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Bioaerosol Sampling: Classical Approaches, Advances, and Perspectives

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Abstract

Bioaerosol sampling is an essential and integral part of any bioaerosol investigation. Since bioaerosols are very diverse in terms of their sizes, species, biological properties, and requirements for their detection and quantification, bioaerosol sampling is an active, yet challenging research area. This paper was inspired by the discussions during the 2018 International Aerosol Conference (IAC) (St. Louis, MO) regarding the need to summarize the current state of the art in bioaerosol research, including bioaerosol sampling, and the need to develop a more standardized set of guidelines for protocols used in bioaerosol research. The manuscript is a combination of literature review and perspectives: it discusses the main bioaerosol sampling techniques and then overviews the latest technical developments in each area; the overview is followed by the discussion of the emerging trends and developments in the field, including personal sampling, application of passive samplers, and advances toward improving bioaerosol detection limits as well as the emerging challenges such as collection of viruses and collection of unbiased samples for bioaerosol sequencing. The paper also discusses some of the practical aspects of bioaerosol sampling with particular focus on sampling aspects that could lead to bioaerosol determination bias. The manuscript concludes by suggesting several goals for bioaerosol sampling and development community to work towards and describes some of the grand bioaerosol challenges discussed at the IAC 2018.

1. Introduction

Bioaerosols, or biological aerosols, consist of airborne particles of biological origin including bacteria, fungi, archaea, viruses, pollen, their fragments, components and byproducts, such as DNA, endotoxin, and mycotoxins (Cox and Wathes 1995; Ghosh, Lal and Srivastava 2015; Lindsley 2017b). Over the past several decades there has been a renewed interest in various aspects of bioaerosol research, including investigation of adverse health effects (Douwes et al. 2003), bioaerosol role in environmental processes, such as cloud formation or ice nucleation (Smets et al. 2016), their presence at high altitudes (Smith et al. 2018), bioaerosol-ecosystems interactions (Fröhlich-Nowoisky et al. 2016), and

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transmission of plant disease (Parker, McDonald and Boland 2013; West and Kimber 2015). The renewed interest in bioaerosols has naturally led to the development and application of new bioaerosol research tools, including the development and evaluation of various bioaerosol sampling devices. The primary goal of any bioaerosol sampling device is to extract a *representative* bioaerosol sample from an air environment being investigated and then preserve bioaerosol sample properties needed for sample analysis by traditional and modern analysis techniques (Griffiths and Stewart 1999).

Bioaerosol sampling and characterization face several challenges:

1. The commercially existing bioaerosol samplers, as well as published bioaerosol sampling concepts, differ in their sampling mechanisms, performance characteristics (e.g., cut-off size, sampling flow rate and time, collection medium, etc.), ability to preserve critical bioaerosol properties and compatibility with various analysis methods. Due to these differences, even bioaerosol sampling instruments that operate concurrently are likely to produce different bioaerosol signals.
2. Because a universal bioaerosol sampler does not exist, very often a selection of a particular sampling technique dictates which analytical tools could be used and vice versa. At the same time, specific analytical tools inform us only about certain aspects of bioaerosol presence, such as culturability and concentration, adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA) amount, presence of specific antigens or gene sequences, or the presence of particular components such as beta-glucans, arabitol, mannitol, or endotoxins; Recent advances in gene sequencing provide us with Operational Taxonomic Units (OTUs) allowing us to derive various richness and diversity indices of airborne microbial assemblages. All these are useful metrics, but, if used in isolation, they will provide only a partial picture regarding bioaerosol presence and function.
3. With few exceptions, bioaerosol concentrations in typical indoor and outdoor environments are relatively low or experience strong temporal fluctuations that cannot be dependably captured by most of the available sampling technologies, primarily because of their low sampling flow rates or short sampling times. As a result, information about bioaerosol presence and behavior is often incomplete.

