Journal of Aerosol Science 124 (2018) 54–67

Contents lists available at ScienceDirect

Journal of Aerosol Science

journal homepage: www.elsevier.com/locate/jaerosci

Performance of personal electrostatic bioaerosol sampler (PEBS) when collecting airborne microorganisms

Taewon T. Han, Nirmala M. Thomas, Gediminas Mainelis*

Department of Environmental Sciences, Rutgers, The State University of New Jersey, 14 College Farm Road, New Brunswick, NJ 08901, USA

ARTICLE INFO

Keywords:
Electrostatic precipitator
Personal sampler
Wire-to-Wire charger
Collection efficiency
Biological efficiency

ABSTRACT

We recently developed a new personal electrostatic bioaerosol sampler (PEBS) for determining exposures to airborne microorganisms. PEBS was shown to collect airborne non-biological particles with efficiencies approaching 80% while producing very low ozone concentrations. In this work, we analyzed the performance of this sampler when collecting airborne *Bacillus atrophaeus* bacterial cells and *Penicillium chrysogenum* fungal spores as a function of sampling flow rates (e.g., 10 and 20 L/min) and sampling time (e.g., 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 BioSampler (SKC Inc., Eighty Four, PA) when the samplers were operated at 10 and 12.5 L/min, respectively. PEBS achieved physical collection efficiency as high as 83%, and its physical performance in terms of measured bioaerosol concentration was better than that of BioSampler. In addition, a 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. Overall, we show that PEBS is a viable and efficient technology to determine *personal* exposures to airborne microorganisms using multiple sample analysis techniques.

1. Introduction

Bioaerosol are airborne particles of biological origin (e.g., bacteria, viruses, fungal spores, pollens), their by-products (e.g., endotoxins and mycotoxins), and fragments (e.g., insect parts and excreta, skin scales, hair) (Douwes, Thorne, Pearce, & Heederik, 2003; Dungan, 2010; Morawska, 2003). Bioaerosols have been shown to cause adverse health effects such as infections, hypersensitivity pneumonitis, and toxic reactions (Douwes et al., 2003) due to their ubiquitous nature (Zucker & Müller, 2004) and a combination of airborne transmission (Yu et al., 2004) and inhalation exposure (Burger, 1990). Due to these potential health effects bioaerosol exposure is one of the crucial health issues in many occupational environments including waste recycling industry, composting sites, as well as indoor environment, especially in buildings with poor ventilation (Domingo & Nadal, 2009; Niven et al., 2000; Poulsen et al., 1995a, 1995b). While the health effect-causing potential of bioaerosol exposures was recognized long time ago, dose-response relationships between bioaerosol exposure and respiratory effects (Walser et al., 2015) have not been established (Eggleston et al., 1998; Ivens et al., 1999) and exposure threshold values have not yet been defined (Nadal, Inza, Schuhmacher, Figueras, & Domingo, 2009). One of the key elements needed to establish dose-response relationships is accurate exposure

* Corresponding author.
E-mail address: mainelis@envsci.rutgers.edu (G. Mainelis).

https://doi.org/10.1016/j.jaerosci.2018.07.004
Received 30 April 2018; Received in revised form 2 July 2018; Accepted 16 July 2018
Available online 19 July 2018
0021-8502/

