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Development and optimization of Stationary Electrostatic Bioaerosol Sampler (SEBS) for viable and culturable airborne microorganisms

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ABSTRACT

This study focused on developing and testing a stationary electrostatic bioaerosol collector with a high concentration rate while preserving viable and culturable microorganisms. Using our earlier advances in the electrostatic collection of airborne microorganisms, we designed and optimized a Stationary Electrostatic Bioaerosol Sampler (SEBS), which incorporates our previously developed wire-to-wire charger and a newly-designed removable particle collector. The sample elution system was also redesigned and optimized to achieve a practical solution yielding a high sample concentration rate. The sampler's collector and its hydrophobic coating methods, collection voltages, and material of sample removal tubes were optimized through an iterative process. SEBS is a two-stage electrostatic sampler with a wire-to-wire charger and a stainless steel collection electrode coated using polydimethylsiloxane (PDMS) coating technique and ultraviolet/ozone surface treatment method. The entire collected sample is eluted into 0.2 or 1 mL of sterile phosphate-buffered saline (PBS) using a customized particle removal system made of glass. The developed SEBS was operated at 20 L/min; its physical and biological performance was compared to the Button filter sampler (SKC, Inc.) when sampling two airborne bacteria and two fungi in the laboratory. On average, SEBS showed actual physical collection efficiency of ~50% when samples were eluted into 0.2 mL; the efficiency increased to ~75% when 1 mL elution liquid was used. The average relative viability efficiency reached ~80%, suggesting that the sampling stress was reduced compared to the filter sampler. The relative culturability efficiency was ~60%. The use of 0.2 mL elution liquid resulted in a sample concentration rate of ~5×10^4 min^{-1}, which will enable faster detection and determination of viable and culturable bioaerosols, especially when sampling in low concentration environments. Future studies will evaluate SEBS's performance and utility in field studies.

1. Introduction

Airborne microorganisms (i.e., bioaerosols) are ubiquitous in our environment and daily life (Madhwal, Prabhu, Sundriyal, & Shridhar, 2020). In addition to benign species, some bioaerosols could cause negative human health effects, including respiratory and pulmonary diseases (Górny, 2020; Humbal, Gautam, & Trivedi, 2018). The role of bioaerosols in causing negative health effects has been known for years due to infectious viruses such as Ebola, MERS-CoV, as well as other bioaerosol particles and complex bioaerosol

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mixtures, including viable and non-viable microorganisms, their components, and metabolites (Douwes, Thorne, Pearce, & Heederik, 2003; Griffiths & DeCosemo, 1994; Mandal & Brandl, 2011). The COVID-19 pandemic caused by the SARS-CoV-2 virus, however, brought the role of bioaerosols in infectious disease transmission into the fore of our daily lives (Shammi, Rahman, & Tareq, 2021; Noorimotlagh, Jaafarzadeh, Martínez, & Mirzaee, 2020).

Dose-response relationships for bioaerosol exposures are complex and not yet well understood (Srikanth, Sudharsanam, & Steinberg, 2008; Pearson et al., 2015). One of the obstacles to establishing such a relationship is a lack of performance standards and varying performance among existing bioaerosol sampling and analysis technologies. Therefore, collecting and analyzing representative bioaerosol samples is critical for developing and understanding dose-response relationships (Brągoszewska, 2020; Dean & Mitchell, 2020). To this end, the bioaerosol monitoring system should quantify total, viable, and culturable bioaerosol fractions (Cho et al., 2020). Various devices, including filters, impactors, impingers, and electrostatic precipitators, have been used for bioaerosol sampling (Mainelis, 2020; Kabir et al., 2020; Xu et al., 2011). Depending on the study design and detection limit of the selected analytical tool, each method or device has to collect a certain amount or concentration of biological material for a quantifiable analysis; this means that the chosen collectors have to operate at a certain flowrate and collection time or be able to provide a sufficiently concentrated sample. Also, the analysis of viable (metabolically or physiologically active yet not culturable on media) or culturable microorganisms presents an additional challenge due to sample desiccation, poor sample recovery, exposure to ozone, and other environmental and sampling stressors (Han & Mainelis, 2012; Mainelis et al., 1999; Nevalainen, Pastuszka, Liebhaber, & Willeke, 1992; Polydorou, Halili, Wittmer, Pelz, & Hahn, 2012). Despite recent advances in bioaerosol sampling, there is still a lack of samplers that facilitate bioaerosol detection by utilizing a high sample concentration rate while at the same time preserving biological particle viability and culturability (Han & Mainelis, 2008; Han, Zhen, Fennell, & Mainelis, 2015).

Over the past years, several electrostatic precipitators (ESPs) have been used for sampling of non-viable or non-culturable bioaerosols, typically at relatively low sampling flow rates (e.g., less than 10 L/min) (Roux, Kaspari, Heinrich, Hanschmann, & Grunow, 2013). Since preserving microbial viability/culturability in such samplers is not the main concern, developers focused on collection efficiency and efficient sample elution. Because electrostatic samplers feature lower pressure drop, impaction stress (Mainelis et al., 1999), and power consumption (Tan, Shen, Yao, & Zhu, 2011) compared to conventional inertia or filtration-based bioaerosol samplers, they could be highly suitable to capture and analyze not only total but also culturable and viable bioaerosols. At the same time, it has long been known that electrostatic stresses (e.g., electrostatic discharge, ozone production, and electrostatic field) during the collection process may affect sample viability/culturability (Ichim, Creanga, & Rapa, 2007). Therefore, rather few ESPs are available to capture and preserve culturable bioaerosols. For example, the efficiency of the electrostatic device (Gast, Mitchell, & Holt, 2004) when capturing culturable microorganisms was similar to that of an impaction sampler (SAS Super 90, Bioscience International, Rockville, MD). The Electrosampler (Yao & Mainelis, 2006) measured 9× higher culturable bioaerosol concentration than the BioStage impactor (SKC, Inc., Eighty Four, PA); however, this sampler collects aerosols on an agar medium, and that limits their analysis to culture-based methods. An automated electrostatic sampler was also developed and integrated with a peristaltic pump and microfluidic channel for near-real-time analysis, but the recovery of culturable samples was about 2–3 times lower than in the Button sampler (Tan et al., 2011). A recently developed Personal Electrostatic Bioaerosol Sampler (PEBS) showed similar or higher airborne concentrations of culturable bacteria compared to BioSampler (SKC, Inc.), but lower airborne concentrations of culturable spores (Han, Thomas, & Mainelis, 2018).

One of the performance metrics of bioaerosols samplers is their concentration rate (a ratio of particle concentration in collection liquid versus the airborne particle concentration per time unit) since highly concentrated samples allow the detection and quantification of low bioaerosol levels. In our earlier work, we presented Electrostatic Precipitator with Superhydrophobic Surface (EPSS), which achieved a concentration rate as high as $\sim 1 \times 10^6$ (Han, An, & Mainelis, 2010; Han & Mainelis, 2008; Han, Nazarenko, Lioy, & Mainelis, 2011), yet it provided low recovery of culturable organisms (Han, Zhen, et al., 2015). Due to its high concentration rate, the EPSS technology could be used in low concentration environments or as a sentry to quickly detect intentionally released airborne biological agents. However, additional research was needed to advance this sampling concept to collect and detect viable and culturable microorganisms.

Thus, the main goal of this study was to utilize the EPSS concept and develop a novel Stationary Electrostatic Bioaerosol Sampler (SEBS) that would provide a high sample concentration rate while preserving sample viability and culturability. This paper describes the iterative design process for SEBS and its physical and biological efficiency when collecting airborne bacteria and fungi in a laboratory setting. We investigated and modified the sampler's collection electrode, its surface coating type, collection voltage, and sample elution options during this process. This laboratory evaluation of SEBS was carried out with commonly used Gram-positive bacteria of *Bacillus atrophaeus,* Gram-negative bacteria of *Pseudomonas fluorescens*, and fungal spores of *Penicillium chrysogenum and Penicillium melinii*. The physical and biological performance of SEBS was compared against filter samplers due to their high collection efficiency.

