pore size Teflon filter (Millipore, Billerica, MA) and its nominal sampling flow rate of 4 L/min. The BioSampler was operated with 5 mL of collection fluid and an appropriate sampling cup at its nominal sampling flow rate of 12.5 L/min. In order to account for liquid losses in the BioSampler due to evaporation, it was refilled every 15 min to 5 ml of collection fluid volume.

All samplers were operated simultaneously, and once the sampling was completed, the particles collected on the Button sampler's filter were eluted into sterile deionized water (5 mL) using a previously described procedure (Wang et al., 2001). The collection liquid remaining in the BioSampler cup was transferred to a 50 mL centrifuge tube, and then sterile DI water was added to increase its sample volume to 5 mL for subsequent analysis. The collection plate of the FD-PEBS was removed, and the collected particles were removed into 5 mL of autoclaved water by vortexing for approximately 30 sec. Bioaerosol content of the samples was analyzed

using the adenosine triphosphate (ATP)-based bioluminescence method, flow cytometry (Live/Dead method), and culture method.

All types of viable cells have a basic energy molecule, ATP, which, when combined with appropriate reagents, produces luminescence. The amount of light emitted during the reaction is directly proportional to the ATP content, i.e., viable bioaerosol mass. Here, 100 µL from each sample was combined with an equal volume of Bactiter-Glo reagent (Promega Corp., Madison, WI). The contents were briefly vortexed and then left at room temperature for 1 min. The luminescence intensity of the resulting suspension was measured by a luminometer (model 20/20^n, Turner Biosystems Inc., Sunnyvale, CA) and recorded as relative luminescence units (*RLU*). The total airborne ATP concentration, C_{ATP} (*RLU*/m^3), was then determined for each tested device:

$$C_{ATP} = \frac{RLU \times V \times 1000}{0.1 \times Q_S \times t} \qquad [5.1]$$

where *RLU* is the ATP bioluminescence reading per 0.1 mL, *V* is the total volume of liquid sample (5 mL), Q_s is the sampling flow rate (L/min), *t* is the sampling period (240 min), and 1000 is a conversion factor from L into m^3. Background RLU values for sterile deionized water (typically 1.5 ~ 2.0 × 10^3 $RLU/100$ µL) were subtracted from the *RLU* readout (Seshadri et al., 2009).

The procedures to determine the total number concentration, the culturable concentration and the physiological cell fractions were the same as during laboratory tests with airborne microorganisms. The results from the field tests are shown in Fig. 5.3.

5.2 Results of field testing

The concentrations of total bacterial cells, fungal spores and their sum collected by the samplers and determined by microscopy are shown in Fig. 5.3a. The highest number concentrations in all three categories were collected by the Button sampler owing to its highest physical collection efficiency. The difference in total bioaerosol number concentration determined by Button and the other two samplers was statistically significant. The concentration of fungi number concentration collected by the Button sampler was different from that collected by BioSampler but not from PEBS. The concentrations of bacteria measured by all three samplers were not different. The airborne concentrations of total bioaerosols, as well as bacteria and fungi concentrations measured by BioSampler and PEBS, were not different.

The airborne ATP concentrations expressed as RLU/m^3 are shown in Fig. 5.3b. The Button sampler showed the highest concentration due to its high physical collection efficiency. However, the airborne ATP concentrations measured by FD-PEBS and BioSampler were not statistically different.

The concentrations of culturable bacterial cells, fungal spores and total bioaerosols collected by the samplers and determined by culture techniques are shown in Fig. 5.3c. The total culturable concentrations were in 1000-2000 CFU/m^3 range, while the concentrations of culturable bacteria were slightly lower, and the concentrations of culturable fungi were approximately 400-500 CFU/m^3. The most important result for this project is that culturable concentrations for all three metrics (total culturable, culturable bacteria and culturable fungi) were not statistically different among the samplers. This result suggests that FD-PEBS, which is an electrostatics-based

collector, did not inactivate collected culturable microorganisms any more than traditional filter-based and liquid-based samplers. Furthermore, Button filter sampler showed a higher concentration of total microorganisms but the same concentration of culturable microorganisms as FD-PEBS and BioSampler. This result indicates that field sampling on a filter inactivates culturable microorganisms over prolonged sampling times, while FD-PEBS is able to recover relatively high culturable microorganism fraction among the collected microorganisms. To the best of our knowledge, FD-PEBS is the first self-contained electrostatics-based sampler that is able to measure culturable microorganisms on the same level as traditional filter-based and liquid-based samplers.