determination, and here the use of efficient bioaerosol sampling and analysis methods yielding representative results is of critical importance, especially when determining long-term personal exposures. While many stationary and portable bioaerosol samplers have been developed and evaluated (Agranovski, Agranovski, Reponen, Willeke, & Grinshpun, 2002; An, Mainelis, & Yao, 2004; Uhrbrand, Koponen, Schultz, & Madsen, 2018; Usachev, Pankova, Rafailova, Pyankov, & Agranovski, 2012), only personal sampling is considered a suitable method for assessing personal bioaerosol exposures (Tarigan et al., 2017; Viegas, 2018). Thus a personal bioaerosol sampler should feature good physical and biological performances for both short-term and long-term sampling (Yao & Mainelis, 2006, 2007) and for the user convenience it should be lightweight and self-contained (i.e., without any external components such as pumps, power supplies, and tubes) (Görner, Fabriès, Duquenne, Witschger, & Wrobel, 2006; Macher, Chen, & Rao, 2008). In addition, to be practical, a personal bioaerosol sampler should be applicable in various environments that feature low and high bioaerosol concentrations; have simple sample handling procedures with minimal sample extraction and transfer losses; allow sample analysis by a variety of traditional and advanced sample analysis methods (Baron, 1998; Chen, Feather, Maynard, & Rao, 2004; Fabian, McDevitt, Houseman, & Milton, 2009; Thompson, Donnelly, Grinshpun, Juozaitis, & Willeke, 1994), including microscopy, flow cytometry, quantitative polymerase chain reaction, adenosine triphosphate (ATP)-based bioluminescence, culture-based techniques, and other methods (Lunau, Lemke, Walther, Martens-Habbena, & Simon, 2005; Noble, Blackwood, Griffith, McGee, & Weisberg, 2010; Seshadri, Han, Krumins, Fennell, & Mainelis, 2009; Stetzenbach, Buttner, & Cruz, 2004; Veal, Deere, Ferrari, Piper, & Attfield, 2000). It is important to note that due to the different nature of those analysis techniques, the same sampler may show different performance characteristics when its sample is analyzed by different techniques (e.g., flow cytometry versus ATP) (Siebel, Wang, Egli, & Hammes, 2008). At the same time, application of different techniques yields a more comprehensive picture of the bioaerosol presence and exposures.

Due to the need for personal bioaerosol exposure assessment, there have been advances in the development, testing and application of various personal bioaerosol samplers (Agranovski et al., 2002; Lindsley, Schmechel, & Chen, 2006; Su, Tolchinsky, Chen, Sigaev, & Cheng, 2012), including adaptation of existing personal and area aerosol samplers for personal bioaerosol sampling needs (Haatainen, Laitinen, Linnainmaa, Reponen, & Kalliokoski, 2009; Kenny et al., 1998). However, these devices require a separate air mover (e.g., pump) and cumbersome sampling lines to pull air through or onto a collection medium, and the need for a power source may limit their operation time; these requirements cause inconvenience to the user and may limit user's mobility while wearing a sampler. Thus, there is still a need to develop a sampler that satisfies criteria listed above.

Our earlier study presented Personal Electrostatic Bioaerosol Sampler (PEBS), which is an electrostatics based collection device. It combined a novel wire-to-wire charger and a dual-sided collector plate and featured a high particle collection efficiency when tested with a broad size range of non-biological particles (26 nm to 3.1 μm) (Han, Thomas, & Mainelis, 2017). Its unique charger design results in very low ozone emissions (Han et al., 2017) - a critical step for applying electrostatics-based collectors for bioaerosol sampling. However, the performance of PEBS has not yet been investigated with biological particles. Therefore, the main goal of this study was to evaluate its physical and biological (i.e., sample viability and culturability) performance when collecting airborne bacteria and fungal spores in laboratory setting as a function of sampling time (10, 60, and 240 min) and sampling flow rate (10 and 20 L/min); in addition, as part of its performance evaluation, the collected particles were analyzed by multiple analysis techniques: acridine orange epifluorescence microscopy (AOEM), direct microscopic counting, adenosine triphosphate (ATP) bioluminescence, flow cytometry (Live/Dead test), and culture techniques. The obtained data were compared with those obtained by a BioSampler (SKC Inc., Eighty Four, PA) as a reference sampler. The default operational settings of PEBS's charging and collection voltages were + 5.25 kV/− 7 kV as determined by our previous study (Han et al., 2017). The experiments were carried out with commonly used test microorganisms: Gram-positive *Bacillus atrophaeus* bacterial cells and *Penicillium chrysogenum* fungal spores.

2. Materials and methods

2.1. Design features of Personal Electrostatic Bioaerosol Sampler (PEBS)

The design features of PEBS have been described in detail elsewhere (Han et al., 2017). Briefly, 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. 1). The entire PEBS has a shape of a cylinder of 2.54 cm (1 in.) in diameter and ~14 cm (5.5 in.) 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. The stainless steel collection plate of PEBS was coated with a superhydrophobic substance (HIREC-1450, NTT Corporation Inc., Japan).