The challenges listed above are some of the reasons why we have not been able to standardize bioaerosol sampling protocols and determine dose-effect relationships. In spite of or perhaps inspired by these challenges, bioaerosol sampling technology has made tremendous strides over the past 20 years or so. We have seen the introduction of portable, personal, high flow rate, and automated bioaerosol samplers. There have been active developments to integrate samplers with advanced detection techniques such as laboratories-on-the-chip and/or application of microfluidics (Ma et al. 2016; Pardon et al. 2015). At the same time, advances in bioaerosol analysis such as gene sequencing present new and urgent challenges for bioaerosol sampling as the effect of sampling bias, e.g., preferential capture and preservation of certain species remains mostly unexplored.

This manuscript was inspired by the discussions during the International Aerosol Conference (IAC) of 2018 (St. Louis, MO) regarding the need to summarize the current state of the art in bioaerosol research, including bioaerosol sampling, and the need to develop a more standardized set of guidelines for various protocols used in bioaerosol research. Thus, this paper is an overview of the primary sampling techniques and concepts, current trends and developments in the field, followed by suggestions on streamlining bioaerosol sampling protocols and reporting guidelines, as well as a discussion of the grand challenges in the field.

2. Main sampling principles

The main goal of bioaerosol sampling is to extract a *representative* bioaerosol sample from an air environment being investigated, deposit the sample into/onto a sampling medium and then make it available for analysis by culturing (Herr et al. 2003), microscopy, flow cytometry, ATP-based bioluminescence, quantitative polymerase chain reaction (qPCR), gene sequencing and other methods (Górny 2020; Reponen, Willeke and Grinshpun 2011). Specific aspects of bioaerosol analysis are addressed in another manuscript of this special issue (King 2019).

There are numerous commercially available bioaerosol samplers (Lindsley 2017b), and many new sampler concepts are under development, but the vast majority of samplers rely on filtration, impaction, impingement and electrostatic precipitation, or a combination thereof, to capture particles of interest. Physical aerosol sampling steps, such as aspiration, transport, and deposition of particles onto the collection medium, could lead to particle losses and affect the representativeness of the collected sample. In addition, when sampling bioaerosols, potential damage to bioaerosol particles, including loss of culturability or physical integrity, caused by the sampling process itself (Zhen et al. 2013) is another source of bias and a unique aspect of bioaerosol sampling.

Sampling methods can be discussed as being active or passive. Active methods allow to quantify bioaerosol concentration, however the need to use an air mover and the related power requirements can lead to restricted sampling mobility, especially where high pressure drops and long sampling times are encountered. Battery-operated bioaerosol samplers do not have the mobility issue, but their operational time depends on battery capacity and power requirements. On the other hand, passive samplers do not use air movers, and particles are collected due to gravity and/or electrostatic attraction. Passive samplers are easy to use and inexpensive compared to active samplers. They are also useful when determining the contamination of surfaces by settled particles, such as the contamination of sterile surfaces during surgery or contamination of food product production lines. However, passive sampler data can be treated only as qualitative as the sampled air volume is unknown. Some studies suggested that when passive samplers are used in parallel to active samplers their equivalent sampling flow rate, i.e., a flow rate of a sampler with 100% collection efficiency resulting in the same quantity of bioaerosols as determined by the active sampler, could be determined (Therkorn et al. 2017b; Yamamoto et al. 2011). At the same time, equivalent sampling flow rates are likely to depend on a reference sampler, analysis method, and environmental conditions. It has also been suggested that passive and active sampling could yield very

different species diversity based on size of the microorganisms, deposition rates, ventilation in the room, and other parameters (Cox et al. 2017).

3. Bioaerosol capture mechanisms and representative samplers

3.1. Filtration and filter-based samplers

Filtration is one of the most commonly used methods to capture airborne particles, including bioaerosols, because it is convenient and easy to use. Once the bioaerosol particles are collected on a filter, they can be eluted into liquid for subsequent analysis by various techniques. Particles deposited on a filter can also be examined directly using microscopy, including electron microscopy (Crook 1995) or be directly placed directly on agar for cultivation.

Polycarbonate, mixed cellulose ester, polytetrafluoroethylene (Teflon, PTFE), polyvinyl chloride (PVC), nylon, gelatin, and other filter types have been used for bioaerosol sampling depending on the selected analysis technique (Burton, Grinshpun and Reponen 2007; Li et al. 2018; Van Droogenbroeck et al. 2009). When using flat filters, such as Nuclepore™ or Nuclepore-type, particles are collected on the membrane surface, which is especially conducive for microscopy analysis (Crook 1995). Washable filters have also been used (Choi et al. 2018). Gelatin filters have been developed to preserve sample culturability better, and they can be dissolved in a buffer (Cox and Wathes 1995) or placed directly on agar for culturable analysis (Yao and Mainelis 2007a). Gelatin filters have also been used for the collection of viruses (Li et al. 2018).

