Development and initial testing of an active low-power, ferroelectric film-based bioaerosol sampler

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ABSTRACT
This article introduces REAS (Rutgers Electrostatic Active Sampler), a new active bioaerosol sampler using permanently polarized ferroelectric film (e.g., PVDF) to capture charge-carrying bioaerosol particles. While REAS operates on an electrostatic collection principle, due to its unique materials and design, it does not require external power to charge incoming particles or to create an electrostatic collection field. The sampler consists of a polarized film wound in a spiral configuration with oppositely polarized film sides positioned 2.25 mm apart. The film and its holder are inserted into a 3D-printed housing cylinder to connect to a pump. The device has an open channel design, creating virtually no pressure drop, which allows for longer sampling times on the same battery charge compared to filter samplers. When REAS was tested in different field environments, the physical collection efficiency ranged from 19 ± 2% in a laboratory environment at 1 L/min to 41 ± 0.1% in residence at 0.1 L/min. When REAS was used to capture culturable bacteria and fungi over a 24-hr period, the concentrations determined by REAS were not different from those determined by an Institute of Medicine sampler (IOM, SKC, Inc.). The concentrations determined by both samplers were lower than those measured by a SAS Super 180 Sampler (SAS, Bioscience International), except for outdoor fungi. However, the SAS was used as a grab sampler to avoid overloading or desiccating the plates, while both REAS and IOM continuously sampled for 24 hrs. Further studies will explore improvements to the REAS sample elution protocols.

GRAPHICAL ABSTRACT

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1. Introduction

Bioaerosols are airborne particles of biological origin, including bacteria, fungi, archaea, viruses, pollen, their fragments, and byproducts (Fröhlich-Nowoisky et al. 2016; Ghosh, Lal, and Srivastava 2015; Haig et al. 2016; Gediminas Mainelis 2020; Cox and Wathes 1995). Bioaerosols can negatively impact human health by transmitting disease, causing toxic reactions, and exacerbating respiratory conditions such as asthma and allergies (Ghosh, Lal, and Srivastava 2015; Haig et al. 2016; Gediminas Mainelis 2020). SARS-CoV-2, the virus causing the current COVID-19 pandemic, is an example of a disease that could be transmitted by airborne biological particles (van Doremalen et al. 2020; Passos, Silveira, and Abrahao 2021; Samet et al. 2021).

Because bioaerosols are relevant to human health and atmospheric processes such as ice nuclei formation (Carotenuto et al. 2017; Fröhlich-Nowoisky et al. 2016; Gong et al. 2019; Matthias-Maser and Jaenicke 1994), it is important to be able to obtain representative samples of bioaerosols in different air environments. Obtaining such samples in the indoor environment is especially critical because people spend most of their time indoors (Klepeis et al. 2001). There are many commercially available bioaerosol samplers, and they use filtration, impaction, impingement, cyclonic forces, electrostatic precipitation, gravity, or their combination to collect bioaerosol particles. At the same time, there is no “one-size-fits-all” sampler or analysis method. Instead, when choosing a bioaerosol sampler, research question(s), bioaerosol particles of concern, method(s) of analysis, sampling conditions, and other parameters must be considered. For example, different sampling conditions such as heating have been shown to reduce the collection efficiency of electrostatic dustfall collectors (Kilburg-Basnyat, Metwali, and Thorne 2016), while high humidity environments may negatively impact gelatin filter performance (Haig et al. 2016). Other considerations, such as sampler noise, might be factors for human-occupied environments or personal sampling.

Among the existing sampling methods, electrostatic precipitation has been demonstrated as an effective means for collecting culturable and total bioaerosol particles (viable and non-viable) (T. Han and Mainelis 2008; T. Han et al. 2011; T. Han, Zhen, et al. 2015; T. T. Han, Thomas, and Mainelis 2017; G. Mainelis 1999; G. Mainelis et al. 2002; Priyamvada et al. 2021; Rufino de Sousa et al. 2020; Zhen et al. 2013; Miksch et al. 2009; T. T. Han et al. 2022). However, typical electrostatic precipitators (ESPs) require both an air mover to aspirate particles and a power supply(-ies) to provide power to the particle charger and collector. The ESP charger imparts an electrostatic charge to incoming particles, while the collection surface connected to a power supply collects the charged particles. However, corona discharge used to charge particles in classical ESPs generates ozone and reactive oxygen species (ROS) (Goheen, Larkin, and Bissell 1984; T. T. Han, Thomas, and Mainelis 2017; Li and Wen 2003). Ozone and ROS can cause the inactivation of viable bioaerosol particles during sampling (T. T. Han, Thomas, and Mainelis 2017; G. Mainelis 1999; G. Mainelis et al. 2002; Shen, Kai, and Yao 2013; Priyamvada et al. 2021). Recent developments in ESP technology, such as the specially designed charging section, result in localized electrostatic fields and significantly reduce ozone production (T. T. Han et al. 2022; T. T. Han, Thomas, and Mainelis 2017). On the other hand, an earlier study demonstrated that bioaerosol particles may carry a sufficient charge to be captured electrostatically without any additional charging (Yao and Mainelis 2006). Such an approach simplifies the ESP design and eliminates drawbacks associated with potential ozone production. Still, the collection section of the ESP requires a power source to create an electrostatic field.