2.2. 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, Martin, & Resnick, 1994; Nadkarni, Martin, Jacques, & Hunter, 2002). The preparation of microorganisms used in this study is described in previous publications (Han et al., 2017; Han, Zhen, Fennell, & Mainelis, 2015). Briefly, *Bacillus atrophaeus* cells were inoculated in Nutrient Broth (Becton, Dickinson and Co., Sparks, MD) and incubated for 18 h at 30 °C prior to experiments (Han,

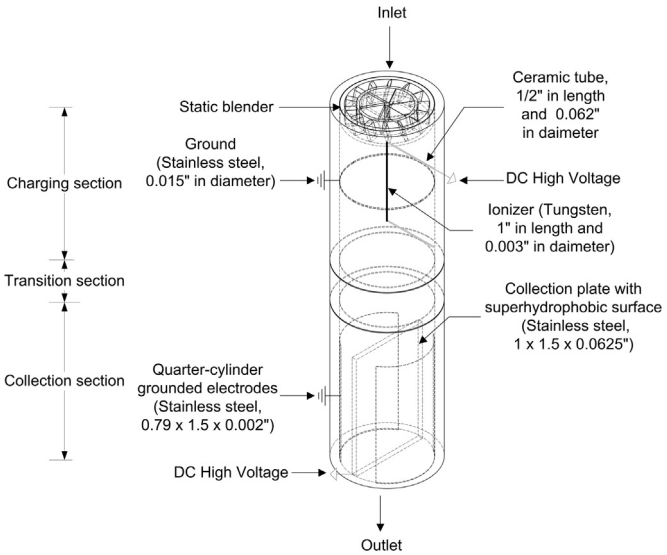

Fig. 1. Schematic diagram of the personal electrostatic bioaerosol sampler (PEBS) with a wire-to-wire charger.

Zhen, et al., 2015); *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, Nazarenko, Lioy, & Mainelis, 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 & Mainelis, 2006). All microorganisms 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 $\sim 10^7$ (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). A polycarbonate jar was used instead of the glass jar to minimize potential damage to the microorganisms during aerosolization (Zhen, Han, Fennell, & Mainelis, 2013, 2014).

2.3. Experimental setup for testing PEBS in laboratory

The test system is shown in Fig. 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. 2): two reference samplers (a tube of the same diameter as PEBS and connected to a 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, Zhen et al., 2015; Zhen et al., 2013). The flow stream passed through

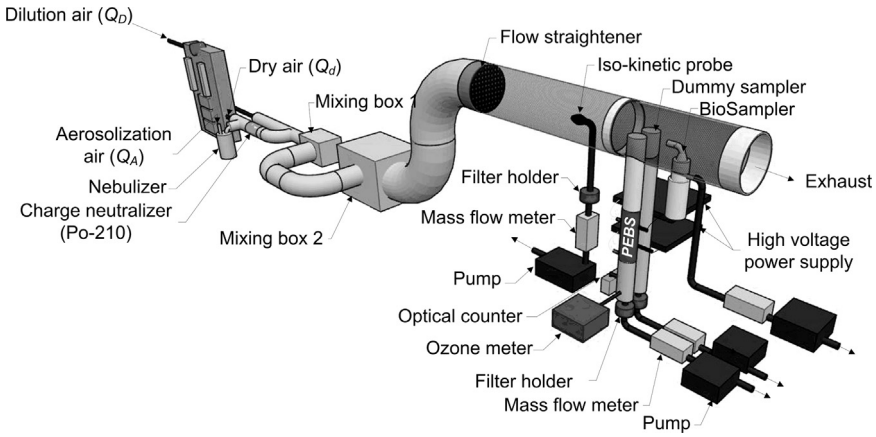

Fig. 2. Schematic diagram of the experimental setup.

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, O'Neal, McFarland, Haglund, & Ortiz, 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.

2.4. Operational conditions of samplers

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 min, 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, Mainelis, & An, 2005).

The stainless steel collection plate of PEBS was coated with a superhydrophobic substance to ensure easy and efficient removal of collected particles. The coating substance and its application procedure were described previously (Han & Mainelis, 2008; Han et al., 2011; Han, Zhen et al., 2015; 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, Fennell et al., 2015). 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 captured on the collection plate were extracted by vortexing for 30 s; the particles collected on the reference filter were eluted into 5 mL of sterile DI water using a previously described procedure (Han, Zhen et al., 2015; Wang, Reponen, Grinshpun, Górny, & Willeke, 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. 2). For 60 and 240 min sampling, the collection fluid of BioSampler was refilled to 5 mL every 15 min.