2. Materials and methods

2.1. Design features of Stationary Electrostatic Bioaerosol Sampler (SEBS)

SEBS was developed to have high biological collection efficiency while also maintaining high physical collection efficiency. It incorporates several developments from our earlier work, including a wire-to-wire charger (Han, Thomas, & Mainelis, 2017) with low ozone production (<10 ppb) and a collection electrode coated with a hydrophobic substance (Han, Zhen, et al., 2015). SEBS is a two-stage electrostatic precipitator consisting of an omnidirectional inlet, a charging section, a transition section, and a collection

section (Fig. 1). The collection section consists of a stainless steel removable collection rod (either a rectangular electrode with dimensions 7/64 × 7/64 × 5.1 inches or a rod 3/16 inches in diameter and 5.1 inches in length) and two grounded stainless steel plates having the shapes of the quarter cylinder (0.78 × 1.5 × 0.002 inches). The core part of SEBS has a shape of a cylinder of 2.54 cm (1 inch) in diameter and ~21.2 cm (8.35 inches) in length. The omnidirectional inlet minimizes the wind effect on the aspiration efficiency. A high-voltage power supply provides the charging and collection voltages, while the airflow is provided by an air mover.

Once the sampling is completed, the collection electrode is removed and transferred into a sample removal system (Fig. 2), and the sample is eluted as described below.

2.2. Preparation of test particles

The physical and biological performance of SEBS was determined using four microorganisms: gram-positive *Bacillus atrophaeus* (ATCC 49337, American Type Culture Collection, MD) and gram-negative *Pseudomonas fluorescens* (ATCC 13525) bacterial cells, and *Penicillium chrysogenum* (ATCC 10135) and *Penicillium melinii* (ATCC 10469) fungal spores. Not all experiments were performed with all microorganisms. These microorganism species are often used in bioaerosol studies (Han et al., 2010; Han et al., 2011; Hill et al., 1999; Johnson, Martin, & Resnick, 1994; Nadkarni, Martin, Jacques, & Hunter, 2002). We measured the aerodynamic diameter of several of these organisms in our earlier studies. The diameters were approximately 0.89 μm (*B. atrophaeus*), 0.82 μm (*P. fluorescens*), and 2.1 μm (*P. melinii*) (Han and Mainelis, 2010; Han et al., 2011). In another set of experiments, we measured the size distribution of aerosolized *Penicillium chrysogenum* spores using Grimm OPC (Han et al., 2018), and the spore mode diameter was 2.5 µm. The bacterial cells were inoculated in Nutrient Broth (Becton, Dickinson and Co., Sparks, MD) and incubated for 18 h at 30 °C. All cells were washed four times by repeated centrifugation for 5 min at 7000 rpm (BR-4 centrifuge, Jouan, DEC Inc., Lorton, VA) and resuspended in 20 mL of sterile PBS (Han et al., 2017, 2018). The fungal spores were streaked on Malt Extract Agar (Becton, Dickinson, and Co.) and incubated for 7 day at room temperature before sampling (Han et al., 2010; Han et al., 2011). The spores were harvested by adding sterile Milli-Q water (EMD Millipore Corp., Billerica, MA), gently scraping the surface with a scraper, and then filtering the suspension using a sterilized fabric screen. The final liquid suspensions were diluted as needed to obtain airborne cell concentration of ~1×10^7 cells/m^3 as determined by a Grimm optical particle counter (OPC) (model 1.108, Grimm Technologies Inc., Douglasville, GA, USA) connected to

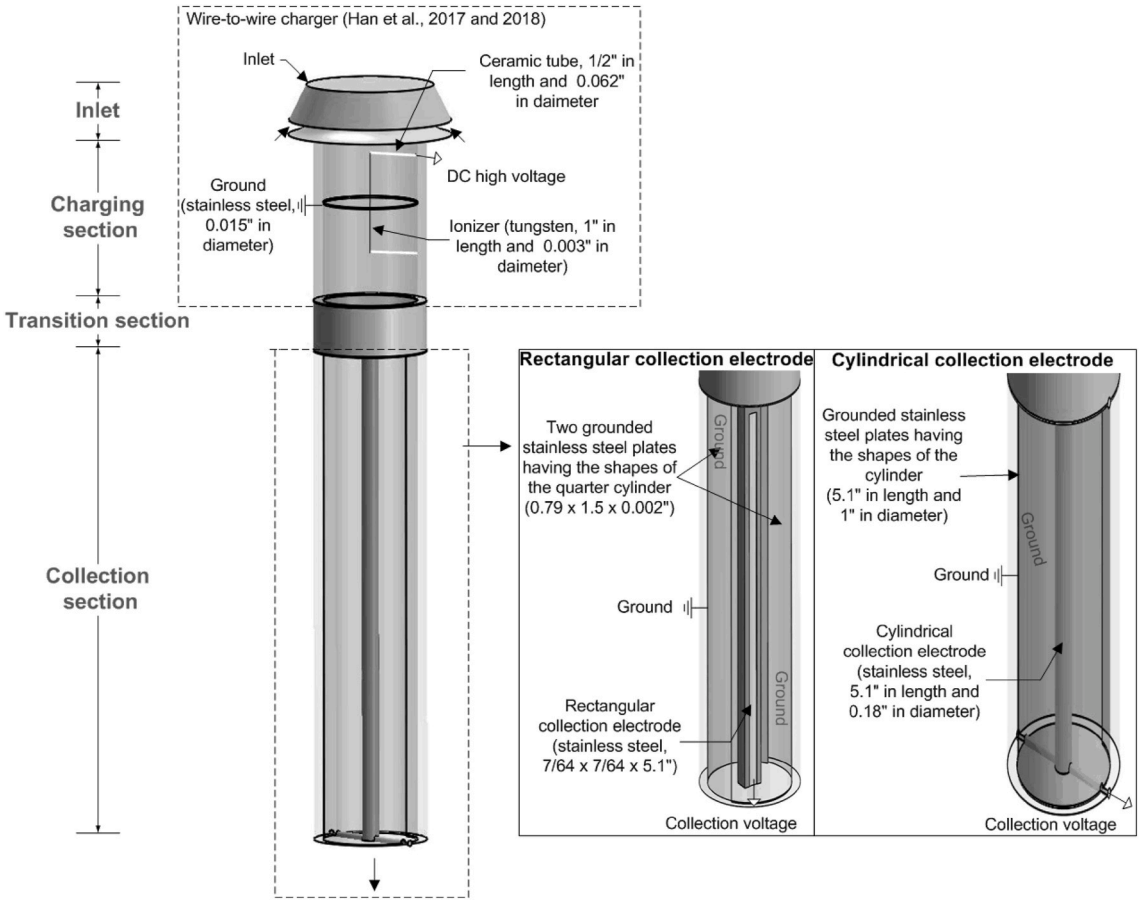

Fig. 1. Schematic diagram of the Stationary Electrostatic Bioaerosol Sampler (SEBS) with a wire-to-wire charger.