One potential solution is to use advanced materials that create static electrostatic fields. That was the idea behind the Rutgers Electrostatic Passive Sampler (REPS)—a passive bioaerosol sampler that uses electrostatic attraction (in addition to gravitational settling) to collect bioaerosol particles carrying electrostatic charge (Therkorn, Thomas, Calderon, et al. 2017; Therkorn, Thomas, Scheinbeim, et al. 2017). REPS uses a permanently polarized polyvinylidene fluoride film (PVDF, Kureha America LLC, New York, NY) wound in a spiral configuration to generate an electrostatic field between film layers as well as a fringing electrostatic field that both attracts and collects particles onto the film (Therkorn, Thomas, Calderon, et al. 2017; Therkorn, Thomas, Scheinbeim, et al. 2017). In this configuration, the PVDF film is 40 μm thick, 70 mm tall, and 130 mm wide. The film is wound into a 3-D printed film holder, which forms three concentric film layers spaced 2.25 mm apart—the distance determined to be optimal for bioaerosol collection by this design (Therkorn, Thomas, Calderon, et al. 2017). REPS performed well in outdoor environments (Therkorn, Thomas, Scheinbeim,
et al. 2017; Manibusan and Mainelis 2021), including analyzing bioaerosol diversity via next-generation sequencing (Metaxatos, Manibusan, and Mainelis 2021). However, REPS performed less efficiently in indoor settings, which may be due to lower air movement, thus reducing the “supply” of biological particles near the sampler (Manibusan and Mainelis 2018, 2021).

To overcome this shortcoming, we propose converting REPS into an active sampler (Rutgers Electrostatic Active Sampler, REAS) while retaining its key innovative feature, i.e., advanced materials to create and maintain an electrostatic field for capturing particles. This sampler has an open channel design with minimal in-line pressure drop, which minimizes power requirements for the pump, thus maximizing sampling times. Thus, the new device is a low-power, active sampler technology that can be used in various air environments, including both indoors and outdoors. The new sampler collects particles by an electrostatic attraction like the original passive REPS design. However, unlike traditional ESPs, it does not employ a particle charger, thus removing the ozone issue and the need for a power supply. This new design provides the same open channel airflow efficiency and electrostatic attraction properties as traditional ESP devices but without the additional and undesirable stress on organisms. The active sampler design also overcomes challenges experienced by passive REPS when sampling in environments with limited air movement or when quantitative, volumetric airborne microorganism concentration must be determined.

Thus, the overall objective of this project was to develop REAS and perform its initial evaluation. First, REAS was tested in a chamber with aerosolized bacteria and fungal spores to determine its physical collection efficiency; second, the collection efficiency was tested in several field environments to determine the optimal sampling flow rate; and third, a short-term sampling for culturable biological particles was performed to demonstrate the feasibility of the method.

2. Materials and methods

2.1. Design and construction of REAS

This new low-power active sampling device REAS is shown in Figure 1. REAS utilizes the same 3D-printed open-channel film holder as the original REPS (Figure 1a). In addition, it features a housing cylinder for the film holder (Figure 1a). Both parts are printed by Shapeways Inc. (New York, NY) using a Polyamide PA 2200 material (CAS-No. 25038-74-8, EOS GmbH—Electro Optical Systems, Krailling, Germany) with a Selective Laser Sintering (SLS) printer. When assembled, the film holder with the spiral-wound 70 mm × 130 mm PVDF film with 2.25 mm spacing between film layers is placed in the housing cylinder (Figure 1b). The internal diameter of the housing cylinder is 22.5 mm. For sampling, the housing cylinder is connected to a pump or other air mover (Figure 1c). As the air with charged particles passes through the sampler, the electrostatic field created by the oppositely polarized PVDF film sides deposits particles onto the film. The captured bioaerosol particles can then be eluted into a liquid for analysis (Materials and Methods 2.4.1).

**Figure 1.** REAS sampler design: (A) 3D-printed film holder and its housing cylinder; (B) a view of an assembled REAS film holder placed inside the housing cylinder; and (C) the assembled sampler connected to a pump. The film holder and housing cylinder are 3D printed using Polyamide PA 2200. The 40 μm-thick polyvinylidene fluoride (PVDF) film is 70 mm × 130 mm. The film is wound in a spiral configuration with layers spaced 2.25 mm apart.
2.2. Testing of physical collection efficiency in a chamber

Initial testing of REAS was conducted by determining its physical collection efficiency in controlled chamber tests (Figure 2). The test chamber was assembled in a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN). This chamber setup was used in previous studies (T. T. Han, Thomas, and Mainelis 2017; T. T. Han et al. 2022). Test particles were aerosolized by a 3-jet Collison nebulizer (CH Technologies, Westwood, NJ) at a 5 L/min flow rate and 14 psi pressure. The physical collection efficiency was tested with and without a charge neutralizer (2-mCi Po-210, Amstat Industries Inc., Glenview, IL) in the air stream to represent aged particles (with neutralizer) and freshly aerosolized particles (without neutralizer). The charge neutralizer reduces the particle charge to its Boltzmann charge equilibrium. An air compressor with HEPA filter supplied dilution air (33 L/min, CAT-8010DSPC, California Air Tools, Inc., San Diego, CA). A flow straightener was used to provide uniform distribution of the test particles in the sampling chamber (T. T. Han et al. 2022; T. T. Han, Thomas, and Mainelis 2017).