2.5. Methods used to determine physical collection efficiency, viability, and culturability

2.5.1. Optical particle counter

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

2.5.2. 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 (10–30 microorganisms per view field). 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, Zhen et al., 2015). 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:

$$C_{sample,bacteria} = n \times X \times D_{10-fold}, \#/mL \quad (1)$$

Here, n is the average cell count in each microscope view field, X is the number of fields ($X = 6125$) for the entire 25 mm 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, Jakob, Aebi, & Tuor, 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. In order 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)

$$C_{sample,fungi} = n \times 10000,\ \#/mL \text{(when spores in thefour corner squares plus the center square of the hemocytometer were counted)} \quad (2)$$

Here, n is the total counted spore number and the factors 10,000 and 50,000 are based on internal dimensions of the hemocytometer and the number of squares counted.

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

$$C_{N,i} = \frac{C_{sample,i} \times V_s}{Q \times t} \quad (3)$$

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

2.5.3. 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 & Pedersen, 2007; Han & Wren, 2015). When applying this method to our samples, we followed procedures developed in our previous studies (Han, Wren, DuBois, Therkorn, Mainelis, 2015; 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 s and then left at room temperature for 1 min. The luminescence intensity of the resulting aliquot was measured by a luminometer (model 20/20 n, Turner Biosystems Inc., Sunnyvale, CA) and recorded as relative luminescence units (RLU).

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

$$C_{RLU,i} = \frac{\frac{RLU}{V_a} \times V_s}{Q \times t} \quad (4)$$

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, liters/min; and t is the sampling time in min; subscript i refers to either the bacterial cells or fungal spores.

2.5.4. 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 & 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 1 mg 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 1 mg/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, Carter, Pearson, Paul, & Wilkinson, 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 h 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 & 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 excitation wavelength for both dyes was at 488 nm; the emission wavelength of cFDA-AM was at 530 ± 30 nm, and the emissions were captured with FL1; the emission wavelength of Propidium Iodide was at > 660 nm, and the emissions were captured with FL3 (Banin, Brady, & Greenberg, 2006; Lee, Chen, & Chiu, 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.

2.5.5. 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 h 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 h at room temperature. New colonies counted every 24 h (Yao & 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/L) was determined as follows:

$$C_{CFU,i} = \frac{\frac{N_{CFU}}{V_a} \times V_s}{Q \times t \times C_{Initial}} \quad (5)$$

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, liter/min; and t is the sampling time in min; and $C_{Initial}$ is the culturability of the initial suspension prior to its aerosolization. The $C_{Initial}$ was determined by dividing the total number of CFUs by the total number of cells in 1 mL of the initial suspension using the same procedures as described above.

2.6. 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:

$$\eta_{OPC} = \frac{C_{CHARGER\_ON} - C_{CHARGER\_ON\&COLLECTOR\_ON}}{C_{OFF}} \quad (6)$$

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. (1):

$$\eta_{Microscopy} = \frac{C_{PEBS}}{C_{reference\ filter}} \quad (7)$$

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 1 mL of elution liquid (Eq. (1)).

In addition to the absolute physical collection efficiency of PEBS determined by Eqs. 6 and 7, 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. (3), and the resulting relative physical efficiency, R_P, was calculated as:

$$R_P = \frac{C_{N,i,PEBS}}{C_{N,i,BioSampler}}, \ (\text{subscript} p \text{for physical performance}) \quad (8)$$

Where subscript i refers to either the bacterial cells or fungal spores.

Similarly to the relative physical collection efficiency, biological efficiencies of PEBS relative to those of BioSampler were determined for ATP, flow cytometry, and culture methods.

When using ATP analysis, the resulting airborne ATP concentrations (RLU/L) determined by Eq. (4) were compared as:

$$R_V = \frac{C_{RLU,i,PEBS}}{C_{RLU,i,BioSampler}}, (\text{subscript} V \text{for viability}) \quad (9)$$

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:

$$R_{VF} = \frac{F_{Live\ cells,i,PEBS}}{F_{Live\ cells,i,BioSampler}}, \quad (\text{subscript} VF \text{for a viable fraction}) \quad (10)$$

When using culture analysis method, the resulting airborne culturable microorganism concentrations (CFU/L) determined by Eq. (5) were compared:

$$R_C = \frac{C_{CFU,i,PEBS}}{C_{CFU,i,BioSampler}}, \quad (\text{subscript} C \text{for culturability}) \quad (11)$$

2.7. Statistical analysis

The physical and biological performances were compared as a function of sampling time, flow rate, microorganism type, and