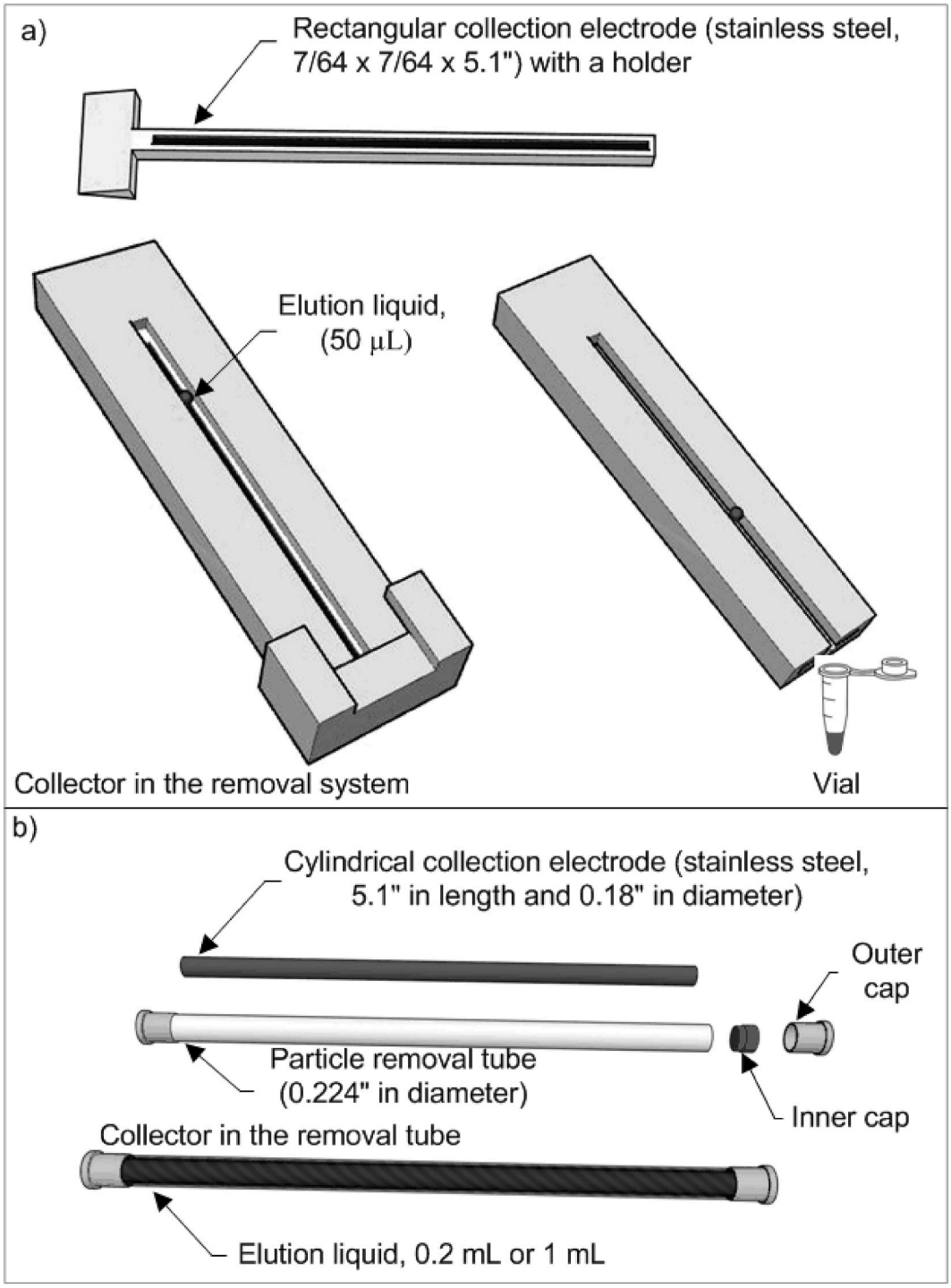

Fig. 2. A system to remove particles collected by SEBS: a) for a rectangular collection electrode and b) for a cylindrical collection electrode.

an isokinetic probe (Apex Instruments Inc., Fuquay-Varina, NC). A fresh liquid suspension of 10 mL of each species was prepared for each test.

2.3. Experimental setup for testing SEBS in laboratory

The test system was based on a previous study (Han et al., 2017) consisted of a flow controller, particle generators, air-particle

mixing elements (i.e., mixing boxes and a static blender), a flow straightener, a test chamber, and a particle counter (Fig. 3) (). The test chamber was housed inside a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN). A three-jet Collison nebulizer (Mesa Laboratories Inc., Butler, NJ) with a polycarbonate jar was used to aerosolize biological 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 airflow, Q_d (10 L/min) (Han, Zhen, et al., 2015; Zhen, Han, Fennell, & Mainelis, 2013). The polycarbonate jar was used to minimize damage to the microorganisms (Zhen et al., 2013, 2014).

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. Such a neutralizer is effective in substantially reducing particle charge. For example, in our measurements with 1 μm polystyrene latex (PSL) particles, freshly generated aerosol induced 58.3 fA current in an electrometer (TSI Inc., Shoreview, MN) when no neutralizer was used. Using a 1-year-old neutralizer reduced the current to 3.1 fA, while a new neutralizer further reduced the current to 1.5 fA, a more than 97% reduction.

A HEPA-filtered and humidity-conditioned dilution airflow, Q_D (90 L/min), was provided by an air compressor (CAT-8010DSPC, California Air Tools, Inc., San Diego, CA). The samplers were positioned four duct diameters downstream of the flow straightener to have a uniform cross-sectional profile of test particles (Han et al., 2017). To check the uniformity of the cross-sectional particle profile in the sampling location, two empty tubes with the same diameter as the inner diameter of SEBS were positioned side by side and in preparatory experiments measured particle number concentrations using a Grimm OPC; they agreed within 3.2 ± 0.5% for *B. atrophaeus*.

In the iterative part of SEBS development, an isokinetic probe with a 25 mm filter holder (VWR Corp., Radnor, PA) was used as a reference, and it sampled air at 2 L/min. The completed SEBS was compared against a Button sampler (SKC, Inc.) operated at its nominal 4 L/min sampling flow rate. The probe was positioned 1 duct diameter upstream of SEBS, and the Button sampler was positioned side by side along with SEBS. The two reference samplers were used with 1-μm-pore 25 mm PTFE membrane filters (Pall Inc., East Hills, NY, USA). SEBS's inlet in the test section was perpendicularly oriented to the airflow (Fig. 3). SEBS was operated at a sampling flow rate of 10 or 20 L/min (Q_S) provided by a vacuum pump. Sampling time was 10 min for all samplers. The ozone concentration downstream of SEBS was measured using a UV photometric ozone monitor (Model 202, 2B Technologies Inc., Boulder, CO) (Fig. 3).

2.4. Optimization and testing protocol

Performance of SEBS was iteratively optimized by adjusting the following parameters:

Step 1: Collection electrode configuration and its hydrophobic coating method

The following four collection electrodes were fabricated and tested:

- R–H: a rectangular collection electrode coated by a commercially available superhydrophobic spray (HIREC-1450, NTT Corporation Inc., Japan).
- R–P: a rectangular collection electrode coated by polydimethylsiloxane (PDMS) and treated with ultraviolet light and ozone (Kim et al., 2008; Lee et al., 2006).
- C–H: a cylindrical (rod-shaped) collection electrode coated by HIREC-1450.
- C–P: a cylindrical (rod-shaped) collection electrode coated by PDMS.

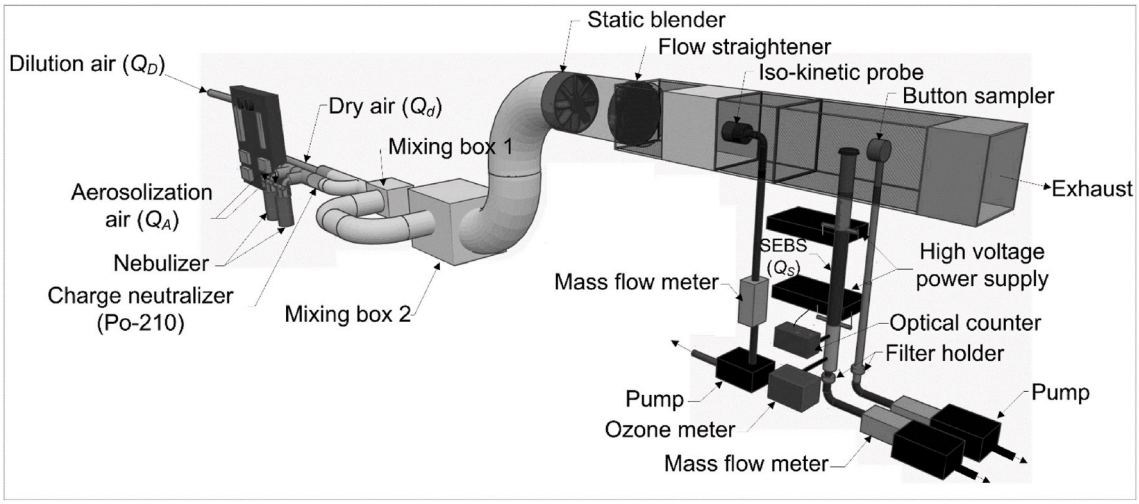

Fig. 3. Schematic diagram of the experimental setup.

The coating of the collection electrode with HIREC-1450 was described previously (Han & Mainelis, 2008; Han, Nazarenko, Y., Lioy, P. J., &; Mainelis, 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. For the PDMS coating method, the electrode was first sonicated with isopropyl alcohol (Sigma-Aldrich Inc., St. Louis, MO) for 10 min and then dried in an oven at 60°C; next, the electrode was exposed to UV-C (254 nm) for 3.5 h, soaked in 0.5 wt% Aminopropyl triethoxysilane (APTES) (Sigma-Aldrich Inc.) and rinsed with DI water (Millipore Corp., Billerica, MA); finally, poly(dimethylsiloxane), monoglycidyl ether terminated (Sigma-Aldrich Inc.) was applied the electrode and annealed in the oven at 80°C for 4 h.