The airborne particle concentrations were determined by a GRIMM optical particle counter (OPC, GRIMM 1.108, Grimm Technologies, GmbH, Ainring, Germany) operating at 1.2 L/min. The OPC also served as the “pump” to draw air through the REAS sampler during testing. Since REAS was operated at a lower flow rate, the difference in airflow was supplied by a Gast compressor (DOA-P704-AA, Cole-Parmer, Vernon Hills, IL) with an air filter (Whatman PolyVENT 1000, GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Y-connectors were used to direct the additional air supply toward the aerosol instruments and minimize the backflow chances. The collection efficiency of REAS was determined based on particle number concentrations downstream of the housing cylinder with and without the REPS film holder inside the cylinder while drawing air at 0.1, 0.3, or 0.5 L/min. The sampling flow rate was measured before and after each condition change using a TSI Mass Flow Meter (4040, TSI, Inc., Shoreview, MN). The measurement duration was 1 min.

Collection efficiency ($\eta_E$) was determined using the following equation:

$$\eta_E = \left(1 - \frac{C_I}{C_{total}}\right),$$

where $C_I$ is the average particle concentration (#/m³) with REAS in-line, and the $C_{total}$ is particle concentration without the film holder inside the housing cylinder. These two sampling conditions were alternated (i.e., $C_{total}$, $C_I$, $C_{total}$, $C_I$, $C_{total}$, etc.). The $C_{total}$ used in calculations is the average of the particle concentrations recorded before and after the $C_I$ measurement.

A concept of sample concentration rate has been previously used to describe bioaerosol sampler performance based on the particle concentration in the collection liquid compared to the airborne particle concentration per time unit (T. T. Han, Thomas, and Mainelis 2017; T. Han and Mainelis 2008). However, for this study, the concentration rate was modified to Capture Factor ($R_{CF}$, L/min), which excludes the liquid volume because REAS does not collect particles directly into the liquid media:

$$R_{CF} = \eta_E \cdot Q_S,$$

where $\eta_E$ is the collection efficiency for particles of a certain size or range of sizes determined in Equation...
inform us about the total number of particles captured per time at different sampling flow rates.

The testing was performed using pure cultures of microorganisms *Penicillium melinii* (fungal spore, ATCC 10469, American Type Culture Collection, Rockville, MD) and *Bacillus atrophaeus* (Gram-positive bacteria, ATCC 49337). Spores from *P. melinii* were streaked onto Malt Extract Agar (MEA, Becton, Dickinson, and Co.) and incubated at room temperature (≈20°C) for seven days before sampling. Spores were harvested by gently scraping the mycelia with a cell spreader into 5 mL of autoclaved Milli-Q water (EMD Millipore Corp., Milli-Q Direct 8, Billerica, MA, USA). Cells of *B. atrophaeus* were cultured in nutrient broth (Becton, Dickinson and Co., Sparks, MD) at 30°C for 18 h using a previously published protocol (T. T. Han, Thomas, and Mainelis 2017; Therkorn, Thomas, Calderon, et al. 2017). Both bacterial cells and fungal spores were harvested by washing their suspensions four times by centrifugation at 7000 rpm for 5 min at 4°C (Model BR4i, Jouan Inc., Lorton, VA). The resulting pellet was resuspended in 20 mL of Milli-Q water. The suspension was aerosolized by a 3-jet Collison nebulizer. The mean aerodynamic diameter of *B. atrophaeus* is about 0.89 μm, and *P. melinii* is about 2.1 μm, according to our previous studies (T. T. Han et al. 2022; T. Han et al. 2011; T. Han, An, and Mainelis 2010). For this test, the size distributions for both microorganisms were measured by a Grimm optical particle counter. The mode diameter for microorganisms were measured by a Grimm optical particle counter (P-Trak Model 8525, TSI, Inc.) and incubated at room temperature (20°C) for seven days before sampling. Spores from *P. melinii* were streaked onto Malt Extract Agar (MEA, Becton, Dickinson, and Co.) and incubated at room temperature (≈20°C) for seven days before sampling. Spores were harvested by gently scraping the mycelia with a cell spreader into 5 mL of autoclaved Milli-Q water (EMD Millipore Corp., Milli-Q Direct 8, Billerica, MA, USA). Cells of *B. atrophaeus* were cultured in nutrient broth (Becton, Dickinson and Co., Sparks, MD) at 30°C for 18 h using a previously published protocol (T. T. Han, Thomas, and Mainelis 2017; Therkorn, Thomas, Calderon, et al. 2017). Both bacterial cells and fungal spores were harvested by washing their suspensions four times by centrifugation at 7000 rpm for 5 min at 4°C (Model BR4i, Jouan Inc., Lorton, VA). The resulting pellet was resuspended in 20 mL of Milli-Q water. The suspension was aerosolized by a 3-jet Collison nebulizer. The mean aerodynamic diameter of *B. atrophaeus* is about 0.89 μm, and *P. melinii* is about 2.1 μm, according to our previous studies (T. T. Han et al. 2022; T. Han et al. 2011; T. Han, An, and Mainelis 2010). For this test, the size distributions for both microorganisms were measured by a Grimm optical particle counter. The mode diameter for *B. atrophaeus* was 0.9 μm and *P. melinii* was 2.5 μm. Airborne cell concentrations for each test were ≈2 × 10^5 cells/m^3, based on the GRIMM OPC. The performance of REAS was tested in triplicate with each organism, flow rate, and particle charge conditions.