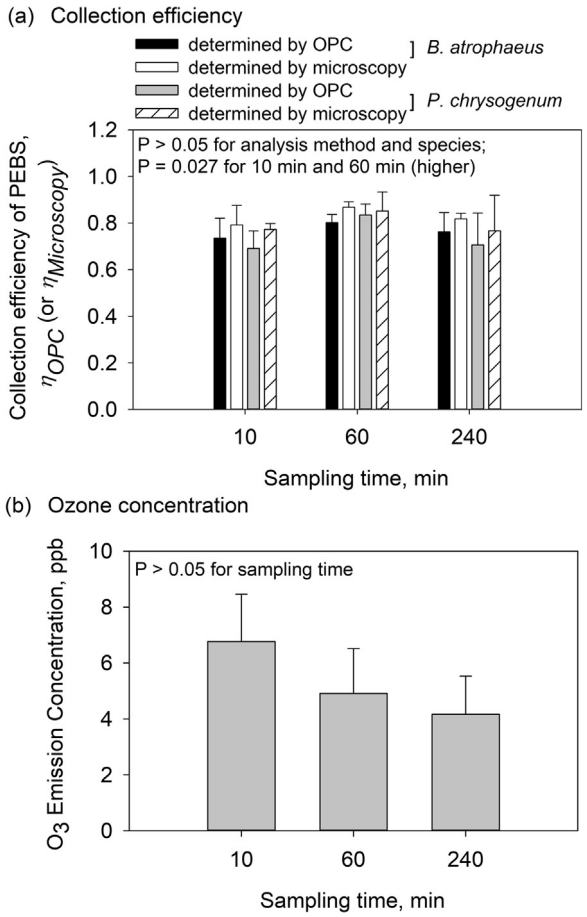

Fig. 3. (a) Physical collection efficiency of PEBS determined by direct particle counting and microscopy as a function of sampling time (10, 60, and 240 min) when sampling *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores; (b) ozone emission concentrations, with background ozone concentrations 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 ~10^4/L. Each data point is an average of least three repeats, and the error bars represent standard deviations.

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 $\alpha = 0.05$.

3. Results and discussion

3.1. Physical performance

Fig. 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. 3a). Airborne concentrations of both test microorganisms were ~$10^7/m^3$. As could be seen in Fig. 3a, the PEBS's physical collection efficiency determined by the OPC versus efficiency determined by microscopy for all sampling times are very similar: for *B. atrophaeus*, 73.6 ± 8.5% vs. 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% vs. 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. 3b shows ozone concentrations emitted during PEBS operation, with ozone background concentrations removed. While the ozone concentration 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 °C, and the relative humidity ranged from 30% to 36%.

When the sampling flow rate increased from 10 to 20 L/min (Fig. 4a), the average collection efficiencies decreased for both

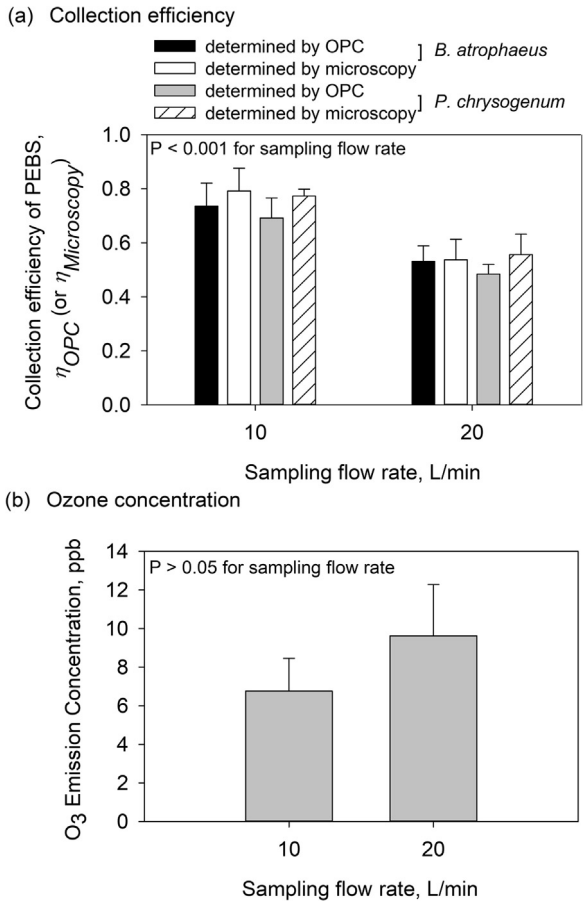

Fig. 4. (a) Physical collection efficiency of PEBS determined by direct particle counting and microscopy as a function of sampling flow rate (10 and 20 L/min) when sampling *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores; (b) ozone emission concentrations, with background ozone concentrations 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.