Post-sampling, electrodes were removed from SEBS, and the collected samples were eluted according to the type of the collection electrode. For example, the rectangular collection electrode was transferred into the removal system (Fig. 2a), and the cylindrical collection rod was moved into a polycarbonate removal tube (Fig. 2b). For the rectangular collection electrode, 0.05 mL of PBS (phosphate-buffered saline) (Sigma-Aldrich Inc.) was manually injected at the top of the inclined removal system. The droplet rolled down, picking up the deposited particles. The procedure was repeated for the other electrode side, resulting in 0.1 mL of elution liquid. For the cylindrical collection electrode, 0.2 mL PBS was added to the elution tube, and the particles were eluted by vortexing the tube for 1 min at the maximum speed. Post-elution, PBS was added to both samples to increase sample volume to 5 mL for subsequent analyses.

This development step used a filter with an isokinetic sampling probe as a reference sampler. First, particles collected on the reference filter were eluted into 5 mL of PBS using a previously described procedure (Han, Zhen, et al., 2015; Wang, Reponen, Grinshpun, Górny, & Willeke, 2001). Then, all resulting 5 mL samples were equally subdivided into five 1 mL microcentrifuge vials to be analyzed by acridine orange epifluorescence microscopy (AOEM) or direct microscopic counting and adenosine triphosphate (ATP)-based bioluminescence.

Tests for this step were carried out with *B. atrophaeus* bacteria and *P. chrysogenum* fungal spores. SEBS was operated at a 20 L/min sampling flow rate and at fixed charging/collection voltages of +5.25 kV/−7 kV. The charging voltage was selected based on our earlier study (Han et al., 2017), and the collection voltage of −7 kV was selected in a preliminary investigation. With this step, we finalized the core parts of SEBS.

Step 2: Collection voltage (−kV)

Once the collection electrode design and coating method were selected, we determined SEBS' performance when sampling airborne *B. atrophaeus* and *P. fluorescens* bacteria cells and *P. chrysogenum* fungal at 1) −4, −7, and −8 kV collection voltages and 2) 10 and 20 L/min sampling flow rates. The charging voltage was fixed at +5.25 kV. In this step, the Button sampler was used as a reference sampler.

Step 3: Material for the sample elution tube

Four different materials were examined for the sample elution tube: polycarbonate, Delrin, Teflon, and glass (McMaster-Carr Co., Elmhurst, IL). First, the selected collection electrode design (determined in Step 1) was placed into the elution tube. Next, the sample was eluted by adding 1 mL of PBS into the tube and vortexing it for 1 min. Then, to determine wall losses in the elution tube and particles remaining on the collection rod, 1 mL of PBS was added to the tube (*sans* electrode), and the tube alone was vortexed for 1 min. Finally, the particles remaining on the collection rod were thoroughly removed by wiping the rod at least three times with a clean 25 mm PTFE filter (Pall Inc.) and then eluting particles from the filter as described above; for the removal, the collection rod was immobilized by tweezers, and the filter was held by another set of tweezers. These tests were carried out with SEBS collecting *P. melinii* fungal spores at 20 L/min. The particle concentration in each of the three fluids was determined by direct microscopy. We used fungal spores because their surface removal is more challenging than for bacteria (Han et al., 2011). A fixed charging voltage of +5.25 kV and an optimal collection voltage determined in Step 2 were applied.

Step 4: SEBS performance

Once all optimization steps were completed, the final version of SEBS was operated concurrently with a Button filter sampler. Its physical and biological performances relative to the Button sampler were determined based on the procedures described below. These tests were performed with all four microorganisms.

2.5. Analytical methods to determine the physical and biological performance of SEBS

2.5.1. Methods to evaluate physical performance

The physical collection efficiency was determined by comparing particle number concentrations downstream of the collector with its charging/collection voltages ON and OFF using a Grimm OPC (Han et al., 2017). This method allows for a quick examination of the collector's performance, and it was used as a reference when analyzing the effect of removal tube material (Fig. 6). For the rest of the experiments, either a filter sampler with an isokinetic probe or a Button sampler (SKC Inc.) was used as a reference. The collected and eluted bacterial cells and fungal spores were counted using epifluorescence microscopy and direct light microscopy, respectively, as described elsewhere (Han et al., 2018). The eluted bacteria were diluted (if necessary), stained, filtered, and then counted. The cell concentration in each sample, C_{sample}, was calculated as follows:

$$C_{sample} = \mathrm{n} \times X \times D,\ \mathrm{mL}^{-1}, \quad [1]$$

where n is the average bacteria count per microscope view field, X is the number of fields viewed in the entire filter ($X = 6125$ for a 25 mm filter), and D is the dilution factor.

Fungal spores were counted using a hemocytometer (Hausser Scientific Company, Horsham, PA) (Freimoser, Jakob, Aebi, & Tuor, 1999), and the concentration of spores in the elution liquid was determined as:

$$\mathrm{C}_{sample} = n \times 10^3,\ \mathrm{mL}^{-1}. \quad [2]$$

Here, n is the average number of spores in the large four corner squares and the center square of the hemocytometer, and 10^3 is a conversion factor.

The airborne number concentration of cells or spores, $C_{N,i}$ (#/m^3), for a particular sampler (e.g., SEBS or reference) was determined as follows:

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

where V_s is the entire sample volume in mL, Q is the sampling flow rate in m^3/min, t is the sampling time in min, and i is either bacterial cells or fungal spores.

2.5.2. Methods to determine biological performance

a) *Cell/spore viability*. The viability of bacterial cells and fungal spores in samples was determined using ATP analysis following earlier published procedures (Han, Wren, DuBois, Therkorn, & Mainelis, 2015; Seshadri, Han, Krumins, Fennell, & Mainelis, 2009). The luminescence intensity of an aliquot taken from the eluted sample was measured using a luminometer (model 20/20n, Turner Biosystems Inc., Sunnyvale, CA), and it yielded the result as relative luminescence units (RLU).

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

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

where RLU is luminescence intensity, V_a is the sample aliquot volume used for analysis (0.1 mL), V_s is the eluate volume in mL (5 mL), Q is the sampling flow rate in m^3/min, t is the sampling time in min, and i is either cells or spores.

b) *Cell/spore culturability*. The concentration of culturable cells and spores in each sample was determined by plating 0.1 mL of the eluate in triplicate on Nutrient Agar (NA; Difco, Becton, Dickinson and Co., Sparks, MD) and Malt Extract Agar (MEA; Difco, Becton, Dickinson, and Co.), and incubating the plates at 30 °C and room temperature respectively. The bacterial colonies were counted every 24 h for 72 h, and fungal colonies were counted every 24 h for 120 h. The airborne CFU concentration $C_{CFU,i}$ (CFU/m^3) determined by a particular sampler was calculated as follows:

$$C_{CFU,i} = \frac{\frac{CFU}{V_a} \times V_s}{Q \times t}, \quad [5]$$

where CFU is the average number of colony forming units per plate, V_a is the aliquot volume used for analysis (0.1 mL), V_s is the entire eluted sample volume in mL, Q is the sampling flow rate in m^3/min, t is the sampling time in min, and i is either cells or spores.

2.6. Performance of SEBS relative to reference samplers

The relative actual collection efficiency of SEBS (R_{CE}) was determined by comparing the airborne particle concentration determined by SEBS relative to a reference sampler:

$$R_{CE} = \frac{C_{N,SEBS}}{C_{N,Reference}} \quad [6]$$

In Step 3, the actual physical collection efficiency of SEBS was determined by comparing the airborne particle concentration determined by SEBS with the particle concentration downstream of SEBS with voltages OFF as measured by an OPC.

$$R_{CE\_OPC} = \frac{C_{N,SEBS}}{C_{N,\ OPC}} \quad [7]$$

where $C_{N,OPC}$ is the airborne particle concentration measured by the OPC.

The concentration rate of SEBS, R_C (min^{-1}), was calculated using the sampler's operational parameters (Han & Mainelis, 2008):

$$R_C = \frac{Q_S}{V_{WD}} \times R_{CE}, \quad [8]$$

where Q_s (m^3/min) is the sampling flow rate, V_{WD} (m^3) is the volume of elution liquid, and R_{CE} is the collection efficiency.