The type, size, and porosity of a filter determine power requirements needed to sample at a particular flow rate. Those power requirements also often dictate how long a sample could be collected, especially when battery-operated air movers are used. Here, one can typically use filters with higher porosity to extend sampling time without a substantial loss of collection efficiency. More information about filter types used for bioaerosol collection, their characteristics and performance could be found in the literature (Lindsley 2017a).

Discussion of filters used for bioaerosol collection would not be complete without discussing filter holders, or filter samplers themselves. Use of an appropriate sampler with specific inlet characteristics or with particle pre-separation allows collecting bioaerosols according to environmental, i.e., PM_{10} or $PM_{2.5}$, or occupational, i.e., inhalable, thoracic and respirable, conventions. For example, the Button Aerosol Sampler and IOM cassette (both by SKC Inc., Eighty Four, PA), which are inhalable samplers, were adapted for bioaerosol collection and used with various filters, including gelatin filters (Aizenberg 2000; Chang and Hung 2012; Wu, Shen and Yao 2010; Yao and Mainelis 2007a). Use of different polyurethane foams (PUF) enables the collection of thoracic and respirable bioaerosol fractions (Kenny et al. 1999), while the use of traditional PM_{10} and $PM_{2.5}$ samplers allowed the collection and analysis of bioaerosols in PM_{10} and $PM_{2.5}$ fractions (Bowers et al. 2013). A filter sampler with a focused collection area for the examination of airborne fungal spores using microscopy has also been presented (Spurgeon 2006).

In an emerging trend, filters from Heating Ventilation and Air Conditioning (HVAC) systems are used to analyze bioaerosol presence indoors (Farnsworth et al. 2006; Hoisington et al. 2014; Maestre et al. 2018) and car filters are used to investigate bioaerosol bioburden in mobile environments (Hurley et al. 2019; Viegas et al. 2018b). This approach provides long-term integrated samples and improves detection limit; at the same time, the pore size and collection substrate of these filters, as well as vehicle operating time and the collected air volume may not be known unless specifically measured. Also, the collection duration by these filters can be several months, which could affect the filter's efficiency, making the comparison of filters even more challenging.

3.2. Impaction based samplers

Impaction-based samplers are among the most commonly used devices in bioaerosol sampling. Here, particles with sufficiently high Stokes number deviate from the streamlines as they change direction and land on the impaction surface (Hinds 1999). The aerodynamic particle diameter at which 50% collection efficiency is achieved is called a cut-off diameter, or d_{50}. Since a particle's Stokes number increases with increasing diameter, density, and velocity, installation of consecutive impactors with progressively smaller nozzle(s) leads to higher air velocities and enables collecting gradually smaller particles. Such an arrangement is called a multi-stage impactor. In many bioaerosol impactors, multiple impaction nozzles are used, and such impactors are termed multi-nozzle impactors, or sometimes sieve samplers due to their resemblance to a sieve.

Based on these principles, a great variety of bioaerosol impactors have been developed (ACGIH 1999; Grinshpun et al. 2015; Lindsley 2017b). The available impactors differ in their flowrates, cut-off diameters, number of nozzles, and collection stages. The technical characteristics of many bioaerosol impactors could be found in review articles listed above and, of course, are provided by the manufacturers. Typical collection surfaces used in impactors are agar plates for direct cultivation of microorganisms, and glass slides, filters and other solid surfaces for microscopy analysis. Impactors available for bioaerosol research could be generally divided into agar-based impactors, spore traps, rotating arm collectors, and other arrangements.