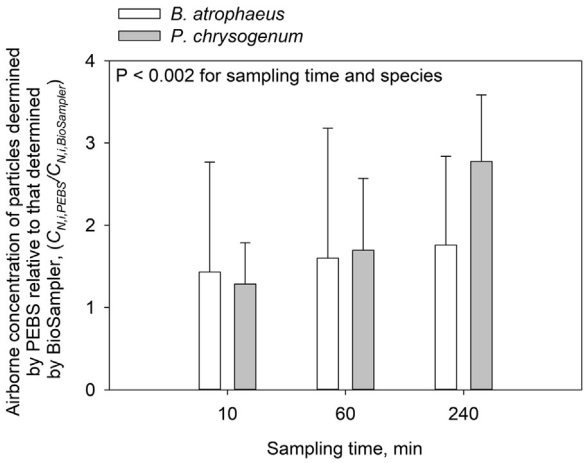

Fig. 5. Physical performance of PEBS presented as a ratio of the airborne particle number concentration (*N/L*) determined by PEBS to the number concentration of BioSampler for different sampling times (10, 60, and 240 min) when sampling *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores. PEBS 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.

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. 3, there was no statistical difference among the sample analysis methods. Fig. 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. 5 presents airborne concentrations of particles (#/L) 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 & 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.

3.2. 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.

3.2.1. Flow cytometry Live/Dead method

The physiological fractions of *B. atrophaeus* (Fig. 6a) and *P. chrysogenum* (Fig. 6b) microorganisms collected by PEBS and

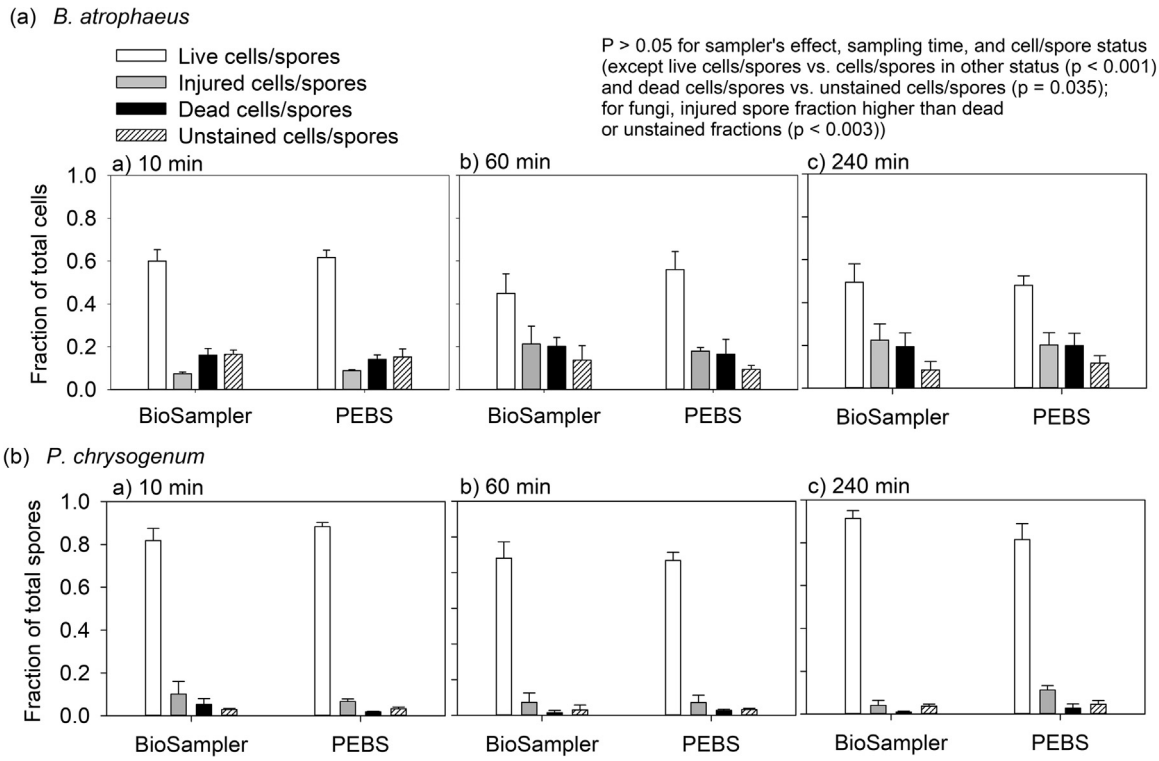

Fig. 6. The physiological status of test microorganisms collected by PEBS and BioSampler as a function of sampling time (10, 60, and 240 min). The presented values are fractions of total cells as measured by flow cytometry (Live/Dead) method. The experiments were performed with biological particles, 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.