### 2.3. Field testing: Physical collection efficiency tests

Following chamber tests, physical collection efficiency was determined in real environments. This is particularly important because naturally occurring bioaerosols might have different electростatic charges and size ranges than particles in the chamber study. Figure 3 illustrates the setup for testing in the field. Briefly, a P-Trak condensation particle counter (P-Trak Model 8525, TSI, Inc.), which counts particles larger than 0.02 μm, and GRIMM OPC (range 0.3 to 20 μm in diameter) were positioned downstream of REAS. The joint size range measured by these two instruments encompasses the sizes of individual viruses, bacteria, fungal spores, and their agglomerates. Since the operational flow rate for the GRIMM OPC is 1.2 L/min, and P-Trak requires 0.7 L/min (total of 1.9 L/min), an adjustable, air-supplying Gast compressor with an air filter was added downstream of REAS to achieve its test flow rates of 0.1 L/min, 0.5 L/min, and 1.0 L/min. The operational flow rate for REAS was checked both before and after each test using a TSI Mass Flow Meter (4040, TSI Inc.).

The collection efficiency (Equation (1)) and capture factor (Equation (2)) were determined for both number and mass concentrations in three different environments: an indoor laboratory, a residence, and outdoors. Particle mass concentration (Cm) was calculated assuming that particles are spherical with the density of water (1 g/cm³):

\[
C_m = \sum \left( C_{ni} \cdot \frac{\pi}{6} \cdot \frac{10^{-6} \mu g}{\mu m^3} \right),
\]

where \(C_m\) is the particle mass concentration (μg/m³), \(C_{ni}\) is the number concentration in the \(i\)th size bin (#/m³), \(d_i\) is the midpoint diameter of the \(i\)th size bin (μm), \(\pi/6\) is a conversion to determine the volume of a sphere (\(d^3 \pi/6\)), and \(10^{-6} \mu g/\mu m^3\) is the density of water. The tests were repeated 5 times per sampling day for each flow rate on three different days for each location. A total of 45 tests were conducted for each flow rate, for a total of 135 tests.

### 2.4. Impact of pressure drop on sampler operation

A pump test was conducted to demonstrate the benefit of the open channel design of REAS compared to a typical filter-based sampler. We predicted that a minimal pressure drop would allow REAS to sample for a longer duration compared to a filter-based sampler when operated at the same flow rate and with the same pump. We used an IOM sampler (SKC, Inc., Eighty Four, PA) sampler with a 25 mm polytetrafluoroethylene (PTFE) filter (FALP02500, hydrophobic 1.0 μm pore PTFE membrane, MilliporeSigma, Burlington, MA) as a comparison device. Both devices operated at 1 L/min flow rates. The 1 L/min flow rate was chosen based on the capture factor results from the physical collection tests. Airflow was provided by an SKC Airchek XR5000 (SKC, Inc.) pump with a fully charged SKC P85004A Li-ion battery (SKC, Inc., 7.4 V, 5.2 Ah, 385 kWh). The flow rate for each sampler was set using a TSI Mass Flow Meter. The pump battery was fully charged before each test and allowed to run until it died (i.e., the flow rate dropped to 0 L/min as per the mass flowmeter). The test was performed to determine the difference in collected air...
volume and sampling time between the REAS and a traditional filter-based sampler (IOM).

2.5. Biological collection tests

REAS was compared to two commercially available bioaerosol samplers: the SAS Super 180 Air Sampler (180 L/min, SAS, Bioscience International, Rockville, MD) and the IOM sampler (2 L/min). The IOM is a filter-based personal sampler for inhalable particles operated at 2 L/min. While it has been designed to capture inhalable particulate matter, it has also been used to collect bioaerosols (Haatainen et al. 2010). It was used with the same 1.0 \(\mu\)m PTFE filter type that was used in the sampling duration test described above. SAS-180 is a high-volume culture-based sampler that collects bioaerosol particles directly onto an agar plate.