Similar to the relative physical efficiency, we also determined the relative biological efficiencies of SEBS based on ATP and culture measurements. Here, the airborne ATP and CFU concentrations determined by SEBS were compared to those determined by reference samplers.

For the ATP method,

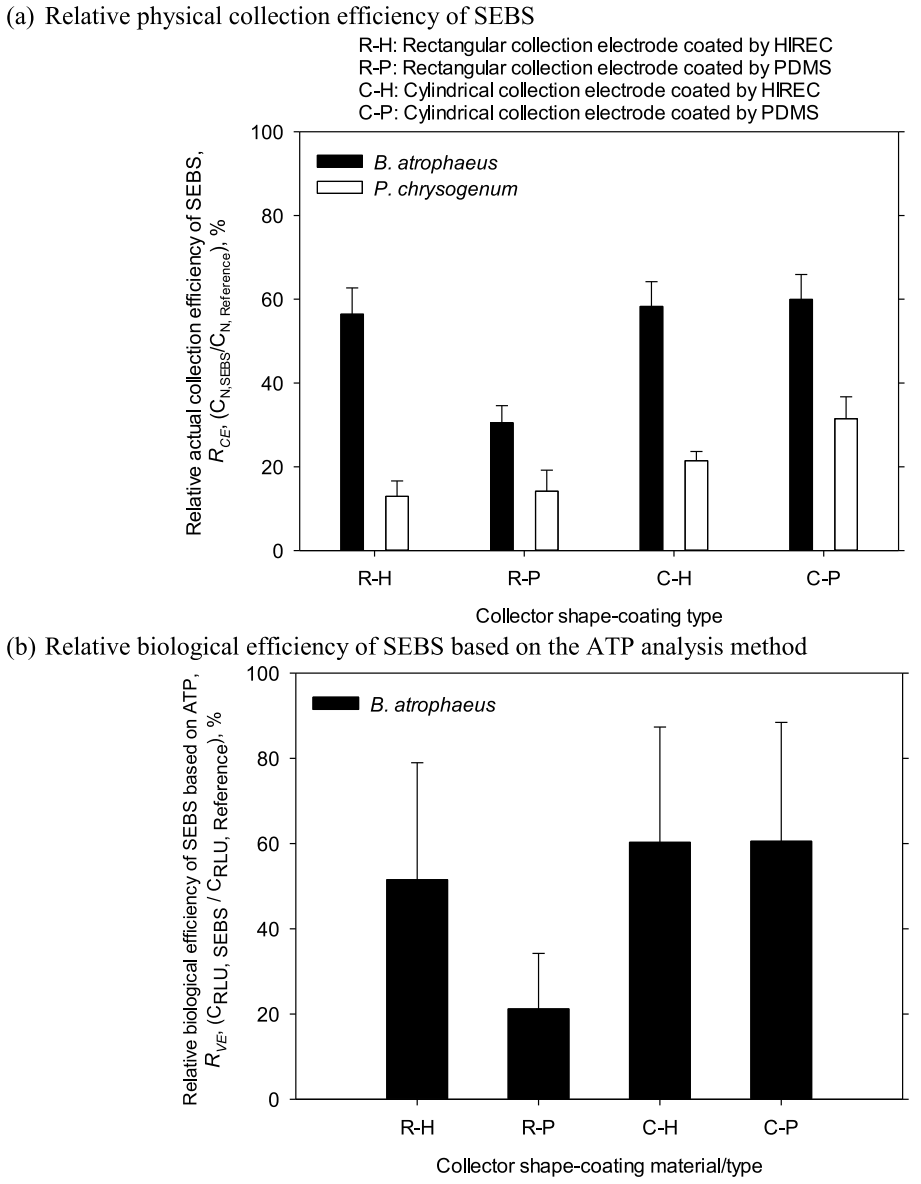

Fig. 4. Performance of SEBS as a function of collector electrode configuration (rectangular electrode and cylindrical rod) and electrode coating material/method (HIREC-1450 and PDMS). a) Relative physical collection efficiency when sampling *B. atrophaeus* cells and *P. chrysogenum* spores; the efficiency was determined by comparing the concentration of airborne particles (N/m^3) determined by SEBS with that determined by a filter sampling through an isokinetic probe. b) Relative viability efficiency when sampling *B. atrophaeus* cells; the efficiency was determined by comparing the airborne ATP concentration (RLU/m^3) determined by SEBS with that determined by a filter sampling through the isokinetic probe. SEBS samples were eluted with 0.2 mL of elution liquid.

SEBS operated at a 20 L/min sampling rate and +5.25 kV/−7 kV charging/collection voltages. The filter was concurrently operated at 2 L/min. The test particle concentration was $\sim 4 \times 10^4$/L. Each data point is an average of at least three repeats, and the error bars represent standard deviations.

$$R_{VE} = \frac{C_{RLU,SEBS}}{C_{RLU,Reference}} \text{ (subscript VE stands for viability efficiency)} \quad [9]$$

For culture method:

$$R_{CUE} = \frac{C_{CFU,SEBS}}{C_{CFU,Reference}} \text{ (subscript CUE stands for culturability efficiency)} \quad [10]$$

2.7. Statistical analysis

The physical and biological performances were analyzed as a function of collection electrode shape, electrode's coating method, collection voltage, sampling flow rate, and microorganism species using one- or two-way ANOVA (Sigmaplot 2011; Version 12.3, Systat Software Inc., San Jose, CA). The differences between individual pairs of variables were examined using the Holm-Sidak method, which considers multiple comparisons. The $p < 0.05$ was considered significant at $\alpha = 0.05$.

3. Results and discussion

3.1. Physical performance

Fig. 4 shows the relative physical and biological collection efficiencies of SEBS as a function of collector configuration and hydrophobic coating method when sampling *B. atrophaeus* bacterial cells and *P. chrysogenum* fungal spores. The concentrations of airborne bioaerosols were ~10^7/m^3. Fig. 4a shows the relative actual physical collection efficiency, R_{CE} (Eq. (6)). The R_{CE} determined for four different collector configurations (i.e., R–H, R–P, C–H, and C–P) when capturing *B. atrophaeus* ranged from 30.5 ± 4.1% (R–P)