The physiological state of collected microorganisms, expressed as a fraction of all collected microorganisms, is shown in Fig. 5.3d. One could observe that viable fraction was approximately 0.7 for all three samplers, while the injured fraction was about 0.07, dead fraction approximately 0.1, and unstained fraction approximately 0.13. More importantly, none of the physiological fractions differed among the samplers. This result suggests again that FD-PEBS is as “gentle” to the microorganisms as commonly filter-based and liquid-based samplers. This is especially important given sampling time of 240 min, which is a half of a typical work shift.

This successful field testing of FD-PEBS clearly demonstrates that the development of this technology is very promising. As it stands now, FD-PEBS can successfully operate as a self-contained sampler for 240 min and obtain results that are on par with those from traditional filter-based and liquid-based samplers. This is achieved without cumbersome external power supplies and pumps. Further research and full-scale field studies will continue improving the technology and the sampler, including making it lighter and more user-friendly. Future studies will also include extensive studies to measure personal exposures in various occupational and residential environments.

5.3 Figures for Chapter 5

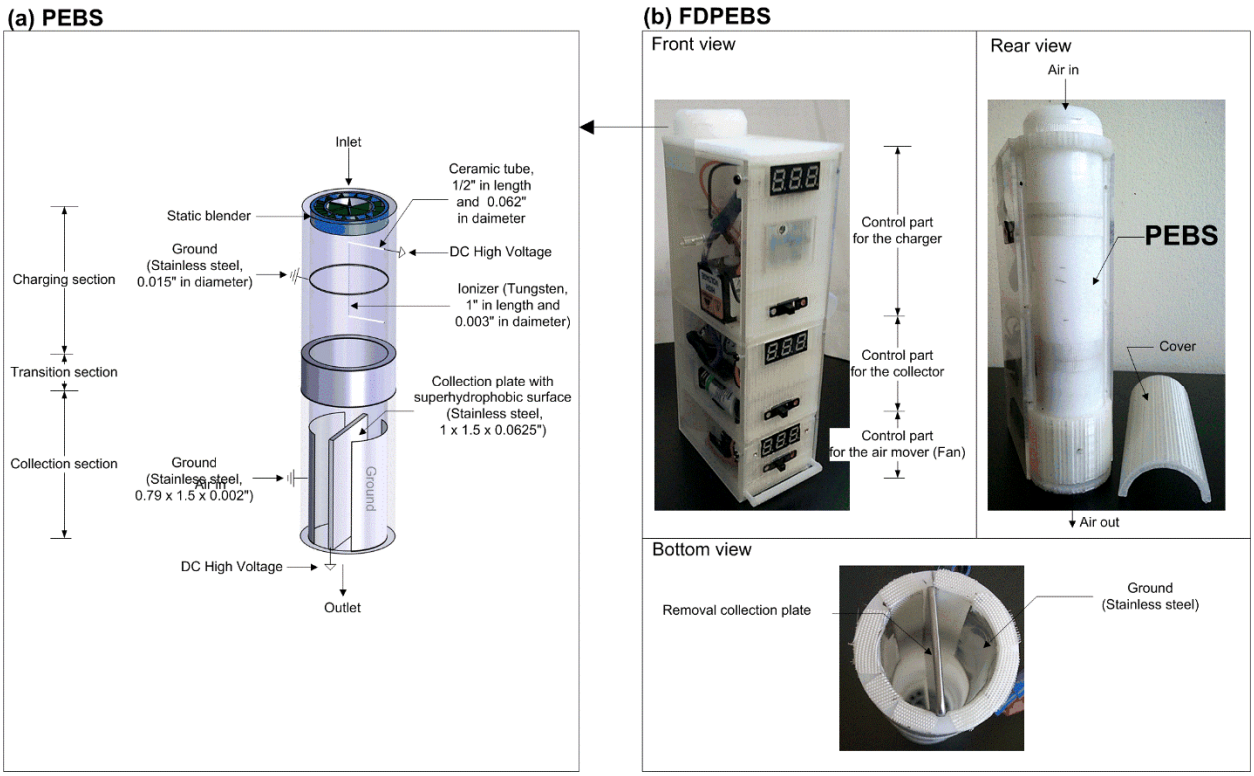


Fig. 5.1. A prototype of the field-deployable version of the personal bioaerosol electrostatic sampler (FD-PEBS) with a superhydrophobic surface made of a static dissipative material by machining and 3D printing. All sampler components are assembled in the control box and integrated with the sampling chamber.