Samples were collected indoors in a residence and outdoors near an organic community garden. Three IOM and three REAS samplers were operated for 24 h in each location, and sampling was repeated on three separate days. Controls included one procedural blank per sample day for both REAS and IOM filters. SAS collected samples on tryptic soy agar (TSA, Difco, Becton, Dickinson and Co., Franklin Lakes, NJ) with cycloheximide (50 \(\mu\)g/mL, Acros Organics, Fisher Scientific Company Ltd., Waltham, MA) for bacteria and malt extract agar (MEA, Difco, Becton, Dickinson and Co.) for fungal spores. Three 1-min SAS samples were collected three times per day (about 4 h apart) for each plate type, resulting in nine plates for bacteria and fungi per sampling day. The average concentration from the nine samples was assumed to represent the average 24-hr concentration. Controls for each sampler included procedural blanks, where samples were handled in the same way as actual samples except for the operation of the pump, accounting for potential contamination due to the handling of samples.

During indoor sampling, temperature and relative humidity were recorded using a Foobot (Airboxlab US, San Francisco, CA). For outdoor samples, a portable weather station collected meteorological data, including temperature, relative humidity, wind speed, wind direction, and precipitation (WS-2080 Wireless Weather Station, Ambient Weather, Chandler, AZ, USA). Additional meteorological data was collected by a nearby air monitoring station within 2 km of the outdoor sampling site.

2.5.1. Sample analysis

REAS samples were eluted into sterile phosphate-buffered saline (PBS) solution. The housing cylinder with a film holder inside was filled with 20 mL of PBS, sealed with paraffin film (Parafilm M, Bemis Co., Inc., Oshkosh, WI), and placed in a 50 mL conical centrifuge tube. Like in the original REPS design, the film holder and housing cylinder fit in a 50 mL conical centrifuge tube. REAS samples were vortexed for 2 min, followed by 15 min of sonication. The eluate was then transferred into a new conical 50 mL centrifuge tube. Samples analyzed from this eluate were labeled “REAS.”

A 15 mL subsample of the eluate was further transferred into a separate tube for concentration by centrifugation at 10,000 rpm for 12 min at 4°C. After centrifugation, 10 mL of the supernatant was pipetted out, leaving 5 mL with a concentrated pellet for analysis. The pellet was then resuspended in the remaining 5 mL. Samples analyzed by this additional concentration step are labeled “REAS Concentrated.”

Each IOM filter and filter holder set was inserted into a 50 mL conical centrifuge tube containing 5 mL
of PBS and soaked for 10 min with the materials submerged by a sterile inoculation wand. The IOM samples were then eluted by the 2-min vortexing and 15-min sonication, the same procedure as used for REAS.

One hundred microliters from REAS and IOM samples were plated onto sterile TSA and MEA plates in triplicate. Samples were spread evenly on individual plates using sterile spreaders and incubated for up to 5 days at room temperature (~20°C). REAS and IOM procedural blanks were eluted and analyzed using the same procedure as actual samples. In addition, blank agar plates and plates with sterile PBS were incubated to identify potential sources of contamination if colonies were observed on procedural blanks. Ten percent of each agar plate type per test were reserved for blanks.

Total colony forming units (CFU) were determined for each plate. The positive-hole correction was used for SAS samples to adjust the total CFUs to account for the probability of multiple bioaerosol particles depositing under the same hole nozzle (Macher 1989).

Culturable bioaerosol concentration (C) for each sampler was determined:

$$C = \frac{R \cdot V_s \cdot n}{Q \cdot t},$$

where $C$ is the bioaerosol concentration (CFU/m³), $n$ is the number of counted colonies (CFU) adjusted for any colonies from blank samples, $V_s$ is the analyzed sample volume (0.1 mL), $V_e$ is the total eluate volume, $R$ is a conversion factor for the concentration step (0.33 mL/mL for concentrated and 1 mL/mL for unconcentrated REAS and IOM samples), $Q$ is the sampler flow rate (m³/min), and $t$ is the sampling duration (min). The 0.33 mL/mL conversion is derived from the 5 mL liquid containing pellet divided from the 15 mL subsample. The term “bioaerosol” in Equation (4) refers to either bacteria or fungi, depending on a test.

Samples were also analyzed using adenosine triphosphate (ATP) bioluminescence analysis to determine the concentration of viable cells (T. Han, Wren, et al. 2015; Seshadri et al. 2009). Briefly, 200 μL aliquots were transferred into 1.5 mL centrifuge tubes. 200 μL of BacTiter-Glo reagent (Promega Corp., Madison, WI) was then added to the tube, and the contents were briefly vortexed for ~5 sec and incubated at room temperature for 1 min. The intensity of each sample was then measured by a luminometer (model 20/20 n, Turner Biosystems Inc., Sunnyvale, CA). Measurements were reported as relative luminescence units (RLU) and converted into concentrations (C, RLU/m³) based on Equation (4) when CFU is replaced with RLU.

2.6. Statistical analysis

Statistical analysis and data presentation were conducted using IBM SPSS Statistics for Windows (Version 27.0, IBM Corp., Armonk, NY) and OriginPro (Version 2021b, OriginLab Corporation, Northampton, MA, USA), respectively. Sample means were compared using two-way (i.e., sampler and sampling day as independent variables) analysis of variances (ANOVA) assuming normality and homogeneity of variances with Bonferroni post hoc analysis to identify specific different variable pairs. Results with $p < 0.05$ were considered significant.