3.2.1. Agar-based impactors—Probably the best-known impactor is the Andersen multistage impactor, which was introduced in 1958 (Andersen 1958). It has been recommended and used as a reference sampler (Chatigny et al. 1989; Griffiths and Stewart 1999; Jones et al. 1985; Yao and Mainelis 2007b) and is the recommended bioaerosol collection method in the NIOSH Manual of Analytical Methods (NIOSH 2017). Once the patent restrictions on the original Andersen impactor expired, several Andersen-type impactors have been introduced by various companies (Lindsley 2017b). A few years ago, a disposable single-stage multi-nozzle agar impactor BioCassette®, became available (EMLab P&K, LLC, Cherry Hill, NJ) (Gallup, Purves and Burge 2004). A disposable agar impactor with slits, BioCapt®, is also commercially available (Particle Measurement Systems, Boulder, CO). This manufacturer also provides multi-use devices based on the same slit design.

One of the main advantages of using impactors to collect airborne microorganisms, especially culturable ones, is the ease of use and convenience: once a sample is collected, the agar plates are transferred directly to an incubator without intermediate steps. Culturable methods are essential to determine microorganisms that are “alive”, but they comprise only a fraction of total microorganisms (Rinsoz et al. 2008) and leave out viable but not culturable microorganisms (VNBC), and non-viable microorganisms which could still cause health effects (Robbins et al. 2000; Speight et al. 1997). Agar is not designed to use with analytical methods other than culturing, although attempts have been made to scrape off the deposited microorganisms using water and analyze them using PCR (Xu and Yao 2011). In addition, impaction subjects airborne microorganisms to sudden deceleration and that damages them, including the loss of culturability (Stewart et al. 1995; Wang et al. 2001) and even membrane integrity (Zhen et al. 2013) thus further reducing the fraction of culturable microorganisms that can be determined. Nonetheless, the convenience of use and a large amount of reference information make agar-based impactors a tool of choice in many studies.

One of the distinct performance and marketing features of bioaerosol impactors is their cut-off size as lowering the d_{50} allows capturing ever-smaller bioaerosol particles. This is especially important when performing laboratory studies with well-defined bioaerosol species. However, the literature suggests that low d_{50} might be not as critical when sampling environmental bacteria as most of them are larger than 2 μm because they tend to be attached to other particles (Lighthart 1997). Field comparison showed that agar impactors with large d_{50} captured similar concentrations of culturable bacteria and fungi compared to impactors with much lower d_{50} (Yao and Mainelis 2007a). One potential reason for similar results is better preservation of microorganism culturability by impactors with larger d_{50} due to their lower impaction velocities.

3.2.2. Hand-held and portable impactors—The Andersen impactor requires an external air mover, e.g., a vacuum pump, to provide the nominal sampling flowrate, which limits the impactor’s mobility and applications in environments where electricity is not accessible or limited. A single-stage Andersen impactor, however, could be coupled with a battery-operated pump (Zhen et al. 2009). To address the impactor portability issue, a number of hand-held Andersen-type impactors, where the impactor is integrated with a pump or a fan in a single unit, have been introduced. Due to power limitations, these impactors are single-stage collectors with d_{50} larger than 1 μm and high as high 8 μm; however, the use of centrifugal fans allows many of them to sample at flow rates exceeding 100 L/min and as high as 180 L/min (Yao and Mainelis 2006). Recently, a portable impactor with a sampling flow rate as high as 1200 L/min and collection efficiency of 10–20% when capturing single bacteria has been described (Chen and Yao 2018). Hand-held impactors typically are agar-based, but some have been adapted to collect microorganisms into liquid (Chang and Chou 2011; Chang and Hung 2012). In addition to traditional jet-to-plate agar impactors, a family of centrifugal agar impactors (Biotest AG, Dreieich, Germany) has been introduced. These Reuter Centrifugal Samplers operate at flow rates ranging from 40 to 100 L/min (Grinshpun et al. 2015); however, there were questions regarding the actual sampling flowrates in early models (Macher and First 1983). A multi-nozzle portable impactor with a Petri dish rotating at 1–4 rpm to increase the sampling surface has been developed (SpinAir

sampler, IUL micro, Barcelona, Spain). Rotation to increase collection surface has also been used in slit impactors (Grinshpun et al. 2015); however, here the rotating part is the impactor's cover with a narrow slit allowing time-resolved sampling as long as 3 hours (BIAP Slit Sampler, Scantago, ApS, Denmark).