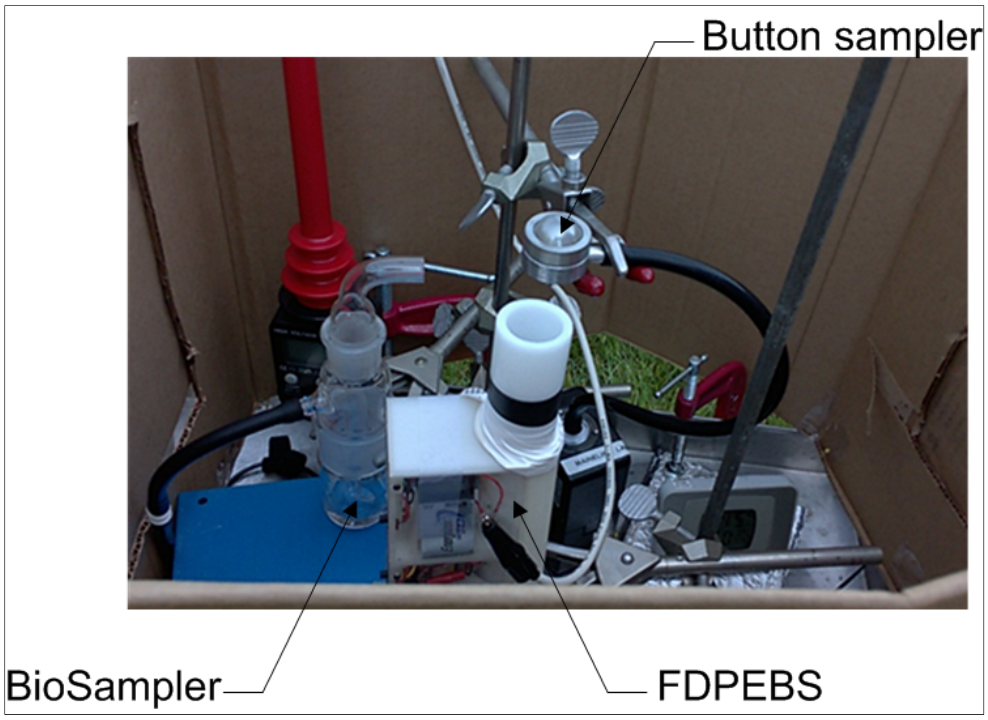


Fig. 5.2. A photo of the experimental setup for the field test.