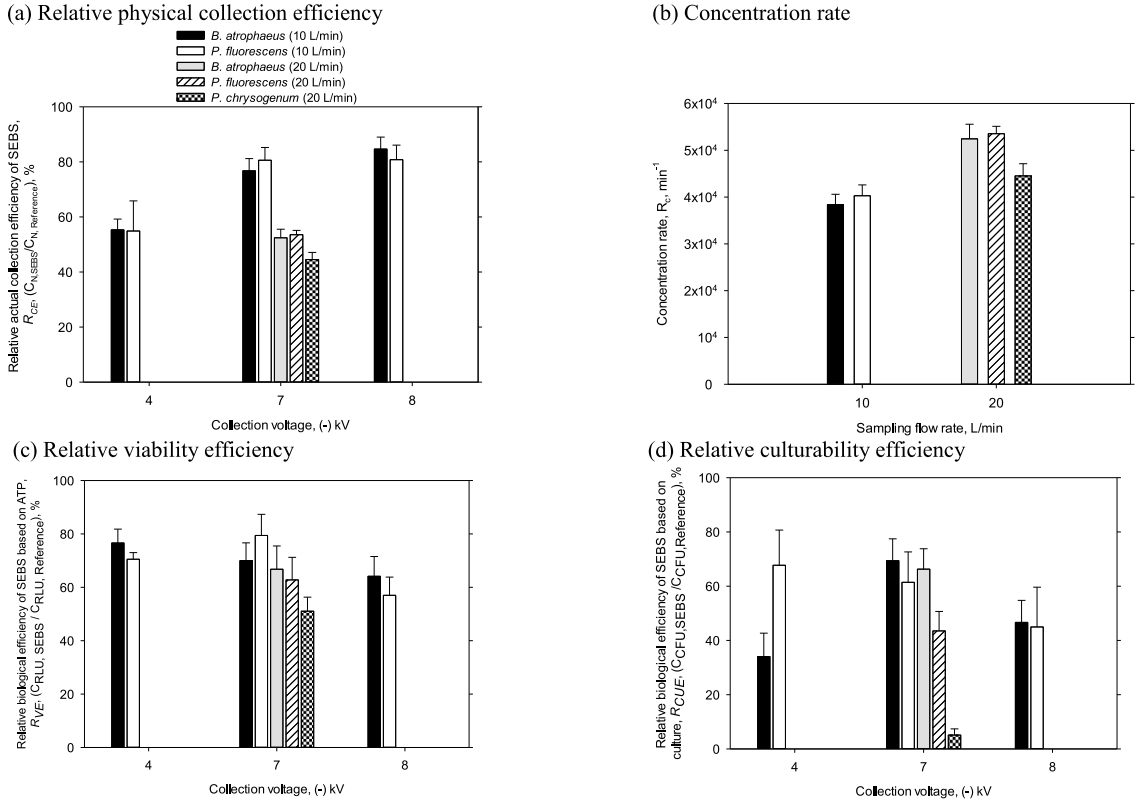

Fig. 5. Performance of SEBS as a function of collection voltage and sampling flowrate when sampling different microorganisms. a) Relative physical collection efficiency; the efficiency was determined by comparing the concentration of airborne particles (N/m^3) determined by SEBS with that determined by the Button filter sampler. b) Concentration rate calculated according to Eq. 8. c) Relative viability efficiency; the efficiency was determined by comparing the airborne ATP concentration (RLU/m^3) determined by SEBS with that determined by Button filter sampler. d) Relative culturability efficiency; the efficiency was determined by comparing the airborne culturable microorganism concentration (CFU/m^3) determined by SEBS with that determined by the Button filter sampler.
SEBS samples were eluted with 0.2 mL of elution liquid. SEBS operated at +5.25 kV charging voltage. The test particle concentrations were ~2 × 10^4/L. Each data point is an average of at least three repeats, and the error bars represent standard deviations.

to 60.0 ± 5.9% (C–P). For *P. chrysogenum*, R_{CE} ranged from 14.2 ± 5.0% (R–P) to 31.5 ± 5.2% (C–P). Table S1 shows the results for each electrode configuration. The collection efficiency for fungal spores was lower for all electrode configurations, most likely due to the hydrophobicity of fungal spores and their less effective removal, as was observed in our earlier work (Han et al., 2011). Based on the one-way ANOVA, for *B. atrophaeus*, the collection efficiency with R–P configuration was significantly lower than that for other configurations ($p < 0.001$); for *P. chrysogenum*, the collection efficiencies were statistically significantly different ($p = 0.002$) for the following pairs: R–H vs. C–P and R–P vs. C–P. Overall, the lowest average collection efficiency for both microorganisms, i.e., 27.8%, was observed with the R–P configuration. Most likely, because for this configuration, it was hard to achieve an even coating of the rectangular stainless steel electrode and the adjoining plastic holder using PDMS (Fig. 2a). As a result, the rolling droplet didn't remove the deposited particles effectively. The average emitted ozone concentration was 1.6 ± 0.8 ppb. During each test, the temperature in the test chamber stayed between 26 and 28°C, and the relative humidity ranged from 42% to 51%.

Fig. 4b shows the relative biological efficiency of SEBS as a function of collector electrode configuration, determined using *B. atrophaeus* cells and ATP-bioluminescence. The pattern of the relative biological efficiency follows that of the physical efficiency, with the R–P configuration yielding the lowest value (21%; $p < 0.05$ compared to other configurations) and the other configurations being similar (52–61%; $p = 0.242$). The similarity of patterns in Fig. 4a and b suggest that the viability of *B. atrophaeus* is not affected strongly by the collector configuration and is governed primarily by the physical collection and sample elution efficiencies.

After considering the results shown above and the collection electrode design and coating challenges, the C–P electrode (a rod with PDMS coating) was selected for further experiments.

In the next step, we investigated the efficiency of SEBS at different collection voltages (i.e., −4, −7, and −8 kV) while the charging voltage was fixed at +5.25 kV (Fig. 5). Since the sampling flow rate affects particle residence time inside the charging and collection sections of SEBS, it also affects the charging and physical collection efficiency and, in turn, the concentration rate; plus, the residence time could also affect particle viability and culturability. Therefore, at –7kV collection voltage, 10 and 20 L/min flowrates were included in the experiments.

When the collection voltage increased from −4 kV to −8 kV, the physical collection efficiency increased for both bacteria (Fig. 5a). For *B. atrophaeus,* it ranged from 55.3 ± 3.9% (−4 kV) to 84.7 ± 4.3% (−8 kV); for *P. fluorescens,* it ranged from 54.9 ± 10.9% (−4 kV) to 80.8 ± 5.3% (−8 kV). Table S2 shows the results for each voltage. The difference was statistically significant ($p < 0.001$) between −4 kV and the other two voltages for both bacteria. However, when the sampling flow rate increased from 10 to 20 L/min at −7 kV (Fig. 5a), the physical collection efficiencies decreased for both bacteria: from 76.8 ± 4.4% to 52.4 ± 3.1% for *B. atrophaeus* and from 80.6 ± 4.6% to 53.5 ± 1.6% for *P. fluorescens.* This decrease was statistically significant ($p < 0.001$), and the difference in the average efficiencies was 25.7%. The decrease was observed because particles spent less time in the collection chamber at the higher sampling flow rate and had a lower chance of being deposited on the collection electrode by electrostatic forces.

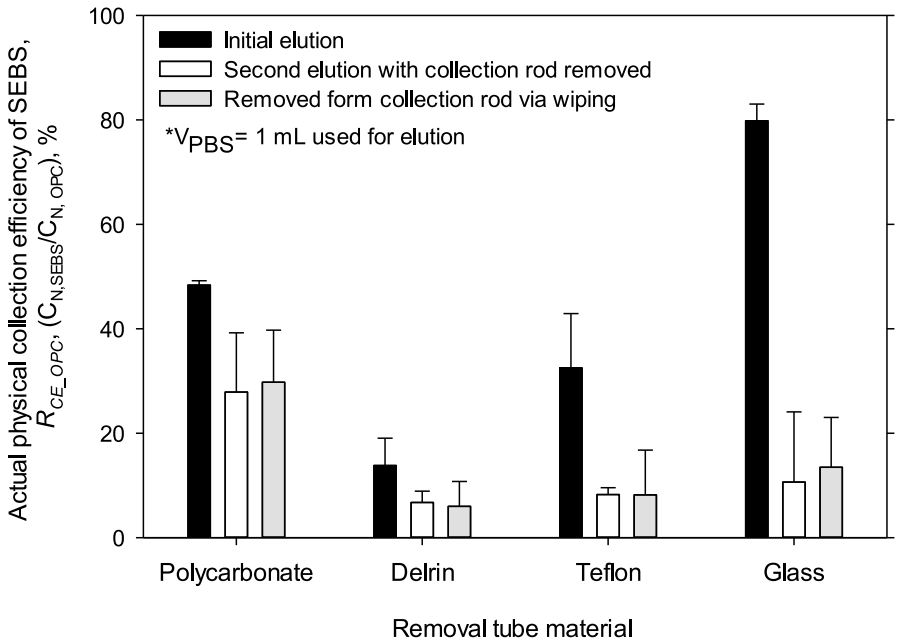

Fig. 6. The relative physical collection efficiency of SEBS determined for individual components of the removal tube as a function of removal tube material: (1) in elution liquid after the first elution, (2) removed from the tube walls in the second elution, and (3) removed from the collection rod by swabbing. The efficiency was determined by comparing the concentration of airborne particles determined by each tube component relative to that determined by OPC at the SEBS's outlet with voltage OFF. The experiments were performed with *P. melinii* fungal spores with SEBS sampling at 20 L/min and charging/collection voltages of +5.25/−7 kV. The concentration of test particles was ~1×10^4/L. Each data point is an average of at least three repeats, and the error bars represent standard deviations.

Before optimizing another parameter, we also tested SEBS when collecting fungus *P. chrysogenum* at −7 kV and 20 L/min. The relative physical collection efficiency was 44.5 ± 2.6% (Fig. 5a). However, the efficiency was statistically significantly lower than that observed with bacteria at 20 L/min ($p = 0.009$) due to the higher hydrophobicity of fungal spores (Han et al., 2011).