Collection efficiency and capture factor in chamber tests were analyzed as a function of flow rate and microorganism neutralization status. For field-based physical collection efficiency tests, collection efficiency and capture factor were analyzed as a function of flow rates and sampling locations. For culture-based tests, the culturable microorganism concentrations were analyzed as a function of sampler and sampling location.

3. Results and discussion

3.1. Physical collection efficiency determined in chamber tests

Collection efficiency of REAS, when tested with charge-neutralized airborne microorganisms, ranged from 25 ± 10% when sampling P. melinii at 0.5 L/min to 71 ± 11% when sampling the same microorganism at 0.1 L/min (Figure 4). Overall, the 0.1 L/min sampling condition yielded the highest collection efficiency for the charge-neutralized aerosol compared to other flow rates, and the difference was statistically significant ($p < 0.05$).

A sampling of non-neutralized bioaerosols resulted in collection efficiencies above 80% for both test organisms and all 3 flowrates. Here, there was no statistically significant difference in collection efficiency as a function of flow rate. Also, collection efficiencies when sampling charge-neutralized aerosol were statistically significantly lower than when sampling non-neutralized aerosol for all tested flow rates and organisms.

The neutralized and non-neutralized aerosol represents a range of condition extremes for particle collection by REAS. For field environments, we hypothesize that collection efficiencies will be closer to those observed at the neutralized condition unless a major bioaerosol source is nearby, producing fresh and, likely, charged bioaerosol. As the aerosol ages, it loses its charge (G. Mainelis et al. 2001). Aged particles are, therefore, less efficiently collected by REAS because the sampler collects using the particle’s existing charge.
Based on the chamber physical collection test with pure cultures, 0.5 L/min yielded the highest capture factor (Figure 4) for both charge-neutralized and non-neutralized organisms, i.e., at this flow rate, REAS would collect the most particles compared to other tested flowrates. Thus, 0.5 L/min and an even higher flow rate of 1 L/min were selected for field testing.  

3.2. Physical collection efficiency determined in the field

Physical collection tests were conducted in a residence, a laboratory environment, and outdoors at 0.1, 0.5, and 1.0 L/min sampling flow rates. Particle size distributions for each location and test are presented in Supplementary Figure S1. The collection efficiencies and capture factors determined based on the particle number concentration are shown in Figure 5. When both CPC and OPC were used, the observed collection efficiencies ranged from 19 ± 2% in a laboratory environment at 1 L/min to 41 ± 0.1% in a residence at a sampling flow rate of 0.1 L/min. The collection efficiencies at the 0.1 L/min sampling flow rate were statistically significantly higher for all test locations compared to the 1.0 L/min flowrate conditions for all size bin conditions based on two-way ANOVA with Bonferroni post hoc analysis, including location and flow rate (p < 0.05). The 0.1 L/min flow rate was also significantly different from the 0.5 L/min condition, but only when GRIMM data were used. Collection efficiencies for the 0.5 and 1.0 L/min sampling flow rates for all locations were approximately 30% and not significantly different from one another. A sampling at the 1.0 L/min flow rate resulted in the statistically significantly highest capture factor.
Figure 6 shows the collection efficiency and capture factor determined based on particle mass concentration. The total collection efficiency ranged from 28 ± 17% outdoors at 1.0 L/min to 61 ± 4% in a residence at 0.1 L/min. The collection efficiency at the 0.1 L/min flow rate was statistically significantly higher than that at the 1.0 L/min sampling flow rate but was not statistically different from the 0.5 L/min condition ($p < 0.05$). These differences were observed across all three sampling environments. The 1.0 L/min condition resulted in the highest capture factor for all test locations among the three flow rates.

Collection efficiency determined based on particle mass and number concentrations was also examined as a function of particle size, and the results are shown in Supplementary Figures S2 and S3, respectively. The results demonstrate collection efficiencies of up to 100% for particle sizes above 5 μm. In addition, particles were generally more efficiently collected at the 0.1 L/min flow rate condition based on both particle number and mass concentrations.

Sampling conducted at 1.0 L/min resulted in the highest capture factor for all test environments based on both particle number and mass concentrations. Therefore, the 1.0 L/min flow rate was selected for further testing to demonstrate the capture and preservation of culturable bacteria and fungi by REAS.

### 3.3. Impact of pressure drop on sampler operation

When allowed to sample unimpeded in a relatively clean laboratory environment, the IOM sampler with a filter operated at 1 L/min ran for 2295 min (38.25 h) for a total sample volume of 2.30 m$^3$. Upon recharging and recalibrating the flow rate, REAS ran at 1 L/min for 3503 min (58.38 h) for a total air volume of 3.50 m$^3$. Thus, REAS collected 1.20 m$^3$ more air and sampled over 1.5 times longer (additional 20.13 h) than the IOM with a filter operating at the same flow rate and the same fully charged pump.