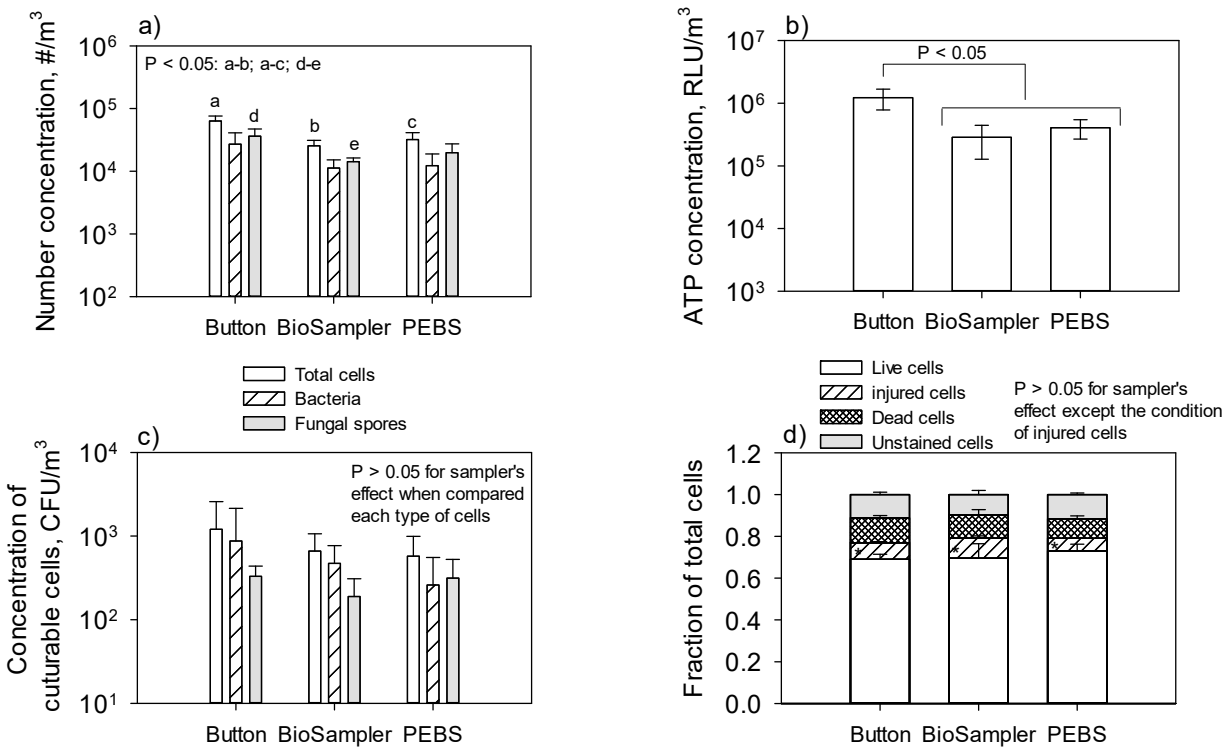


Fig. 5.3. (a) Average microorganism number concentration outdoors (#/m3) determined by Button sampler (SKC Inc., Eighty Four, PA), BioSampler (SKC Inc.), and PEBS when sampling for 240 min; (b) average airborne adenosine triphosphate (ATP) concentration (RLU/m3) determined by the three samplers; (c) average airborne culture concentration (CFU/m3); (d) physiological state of microorganisms collected by the three samplers. The Button Aerosol Sampler was operated at a flow rate of 4 L/min. The BioSampler was operated with 5 mL of collection fluid and at a sampling flow rate of 12.5 L/min. The field-deployable PEBS was operated at 10 L/min flow rates; its charging/collection voltages +5.3 kV/-7.4 kV. Power for all PEBS components was provided by 3.6 V batteries. Each data point is an average of least three repeats, and the error bars represent standard deviation.

6. Overall conclusions and future directions

The main goal of this work was to advance further the development of a new personal sampler for bioaerosols and investigate its performance when sampling two very different airborne microorganisms in a laboratory setting. The data indicate that PEBS achieves actual collection efficiency greater than 80% for sampling periods of up to 4 hours when operating at a 10 L/min sampling flow rate. Also, due to its unique charger design, it produces very low ozone concentrations – less than 7 ppb – which is a welcome level when applying electrostatic collection techniques for bioaerosol collection. When collecting non-biological particles, the sampler also showed good collection efficiency:~77% (Han et al., 2017). This work also shows that PEBS could be used with various sample analysis methods (epifluorescence microscopy, direct microscopic counting, flow cytometry, ATP-based bioluminescence, and culture-based methods), which would provide a variety of sample characteristics to evaluate the presence of and exposures to various bioaerosols better. The data also show that biological performance characteristics of PEBS, which is a self-contained two-stage electrostatic collector, are similar to those of BioSampler, which has become a *de facto* standard for low-impact liquid bioaerosol collectors. Future work will integrate all sampler components into one unit and will investigate the performance of PEBS in various field environments, including its application to determine bioaerosol exposures in different occupational and indoor settings. The used voltage settings for charging and collection yielded satisfactory viability and culturability data; however future work will consider lower voltage settings for the collector (e.g., −3 kV) to investigate if culturability of samples could be increased further.

Further development of this technology will include its miniaturization, and making the sampler “market-ready,” including making it lighter and more user-friendly. Before its wide-scale introduction, the sampler will be broadly tested in various occupational and environmental settings.

Another possible research direction is the sampler’s optimization for the collection of airborne viruses and non-biological nanoparticles. Since the sampler is based on electrostatic technique, it will also be capable of collecting nano-sized airborne particles. A new tool capable of collecting nanoparticles in a breathing zone would enable our better understanding of exposures to ambient and manufactured nanoparticles, including exposures in occupational environments.

7. Publications

Peer-reviewed publications

Han, T., Thomas, N., and Mainelis G. (2017) Design and Development of a Self-Contained Personal Electrostatic Bioaerosol Sampler (PEBS) with a Wire-to-Wire Charger, *Aerosol Science and Technology*, 51(8): 903-915.

Han, T., Thomas, N., and Mainelis G. (2018) Performance of Personal Electrostatic Bioaerosol Sampler (PEBS) when Collecting Airborne Microorganisms, *Journal of Aerosol Science*, submitted.