3.2.3. Traps for fungal spores and pollen—A particular class of bioaerosol impactors is fungal spore traps. Most of such impactors are single-use devices with a single round nozzle or a slit directing airborne particles toward a glass slide with an adhesive surface. Post-sampling, such impactors are disassembled, and the glass slide is examined under a microscope. About a dozen different spore traps, such as Air-O-Cell cassettes (Zefon International, Ocala, FL) and similar are on the market, and they operate at flow rates ranging from 5 to 30 L/min with their d_{50} typically at 2 μm and higher (Grinshpun et al. 2015; Lindsley 2017b). Due to their ease of use and application of standard and even automated microscopy analysis (Wagner and Macher 2012), spore traps are widely used in indoor air quality investigations, especially where the presence of mold is a concern (Godish and Godish 2008), and they became the go-to tool of environmental consulting companies. Disposable spore traps require external pumps that could be operated by electricity or batteries. Recently, several inexpensive (<$100) mold sampling kits that include spore traps and a lightweight pump could be found on Amazon.com and other online stores. The accuracy of such sampling kits has not yet been explored in the literature.

Some spore traps are multi-use devices, such as BioSIS Slit Impaction Air Sampler (EMS Inc., Charleston, SC) and Personal Volumetric Air Sampler (Burkard Manufacturing Co., Ltd.). The latter sampler has a built-in pump. Hirst spore traps (Hirst 1952) offer an automated collection of pollen. Burkard 7-day Volumetric Spore Trap (Burkard Manufacturing Company Ltd., Hertfordshire, UK) uses an adhesive tape supported by a clock-driven drum that could be examined to determine temporal variability of mold spore or pollen concentration over seven days. Rotating arms covered with a sticky substance, also known as a Rotorod, are used to collect larger biological particles such pollen (Li et al. 2019); the captured particles are then examined under a microscope or even using PCR (Calderon et al. 2002). The above-mentioned fungal spore traps could also be used to capture pollen, but their performance was found to be orientation-dependent (Michel et al. 2012). A recent review examined the performance of various pollen capturing devices over the past 50+ years (Jones, Wagle and Bielory 2018).

3.3. Liquid-based samplers

The collection mechanism of many liquid-based samplers is similar to that in impactors: inertia is used to separate particles from the airstream and deposit them into a liquid collection medium. The liquid then can be aliquoted and analyzed by various techniques.

Original liquid impinger designs were presented as early as 1947 (Rosebury 1947), and they led to the development of the classical all-glass impingers (AGI) (Ace Glass, Inc., Vineland, NJ), which operate at 12.5 L/min and collect particles into 20 ml of liquid. This impinger comes in two versions with 4 and 30 mm distances between the sampling nozzle and the bottom of the collection vessel, with the nozzle being submerged into the collection liquid.

The AGI has a long, curved inlet designed to simulate the nasal passage and its capture of inhaled particles (Cox 1987; Grinshpun et al. 1994; Jensen et al. 1994). While most of the impingers are single-stage devices, multi-stage impingers such as Burkard multistage liquid impinger (Burkard Manufacturing Co. Limited, England) capable of collecting particles in three size fractions are also available.

While AGI impingers are efficient collectors with d_{50} of 0.3 μm, the collection liquid evaporates in about 90 min (Lin et al. 1997), and high impingement velocities lead to microorganism damage and reaerosolization (Lin 2000). These concerns led to the development of a liquid-based swirling air sampler - marketed as the BioSampler (SKC Inc., Eighty Four, PA) - which combines impingement with centrifugal motion (Willeke 1998). Here, three collection nozzles are positioned at an angle above the collection fluid and, during sampling, the air stream with particles is directed to the wall of the sampling cup where a liquid film is formed due to the centrifugal motion of the liquid. The collector operates at 12.5 L/min and can be used with either 5 or 20 mL sampling cups. Overall, the BioSampler was found to cause lower microorganism stress (Tseng et al. 2014) compared to traditional impingers and has become a *de facto* reference sampler in bioaerosol studies.