We used the collection efficiency data in Fig. 5a to calculate the SEBS's concentration rates, R_C (Fig. 5b). Here, samples were eluted into 0.2 mL, and this value was used in Eq. (8). Depending on the sampling flow rate, the concentration rates ranged from 3.8×10^4/min to 5.2×10^4/min for *B. atrophaeus* and from 4.0×10^4/min to 5.4×10^4/min for *P. fluorescens*. For *P. chrysogenum* sampled at 20 L/min, the R_C value was 4.5×10^4/min. The 20 L/min sampling flow rate increased the average concentration rate for bacteria by a factor of 1.3 despite the collection efficiency being lower by 33%.

Fig. 5c shows the relative viability efficiency of SEBS for *B. atrophaeus*, *P. fluorescens*, and *P. chrysogenum* as a function of collection voltage at the sampling flow rates of 10 and 20 L/min. For bacteria *B. atrophaeus* and *P. fluorescens*, at 10 L/min sampling rate, the relative viability efficiency ranged from 64.2 ± 7.4% to 76.6 ± 5.2% and from 57.0 ± 6.8% to 79.4 ± 7.9%, respectively. At −7 kV, when flowrate increased from 10 L/min to 20 L/min, the viability efficiency decreased from 70.0 ± 6.7% to 66.8 ± 8.7% for *B. atrophaeus,* and from 79.4 ± 7.9% to 62.8 ± 8.4% for *P. fluorescens*, however, the decrease was not statistically significant. At 20 L/min, the viability efficiency for *P. chrysogenum* was 51.1 ± 5.2%, the lowest among the three microorganisms.

As could be seen, for bacteria, the viability efficiency profile looks similar across collection voltages (the only significant difference is for *P. fluorescens* at 10 L/min, with viability at −7 kV being higher than at −8 kV), even though the higher sampling flow rate of 20 L/min results in lower physical collection efficiency of SEBS.

Fig. 5d presents the relative culturability efficiency of SEBS as a function of collection voltage. The relative culturability of *B. atrophaeus* captured at −4, −7, and −8 kV collection voltages ranged from 34.0 ± 8.6% (−4 kV) to 49.7 ± 8.1% (−8 kV); for *P. fluorescens*, the relative culturability at the same voltages ranged from 67.7 ± 13.0% (−4 kV) to 45.0 ± 14.7% (−8 kV). Table S3 shows the results for each voltage. It is usually assumed that the culturability should decrease with increasing collection voltage due to electrostatic stress. However, we did not observe this case; the lowest culturability for *B. atrophaeus* was observed at −4 kV. When all sampling variables (voltages, flow rates, and species) were considered, the relative culturability was statistically different ($p < 0.001$)

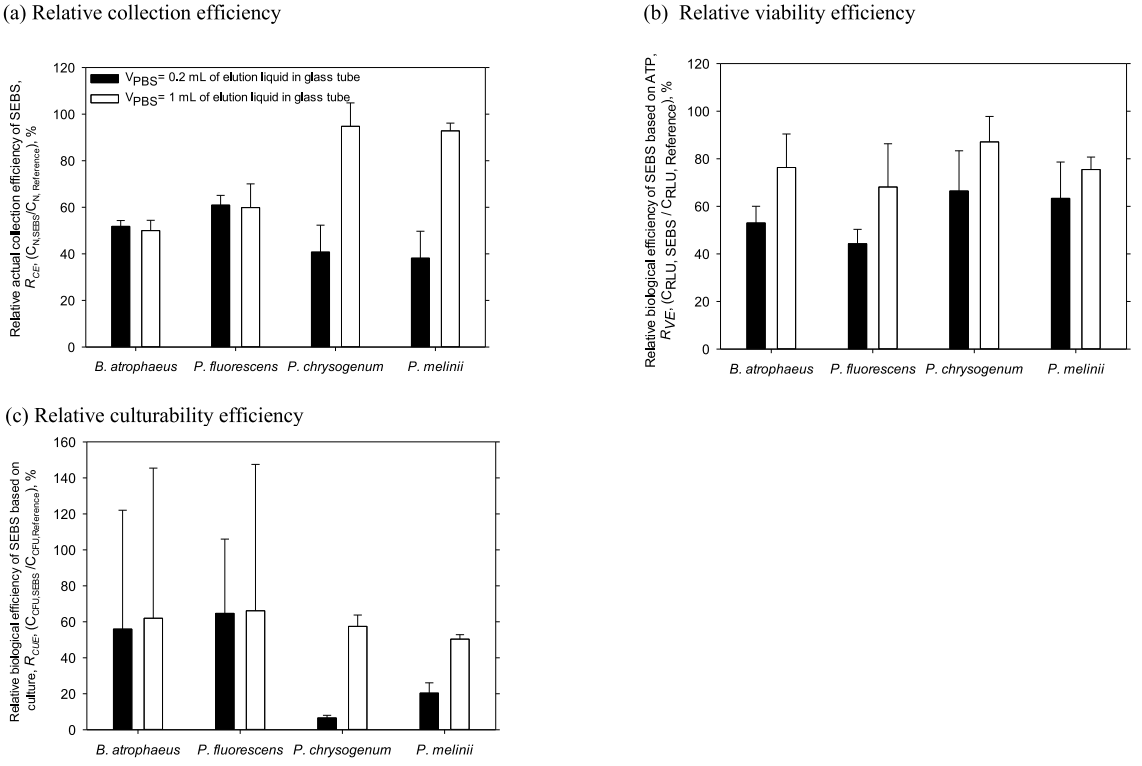

Fig. 7. Performance of finalized SEBS relative to Button sampler when sampling four microorganisms with samples eluted using 0.2 and 1 mL of PBS. a) Relative physical collection efficiency; the efficiency was determined by comparing the number of airborne particles (N/m^3) determined by SEBS with that determined by the Button sampler. b) Relative viability efficiency; the efficiency was determined by comparing the airborne ATP concentration (RLU/m^3) determined by SEBS with that determined by the Button sampler. c) Relative culturability efficiency; the efficiency was determined by comparing the airborne culturable microorganism concentration (CFU/m^3) determined by SEBS with that determined by the Button sampler.

SEBS was operated at a 20 L/min sampling rate and +5.25 kV/−7 kV charging/collection voltages. The Button sampler was concurrently operated at 4 L/min. The test particle concentrations were ~1–2 × 10^4/L. Each data point is an average of at least three repeats, and the error bars represent standard deviations.

for the following pairs: for *B. atrophaeus* at 10 and 20 L/min, it was lower at −4 kV versus −7 kV, for *B. atrophaeus* at –4kV and 10 L/min it was lower versus *P. fluorescens* at −7 kV and 10 L/min, and for *P. chrysogenum* (tested only at −7 kV and 20 L/min) it was lower versus all other sampling combinations. Overall, since the −7 kV collection voltage yielded very good physical and biological performance, it was chosen for further optimization tests. Also, the collection voltage of –7kV yields an electrostatic field of 5.5 kV/cm, similar to 5 kV/cm that was found to preserve microorganism culturability in an electrostatic field in our earlier study (Yao, Mainelis, & An, 2005).

After examining the physical collection efficiency of SEBS obtained using a polycarbonate removal tube with 0.2 mL of elution liquid (Fig. 5a), we hypothesized that not all collected particles are eluted into the liquid; some of them remain on the collection electrode or are lost to the walls of the tube, thus resulting in a reduced actual physical collection efficiency. We further hypothesized that the material of the removal tube plays a role in the extent of losses. Thus, we examined particle presence in three different parts of the removal tube: 1) in elution liquid, (2) remaining on the walls of the removal tube, and (3) remaining on the collection rod, and how the particle presence was affected by the collection tube material (e.g., polycarbonate, Delrin, Teflon, and glass). These experiments were performed with *P. melinii* fungal spores and the collection rod coated by PDMS. Since our preliminary experiments with *P. melinii* indicated that 1 mL of elution liquid had a substantial and positive effect on spore culturability compared to 0.2 mL of elution liquid, 1 mL volume was used for experiments. For easier comparison, the particle presence in each component was expressed as the collection efficiency of the component relative to airborne fungi concentration measured by the OPC with SEBS voltage OFF (Eq. (6)). The OPC was used here to accelerate the analysis process. For all four materials, most of the spores that were captured were found in the eluate (Fig. 6): 48.4 ± 0.8% for polycarbonate tube, 13.8 ± 5.2% for Delrin tube, 32.5 ± 10.4% for Teflon tube, and 79.8 ± 3.2% for glass tube. It is obvious that the amount of spores in the eluate depends on the material of the tube ($p < 0.001$), most likely due to different adhesion forces between the particles and the walls of the tube. When polycarbonate and glass were used, the number of spores in the three components of the removal tube was approximately equal to the number of spores entering the sampler (e.g., efficiency close to 100%). This result highlights the high collection efficiency of SEBS while at the same time illustrating the challenge of eluting the captured particles. Since the glass removal tube yielded a higher spore fraction after the first elution, it was selected for further evaluation of SEBS.