BioSampler for 10, 60, and 240 min and determined by flow cytometry Live/Dead method are shown in Fig. 6. The Y-axis in Fig. 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 ($p < 0.003$).

3.2.2. 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/L. 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, Zhen, 2015; Park, Park, Lee, & Hwang, 2015). At the same time, existing studies show that the strength of ATP signal depends on bioaerosol species and the collection device (Han, Wren et al., 2015). 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/L.

Thus, the ATP signals produced by PEBS and BioSampler after three different sampling times were converted into airborne ATP concentrations (RLU/L) to account for different sampling flowrates of the devices, and the ratios of those ATP concentrations ($C_{RLU,i,PEBS}/\ C_{RLU,i,BioSampler}$) are presented in Fig. 7a. ATP molecules needed for the bioluminescence reaction are produced by viable cells (Venkateswaran, Hattori, La Duc, & Kern, 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. 7b for comparison.

As could be seen from Figs. 7a and 7b, these ratios determined by two different methods are quantitatively and qualitatively different. 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). The increasing ATP concentration ratio over time for *B. atrophaeus* (Fig. 7a) is likely the result of decreasing ATP concentration of this bacterium in BioSampler liquid. The loss of ATP signal of bacteria as a function of time when collecting by BioSampler has been observed in our earlier studies (Han & Mainelis, 2012), including for *B. atrophaeus* specifically (Han, Wren et al., 2015). If the ATP signal from bacteria captured by PEBS remains relatively stable over time, we will observe an increase in ATP concentration ratio.

For *P. chrysogenum* fungal spores, the ratio stayed well below unity but also increased somewhat 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). A possible explanation for this observation is provided below in conjunction with the description of culturability data.

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. 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. 7a that the two investigated species react very differently to sampling by the two devices while data from Fig. 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 Figs. 7a and 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 the 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, Wren et al., 2015).

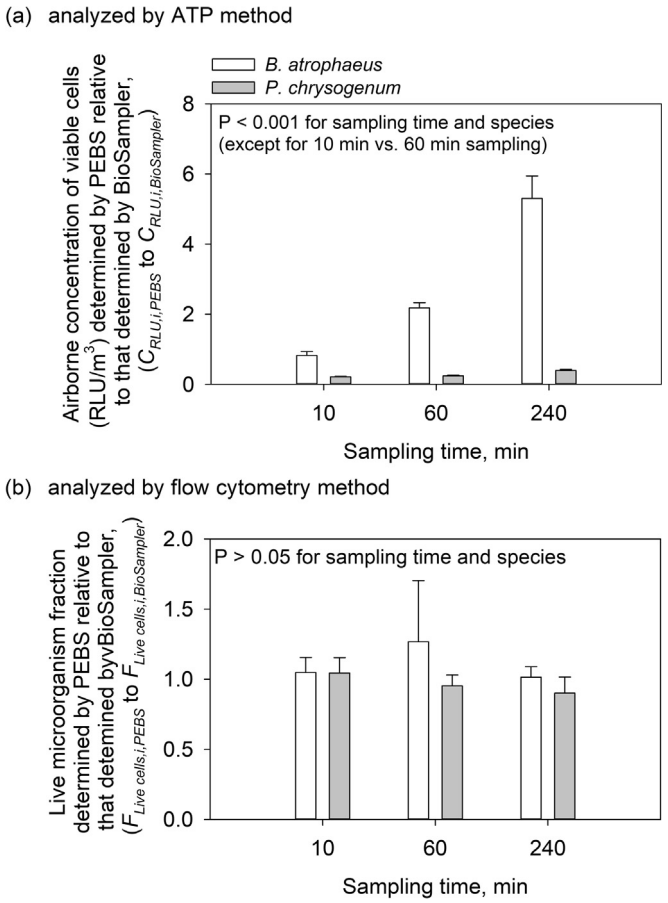

Fig. 7. Relative viability of samples collected by PEBS and presented as (a) a ratio of airborne ATP concentrations expressed as RLU/L relative to those determined by BioSampler (b) the fraction of live cells measured by flow cytometry relative to that in BioSampler samples. The ratios were determined 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.