In addition to traditional impingers, other liquid sampler designs have been introduced. One example of an alternative design uses a porous medium that is submerged into the liquid to divide the aerosol flow into multiple small air streams that create tiny bubbles and remove particles onto the sampler's walls (Agranovski et al. 2002a; Agranovski et al. 2002b; Agranovski et al. 2005). A number of wetted-wall cyclones, which utilize centrifugal impaction or cyclonic collection mechanism and operate at flow rates from 100 to 1000 L/min capturing particles into 10–15 mL of liquid are available (Grinshpun et al. 2015), e.g., Coriolis family of samplers (Bertin Technologies, France) and others. Interestingly, the collection efficiency of the Coriolis was found to be lower compared to that of BioSampler when sampling fungi (Chang, Ting and Horng 2019). Such cyclones can typically operate for several hours, but sampling duration can be extended to several days when control of liquid level is added, e.g., as in the SASS family of samplers (Research International, Inc., Monroe, WA). There are also portable liquid samplers, such as the BioCapture 650 that uses disposable collection cartridges (Thermo Fisher Scientific, Inc.). Yet another approach in liquid sampling it is to use multiple collection vials as in the Burkard Multi-Vial Cyclone Sampler (Burkard Manufacturing Co).

3.4. Electrostatic collection of microorganisms

In a typical electrostatic collector or precipitator (ESP), particles are drawn into the ESP, imparted an electrostatic charge and then deposited onto a collection medium by electrostatic attraction or repulsion. The collected particles are then removed from the collection medium for analysis. One of the main attractive features of electrostatic collectors is their lower particle deposition velocity compared to inertia-based methods (Mainelis et al. 1999), leading to less microorganism damage (Zhen et al. 2013). Second, ESPs are compatible with multiple collection media types, including agar, liquid, and solid surfaces; samples collected into liquid or washed-off from a solid collection surface can be analyzed by a variety of methods. Thus, when designing an ESP, one can choose a collection medium

that is most aligned with project objectives. Third, most of the ESPs are open-channel devices which results in a low pressure drop allowing the use of batteries to operate the device, including its electrostatic components as well as air movers.

One of the earliest adaptations of ESP was used to capture *Serratia marcescens* bacteria (Berry 1941). In the 1960s, an ESP operating at 1000 L/min and designed to collect viruses was described (Gerone et al. 1966). Since then and until about the late 1990s, there seemed to be a reduced interest in applying ESPs for bioaerosols, mostly due to rapid advancement and application of other bioaerosol collection methods. However, interest in low-power sampling techniques and concerns over the negative impacts of inertia-based processes led to a renewed interest in ESPs for bioaerosol collection. In 1999, an existing Electrostatic Aerosol Sampler (model 3100, TSI Inc., Shoreview MN) was modified and used to capture culturable microorganisms in laboratory experiments (Mainelis et al. 1999). The learned principles were then used to develop and apply a stand-alone ESP for laboratory and field investigations of culturable microorganisms (Lee et al. 2004; Mainelis et al. 2002a; Mainelis et al. 2002b). A negative ionizer equipped with a positively-charged collection cup was successfully used to capture airborne cat allergens in more than 80 homes and daycare centers (Parvaneh et al. 2000). An electrostatic capture device was shown to be a dependable instrument for capturing airborne *Salmonella enteritidis* in a poultry house (Gast, Mitchell and Holt 2004). A sampler based on the ionic wind principle had an equivalent sampling flow rate of 113 L/min and was used to analyze the diversity of bacteria and fungi (Gordon et al. 2015). An ESP-based spore trap was found to be compatible with scanning electron microscopy (Schneider, Durr and Giles 2007). The application of ESPs for bioaerosol collection currently is a very active research area, and some of the recent developments are discussed in the following sections.

In addition to the mentioned advantages, there are also a number of concerns regarding the use of ESPs for bioaerosol sampling. First, ozone is produced during particle charging, and it might negatively affect microorganism viability and integrity (Berry 1941). While there is no data suggesting what ozone concentration or ozone exposure (e.g., ozone concentration *x* exposure time) would be safe to most environmental bioaerosols, the consensus is that the ozone concentration should be as low as possible. When performing residential or personal sampling, production of ozone is also unwanted due to its potential health effects and contribution towards the generation of secondary by-products. Some of the new charger designs result in effective particle charging with ozone concentrations below 10 ppb (Han, Thomas and Mainelis 2017). Desiccation of microorganisms over prolonged collection times is also a concern. However, some studies show minimal adverse effects on microorganism culturability and viability after 4 hours of sampling by an ESP compared to the liquid-based BioSampler (Han, Thomas and Mainelis 2018). A third concern, or, rather a drawback at the moment, is a limited number of ESP-based bioaerosol samplers available commercially despite very active and innovative developments in this area.