The pressure drop for the IOM with the filter was $5.47 \times 10^{-1}$ kPa and only $6.22 \times 10^{-3}$ kPa for REAS, i.e., REAS produced only about 1% of the resistance observed in the filter-based sampler. It should be noted that the pressure drop in any filter sampler is highly dependent on the filter choice (Soo et al. 2016). For example, Soo and colleagues observed a pressure drop
of 1.65 kPa for 0.45 μm PTFE filters from SKC operated at 1.7 L/min, but only 0.423 to 0.433 kPa for 2 μm PTFE filters from SKC and Pall also operated at the same sampling flow rate (Soo et al. 2016). Therefore, our findings for the IOM sampler operated with a PTFE filter are similar to the pressure drop results observed for similar filters in other studies. Different filter types, pore sizes, and flow rates will affect the pressure drop in filter-based samplers, affecting power consumption by the pump and the length of sampling as well as its comparison to sampling duration by REAS. Certain filters may have a lower pressure drop; however, this consideration must also be weighed against physical collection efficiency (Hinds 1999; Soo et al. 2016).

Due to the open-channel design of REAS, the pressure drop across it was minimal compared to the filter-based sampler. The observed slight pressure drop was likely caused by the 0.25-inch tubing and connector. Overall, the REAS design allows for extended sampling times with less stress on power supplies than filter-based sampling devices operated at similar flow rates.

**3.4. Collection of culturable organisms**

Culturable bioaerosol particle concentrations were determined by REAS, SAS, and IOM samplers (Figure 7). The discussion below focuses on the REAS Concentrated samples because the colony counts for the unconcentrated samples often were in the single digits, thus potentially reducing detection accuracy. Furthermore, based on the study protocol, there were more replicates for the REAS Concentrated samples.

Within both the indoors and outdoors, the culturable bacteria and fungal concentrations determined by the IOM and REAS Concentrated were not significantly different from each other (p < 0.05), based on a two-way ANOVA, using the sampler and test day as independent variables (i.e., the analysis was based on daily samples, not on three-day averages). No significant differences were observed between bioaerosol concentrations determined by IOM and REAS or between REAS and REAS Concentrated. The SAS sample concentrations for indoor bacteria, indoor fungi, and outdoor fungi were significantly higher.
than concentrations of both bacteria and fungi provided by the IOM and REAS Concentrated. For outdoor samples, the bacteria concentrations determined by the SAS sampler were not significantly different from those determined by the other two samplers ($p < 0.05$).

The average bioaerosol concentrations for individual test days are shown in Supplemental Figure S4. Average culturable bacteria concentrations ranged from 90.7 ± 44.9 CFU/m$^3$ for IOM test #3 indoors to 744 ± 349 CFU/m$^3$ for SAS test #3 indoors. Average culturable fungi ranged from 186 ± 16.5 CFU/m$^3$ for SAS test #3 indoors to 1345 ± 469 CFU/m$^3$ for SAS test #3 outdoors. The two-way ANOVA analysis found significant differences in bioaerosol concentration between individual testing days, supporting our decision to base our statistical analysis on daily samples and not three-day averages. The average temperature, dew point, and total precipitation for each test are shown in Figure S5. The average temperature ranged from 4.72 ± 4.92 $^\circ$C during outdoors test #1 to 24.8 ± 0.38 $^\circ$C for indoors test #3.

As mentioned above, culturable bioaerosol concentrations determined by IOM and REAS concentrated were statistically different from those obtained by SAS, except for bacteria collected outdoors. This may be due to the differences in principle and operation of the samplers. The IOM and REAS samplers used an integrated approach, with the collection lasting 24 h. The SAS is a culture-based, high-volume sampler that was used to collect three replicate grab samples (1 to 3 min) at three different time points throughout the day. The integrated sample might better reflect the average bioaerosol concentration in a particular sampling environment because bioaerosol concentrations fluctuate temporally and spatially as different sources release particles over time (Cox and Wathes 1995). In contrast, the grab sample approach better reflects the bioaerosol concentration at the measured time point. The shorter sampling time may also reduce stress on microorganisms. Still, a high flow rate and impaction forces in an agar impactor might also reduce microorganism viability compared to IOM and REAS. On the other hand, 24-hr sampling with a filter sampler is likely to affect microorganism culturability due to desiccation (Haig et al. 2016; Cox and Wathes 1995). In addition, the IOM and REAS devices require collected particles to be eluted into a liquid for plating and analysis, which might lead to losses from the additional processing steps (e.g., vortexing and sonication). In contrast, SAS collects all particles directly onto the agar plate. However, these potential losses in REAS are negligible according to our previous study using spiked samples and PVDF film (Therkorn, Thomas, Calderon, et al. 2017).