Based on the optimization steps above, the final SEBS configuration included a cylindrical collection rod covered by PDMS, with captured particles eluted using a glass tube (Fig. 2b). SEBS was then operated at 20 L/min to improve concentration rate and compared against the Button sampler (4 L/min) when sampling all four test microorganisms (Fig. 7). Since we found out that the elution liquid volume affected the physical and biological performance of SEBS, the experiments were performed with both elution liquid volumes (0.2 and 1 mL). As could be seen in Fig. 7a, the physical collection efficiency for the two bacteria was very similar when using 0.2 and 1 mL of elution liquid (51.8 ± 2.5% versus 50.0 ± 4.5% for *B. atrophaeus* and 61.0 ± 4.2% versus 59.9 ± 10.2% for *P. fluorescens*). However, for each fungal spore, the difference based on the eluate volume was obvious and significantly higher for 1 mL: 40.7 ± 11.6% versus 94.8 ± 10.1% for *P. chrysogenum* and 38.1 ± 10.5% versus 92.8 ± 3.3% for *P. melinii*.

Based on the data presented in Fig. 7a, we can also estimate the concentration rate. For bacteria eluted using 0.2 mL, the R_C would be approximately 5×10^4; for 1 mL elution liquid, the rate would be approximately 1×10^4. For fungal spores, the rate would be approximately 4×10^4 and 2×10^4 for elution liquid volumes of 0.2 and 1 mL, respectively.

The relative biological performance of SEBS based on microorganism viability determined by ATP-bioluminescence is shown in Fig. 7b. The average relative viability with 0.2 and 1 mL of liquid samples was 56.8 ± 10.1% and 76.8 ± 7.8%, respectively. It is clear that a higher eluate amount yields a stronger ATP signal, thus resulting in the better relative performance of SEBS. We speculate that more liquid ensures a more thorough and contact between the liquid and the collection rod. Interestingly, the viability efficiency did not depend on species and elution volume when all data were analyzed together, except for the following pair: *P. fluorescens* (0.2 mL) vs. *P. chrysogenum* (1 mL) ($p = 0.019$). Additionally, when each elution volume was analyzed separately, the effect of relative viability efficiency was not significantly different for all species: $p = 0.186$ and $p = 0.408$, for 0.2 mL and 1 mL, respectively.

Fig. 7c presents the relative biological performance of SEBS based on microorganism culturability. For the two bacteria, the average relative culturability was similar for both 0.2 and 1.0 mL liquid elution volumes: 55.9 ± 66.1% versus 62.0 ± 83.5% for *B. atrophaeus*, respectively, and 64.7 ± 41.3% versus 66.1 ± 81.4% for *P. fluorescens*, respectively. However, for fungi, the higher eluate volume of 1 mL yielded a statistically higher culturability efficiency compared to 0.2 mL: 57.4 ± 6.3% versus 6.6 ± 1.5% *P. chrysogenum*, and 50.3 ± 2.5% vs. 20.3 ± 5.7% for *P. melinii*, respectively. Also, for 1 mL elution volume, the average relative culturability among all four microorganisms was similar, about 60%. Overall, the bacteria showed high variability in their culturability, as shown by high coefficient of variation values (i.e., standard deviation divided by mean value) (63.9%–134.7%). However, the coefficient of variation of culturability in fungal samples was much lower (4.9%–28.2%). The observed lower variability in culturability of fungal spores could be expected because fungal spores are generally considered hardy and thus less affected by the sampling process (Morris, Kokki, Anderson, & Richardson, 2000).

When the relative viability and culturability efficiencies (Fig. 7b and c) are compared to the relative physical efficiency (Fig. 7a), it is clear that for bacteria eluted with 0.2 mL, the relative viability and culturability are approximately the same as the relative physical collection efficiency, i.e., the bacteria are not subjected to additional stress compared to the Button filter sampler. In fact, when 1 mL of elution liquid is used, the relative viability is higher than the relative collection efficiency suggesting that the stress to bacteria is reduced compared to the filter sampler. The outcomes are more diverse for fungal spores. For 0.2 mL elution liquid, the relative viability is somewhat higher than the relative collection efficiency; however, surprisingly, the relative culturability is much lower. For 1 mL elution liquid, the relative viability is slightly lower, while the relative culturability is somewhat lower, although much better than 0.2 mL elution liquid. The result for fungal spores is surprising because the spores are considered hardy microorganisms (Chatigny, 1986). We speculate that the observed strong dependency on the elution volume has to do with the elution process itself: the

collection rod acts as a beater bar inside the tube during vortexing and damages spores, while the elution liquid acts as a damping material. This would explain much higher relative culturability at 1 mL (more space, less physical contact) than 0.2 mL (less space, more physical contact). The "beater bar" effect is minimal for smaller bacterial cells because they have less inertia and avoid contact with the "beater bar." We also investigated an alternative elution method (e.g., sonication); however, sonication requires higher eluate volume, thus reducing the concentration rate. Our future efforts will explore solutions to minimize the negative effect of beater bar: e.g., immobilization of the collection rod inside the tube during vortexing.

Overall, the main focus of this study was to design and evaluate a new stationary electrostatics-based bioaerosol sampler. The data indicate that this stationary sampler achieves 48 $\pm$ 11% actual collection efficiency at 20 L/min sampling flow rate when 0.2 mL of elution liquid is used; the actual collection efficiency increases to 74 $\pm$ 23% with 1 mL of elution liquid. At the sampling flow rate of 20 L/min, these collection efficiencies translate into the sample concentration rate of 5×10^4 min^{-1} and 1.5×10^4 min^{-1} for 0.2 and 1 mL elution liquid, respectively. Since a large fraction of the eluate is analyzed, such concentration rates will enable faster detection and determination of viable and culturable bioaerosols, especially when sampling in low concentration environments. In addition, we have shown that SEBS is compatible with adenosine triphosphate (ATP)-based bioluminescence and culture-based methods. This feature could be used to determine our exposures to viable and culturable bioaerosols.

The utility and versatility of SEBS and its ability to highly concentrate viable and culturable bioaerosols will be explored in future studies, where it will be applied in various actual indoor and outdoor environments.

4. Conclusions

This study used earlier advances in the electrostatic collection of airborne microorganisms and designed and optimized a Stationary Electrostatic Bioaerosol Sampler (SEBS) focusing on its ability to efficiently capture airborne microorganisms and preserve their viability and culturability. In addition, because electrostatic collectors feature low pressure drop, SEBS could be operated at a relatively high flow of 20 L/min entirely by the battery power and be used as a lightweight and portable field device.

In the laboratory evaluation of SEBS, it was challenged with four bioaerosol species (e.g., *B. atrophaeus*, *P. fluorescens*, *P. chrysogenum,* and *P. melinii*) when sampling at 20 L/min and exhibited physical collection efficiency >60% compared to Button filter sampler. The physical efficiency increased to close to 100% when sampling fungal spores that were eluted into 1 mL of liquid. The use of 0.2 mL elution liquid resulted in the concentration rate of 5×10^4 min^{-1}. Such a high concentration rate will enable faster detection and determination of bioaerosols, especially when sampling in low concentration environments. In addition, SEBS exhibited high viability and culturability efficiency, especially with 1 mL elution liquid. In fact, the relative viability was even higher than the relative collection efficiency, suggesting that the stress to the microorganisms is reduced compared to the filter samplers. Overall, SEBS is one of the few electrostatic collectors, if any, that has been specifically developed and tested to measure airborne culturable microorganisms. The utility and versatility of battery-operated and portable SEBS and its ability to highly concentrate viable and culturable bioaerosols will be explored in future studies with its application in various actual indoor and outdoor environments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaerosci.2022.105951.

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