3.3. PEBS performance when measuring culturable microorganisms

Airborne concentrations of culturable *B. atrophaeus* and *P. chrysogenum* determined by PEBS (expressed as CFU/L, as per Eq. (5)) after 10, 60, and 240 min sampling were compared to those determined by BioSampler as per Eq. (11) and are presented as relative culturability of PEBS samples in Fig. 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 min 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 between the two microorganisms 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 hardy when it comes to their sampling (Morris, Kokki, Anderson, & Richardson, 2000). This result for *P. chrysogenum* in Fig. 8 seems to echo the result presented in Fig. 7a, where the lower relative viability of *P. chrysogenum* was observed when the sample was analyzed by ATP signal – signal produced by viable microorganisms. One possible explanation could be the rugose surface of the spores (Afanou et al., 2014), where the surface wrinkles and ridges result in strong local electrostatic fields when the spores are deposited on the collection electrode thus resulting in their loss of viability (Fig. 7a) and culturability (Fig. 8). This phenomenon definitely warrants further investigation into the effect of electrostatic fields on the culturability and viability of fungal spores and whether the effect depends on fungal species and their surface structure.

The main goal of this work was to advance further the development of a new personal sampler for bioaerosols and investigate its

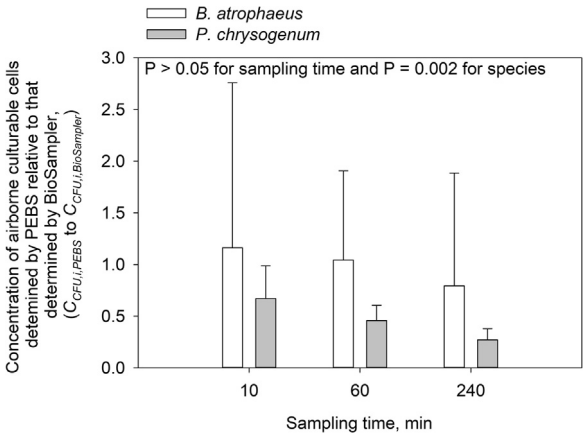

Fig. 8. Relative culturability of microorganism samples collected by PEBS: presented as a ratio of airborne CFU concentration (CFU/L) determined by PEBS to that determined by BioSampler for three sampling times (10, 60, and 240 min). The experiments were performed with biological particles, a) *B. atrophaeus* 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.

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 h when operating at a 10 L/min sampling flow rate. Also, due to its unique charger design, it produces very low ozone concentrations on absolute and relative scale (e.g., less than 7 ppb) compared to other electrostatic collectors (Cardello, Volckens, Tolocka, Wiener, & Buckley, 2002; Kaupp & Umlauf, 1992; Sillanpää, Geller, Phuleria, & Sioutas, 2008), 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 provide are 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 environments. The used voltage settings for charging and collection yielded satisfactory viability and culturability data; however future work will consider lower of voltage settings for the collector (e.g., −3 kV) to see if culturability of samples could be increased further.

4. Conclusions

In this study, we determined the physical and biological performance of PEBS using different sample analysis techniques and compared performance of PEBS against that against of BioSampler. PEBS exhibited physical collection efficiency of approximately 80% when collecting biological particle for up to 240 min. When compared with BioSampler, it showed higher airborne particle concentrations, while the biological performance of the two samplers in terms of the physiological state of captured microorganisms was not statistically different for *B. atrophaeus* and *P. chrysogenum* test species. Based on the results presented here, we conclude that PEBS is an efficient sampler for bioaerosols and that it is compatible with various sample analysis techniques. This technology will be able to provide personal bioaerosol exposure data in various environments. Future studies will investigate the performance of the sampler in the field.

Acknowledgment

The publication was supported by the grant R2110560 “Personal Electrostatic Bioaerosol Sampler (PEBS) with High Sampling Flow Rate” and the grant R01OH009783 “Design of Advanced Electrostatic Sampler for Total Bioaerosols” from CDC-NIOSH. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the CDC-NIOSH.

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