An interplay of these factors was likely responsible for the observed results, illustrating some of the challenges when comparing results between bioaerosol studies that use samplers with different collection principles even within the same study environment. Similarly, Mescioglu et al. (2021) found differences between an electrostatic precipitator and the collocated liquid
impinger and membrane filter samplers used for comparison in both culture and non-culture based analysis. An, Mainelis, and Yao (2004) compared a high volume, culture-based centrifugal impactor and an impinger. The study found that the impactor yielded a lower culturable concentration than the impinger. This difference was thought to be due to the damage to microorganisms caused by the impaction forces and the breakup of agglomerates by impingement, thus yielding more colonies. Mbareche et al. (2018) also found differences in bacterial and fungal aerosol diversity and abundance between collocated liquid cyclonic impactor, liquid impinger, and an electrostatic filter sampler. Therefore, we stress the importance of sampler selection in designing and analyzing a bioaerosol project.

Given that REAS operates using an open channel design, in contrast to a filter placed perpendicular to the airflow, the equivalency of the bioaerosol concentration determined by the two samplers demonstrates the utility of using polarized films to create electrostatic fields inside devices for active bioaerosol collection. This technology clearly shows its ability to capture and maintain the culturability of bioaerosols while imposing minimal pressure drop and battery drain on the pump. Furthermore, the culturable bioaerosol concentrations determined by the two 24-hr samplers, i.e., REAS and IOM samplers were statistically not different, even though REAS has about 30–35% collection efficiency compared to a near 100% by the filter sampler. This result suggests that REAS induces less stress on culturable microorganisms than filter samplers. It is also possible that differences in elution efficiency affected colony counts; however, the elution protocol used in this study was ~80% efficient for PTFE filters (Therkorn, Thomas, Calderon, et al. 2017).

One of the challenges in using REAS is the large initial elution volume (20 mL), which increases the detection limit unless additional steps are taken to concentrate the entire sample or at least part of it, as was done in this study. One application of the REPS technology recently explored fragmenting the film post-sampling and then using only ~2 mL of liquid; the method showed some promise (Metaxatos, Manibusan, and Mainelis 2021). Future studies of REAS applications may explore elution protocols using lower volumes of liquid or alternate sample concentration procedures for already eluted samples (Oh, Han, and Mainelis 2020).

4. Conclusions

The objective of this study was to introduce a novel active bioaerosol sampling device that relies on innovative materials and creates very little resistance to the airflow, thus prolonging sampling time when operating on the battery. This goal was accomplished by developing an active electrostatic film-based bioaerosol sampler, REAS, which adapted the technology previously introduced by the passive REPS device to capture particles by electrostatic attraction using a polarized PVDF film. The use of polarized PVDF film allows for the electrostatic collection of particles without the need to impart charge on the incoming particles or provide power to collection surfaces like in typical electrostatic precipitators. The sampler only requires a power source for a pump, which at the current operational flow rate of 1L/min can easily be supplied by a portable, personal pump. The open channel design of REAS results in a minimal pressure drop, allowing for more extended sampling times compared to filter-based samplers of similar operational flow rates. The compact design will allow for stationary and personal sampling applications in a variety of different environments.

REAS performs well in different sampling environments. For example, the average collection efficiencies for REAS at 1.0 L/min were ~36% based on total particle mass concentration. Furthermore, the culturable bioaerosol concentrations observed by REAS (both in concentrated and un-concentrated samples) matched those of a filter-based sampler operated at 2 L/min. These results are notable because REAS can achieve this concentration equivalency despite its lower collection efficiency, not using any filters or additional power supplies to charge particles or collection plates. Eliminating the need to charge the incoming aerosol also avoids the ozone problem as many ESP chargers generate ozone and nitrogen oxides (Cox and Wathes 1995), which can damage bioaerosols and thus reduce their viability and culturability. High ozone levels generated by industrial-size ESPs may also pose challenges to people nearby as they can cause negative health effects such as eye irritation, asthma exacerbation, or other respiratory effects. Furthermore, REAS can capture and maintain the viability of bioaerosols due to the use of unique materials and their configuration. However, we also recognize that the current REAS design limits its sampling flow rate. This issue will be addressed in further research.

This study demonstrates the REAS technology’s potential applications and bioaerosol sampling opportunities. Recommendations for further development of REAS include analysis of samples with the next-generation sequencing techniques, species identification to compare relative diversity of bioaerosol samples,
optimization of concentration or elution protocols to improve sample detection limits, and design changes to increase the sampling flow rate.

Nomenclature

\( \eta_E \)  
 sampler particle collection efficiency [%]

\( C_i \)  
 average concentration with REAS in-line [#/m³]

\( C_{\text{total}} \)  
 average concentration off-line [#/m³]

\( Q_S \)  
 sampler flow rate [L/min]

\( V_{\text{W/DJ}} \)  
 divided by the volume of the collection liquid [L]

\( \text{RCF} \)  
 sample capture factor [L/min]

\( C_m \)  
 particle mass concentration [µg/m³]

\( C_{\text{ni}} \)  
 number concentration in the ith size bin [#/m³]

\( d_i \)  
 midpoint diameter of the ith size bin [µm]

\( C \)  
 bioaerosol concentration [CFU/m³]

\( V_a \)  
 analyzed sample volume [mL]

\( V_e \)  
 total eluate volume [mL]

\( R \)  
 conversion factor for the concentration step [mL/mL]

\( Q \)  
 sampler flow rate [m³/min]

\( t \)  
 sampling duration [min]

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