Presentations and Proceedings (in reverse chronological order)

Invited presentations

1. Mainelis, G. (2017) Development and Testing of Novel Sampling Tools for Airborne Microorganisms, *Seminar presentation at NASA-JPL* (Pasadena, CA, November 9, 2017).
2. Mainelis, G. (2017) Bioaerosol Encounters: From Exposure Assessment to Environmental Impacts, ***Plenary lecture** at the 10th Asian Aerosol Conference* (Jeju, Republic of Korea, July 2-6, 2017).
3. Mainelis, G. (2017) Advances in Exposure Science: from New Bioaerosol Samplers to Robotic Tools, *Seminar presentation at the Department of Environmental Engineering Sciences*, Université Laval, Quebec (Quebec City, Canada, May 2, 2017).
4. Mainelis, G. (2017) Advances in Exposure Science: from New Bioaerosol Samplers to Robotic Tools, *Seminar presentation at the Department of Environmental Engineering Sciences, University of Florida* (Gainesville, February 3, 2017).
5. Mainelis, G. (2016) Advances in Exposure Science: from New Bioaerosol Samplers to Robotic Tools, *Seminar presentation at College of Environmental Sciences and Engineering, Peking University* (Beijing, China, November 18, 2016).
6. Mainelis, G. (2016) In Search for Better Ways to Measure and Characterize Health-Relevant Aerosols, *Seminar presentation at Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences* (Changchun, China, November 16, 2016).
7. Mainelis, G. (2015) Development of bioaerosol research methods; Exposure and health effects of nanoparticles, S*eminar presentation at University of Denver* (Denver, CO, February 25, 2015).

Conference presentations

1. Mainelis, G., Han, T.T., Thomas, N.M., Therkorn, J., and Scheinbeim, J. (2018) Bioaerosol Samplers for Personal and Distributed Exposure Assessment, *Aerosol Technology* (Bilbao, Spain, June 18-20, 2018), accepted for oral presentation.
2. Han, T., Thomas, N., and Mainelis, G. (2017) Evaluation of a Self-Contained Personal Electrostatic Bioaerosol Sampler (PEBS) for Bioaerosol Collection. *Abstracts of the 36th Annual Meeting of the American Association for Aerosol Research* (Raleigh, North Carolina, October 16-20, 2017).
3. Han, T., Thomas, N., and Mainelis, G. (2017) Advanced Electrostatic Technology for Sampling Airborne Biological Particles, *Abstracts of the US-Korea Conference* (Arlington, Virginia, August 9-12, 2017).

4. Mainelis, G., Han, T., Thomas, N. (2017) New Technology For Assessing Personal Bioaerosol Exposures: Personal Electrostatic Bioaerosol Sampler, *Platform* p*resentation at the 10th Asian Aerosol Conference* (Jeju, Republic of Korea, July 2-6, 2017).
5. Mainelis, G. and Han, T. (2016) Design and Performance of Personal Electrostatic Bioaerosol Sampler (PEBS), *Poster presentation at the 22nd European Aerosol Conference* (Tours, France, September 4-9, 2016).
6. Han, T. and Mainelis, G. (2016) Design and Development of a Self-Contained Personal Electrostatic Bioaerosol Sampler (PEBS). *Abstracts of the 35th Annual Meeting of the American Association for Aerosol Research* (Portland, Oregon, October 17-21, 2016).
7. Han, T. and Mainelis, G. (2015) Initial Development of a Personal Electrostatic Bioaerosol Sampler (PEBS), Poster presentation at the *European Aerosol Conference* (Milan, Italy, September 6-11, 2015).
8. Han, T. and Mainelis, G. (2015) Design and Development of a Portable Electrostatic Bioaerosol Sampler (PEBS) with High Sampling Flow Rate, *Abstracts of the 34th Annual Meeting of the American Association for Aerosol Research (Minneapolis, MN, October 12-16, 2015)*.

8. Inclusion of gender and minority study subjects

NA

9. Inclusion of children

NA

10. Materials available for other investigators

Methodology to apply flow cytometry Live/Dead method
As part of personal bioaerosol sampler development and testing, we developed protocols to use flow cytometry Live/Dead method to analyze live, dead, injured, and unstained microorganisms in collected samples. This method allows for convenient analysis of the physiological state of collected samples. It serves as a useful tool to evaluate the performance of bioaerosol collectors in terms of damage to microorganisms during sampling. This methodology will be presented in peer-reviewed publications and in various conferences and workshops.

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