4. Emerging trends and developments in the bioaerosol sampling field

4.1. Trends in sampler development

4.1.1. Personal bioaerosol sampling—Stationary and portable bioaerosol samplers determine the presence of bioaerosols in our surrounding environments but may not accurately represent personal exposures to bioaerosols, especially given that humans differ in their personal microbial cloud (Meadow et al. 2015). Here, personal samplers, which measure bioaerosol presence in a person's breathing zone, are needed. Development of personal bioaerosol samplers could be divided into two groups: 1) adaptation of existing personal aerosol samplers to sample bioaerosols and 2) development of new personal samplers specifically for bioaerosols. In the first group, as was already mentioned, the Button Aerosol Sampler and IOM cassette (both by SKC Inc., Eighty Four, PA) were used with various filters, including gelatin, to capture inhalable biological particles (Aizenberg 2000; Chang and Hung 2012; Wu, Shen and Yao 2010; Yao and Mainelis 2007a). Polyurethane foams capable of maintaining microorganism viability (Kenny et al. 1998) were placed in series in front of a polycarbonate filter to sample thoracic and respirable bioaerosol fractions in the field (Kenny et al. 1999).

Several new personal bioaerosol concepts and prototypes have been introduced, and most of them are compact and light-weight. The earlier-mentioned sampler with submerged porous medium (Agranovski et al. 2002b) is designed for personal use. Researchers from the National Institute for Occupational Safety and Health (NIOSH) recently developed and successfully used in the field a microcentrifuge-tube cyclone (Lindsley, Schmechel and Chen 2006; Macher, Chen and Rao 2008; Su et al. 2012b). The NIOSH sampler uses a 1.5 mL microcentrifuge tube, which makes it convenient to couple with PCR and other analysis techniques. The latest model of this sampler (NIOSH BC 251) operates at 3.5 L/min and includes a two-stage cyclone with cut-off sizes of 4 μm and 1 μm plus a final 37 mm filter, allowing to determine three bioaerosol size fractions. The airflow in both mentioned samplers is provided by an external pump.

Personal samplers with built-in pumps offer user mobility and convenience, and several such samplers have been introduced. A liquid-based sampler with a rotating sampling cup and featuring sampling heads for different particle fractions is commercially available (Görner et al. 2006). Since it is a liquid-based sampler, the liquid volume should be checked approximately every 30 min during long-term sampling; however, it could be used with non-evaporative fluid extending its sampling time to 8 hrs (Simon et al. 2016). Since the electrostatic collection is a low power method, it is especially suitable for the adaptation to personal sampling. Here, a personal electrostatic sampler based on ionic wind and operating at a sampling flow rate of 5 L/min was described and used to capture fungal spores on a dry collection surface (Roux et al. 2013; Roux et al. 2016); the presence of spores was determined via arabitol analysis. A personal ESP combined with electrowetting-on-dielectric concentrator achieved concentration rate of 1.9×10^5 min^{-1} when sampling *Bacillus atrophaeus* spores at 20 L/min (Foat et al. 2016). Han and Mainelis (2017) introduced a battery-operated self-contained personal electrostatic bioaerosol sampler (PEBS) capable of operating up to four hours at 10 L/min on a single battery charge; in the PEBS, airborne

particles are deposited onto a stainless steel plate and then removed for analysis (Han, Thomas and Mainelis 2017; 2018). Due to its novel charger design, this sampler features minimal ozone production (<10 ppb). Hong et al. demonstrated collection of MS2 and T3 virus particles using personal electrostatic particle concentrator (EPC), and the virus recovery rates were much higher than those with BioSampler (Hong et al. 2016). As discussed below, the use of electrostatic collection principles allows concentrating personal samples thus improving the detection limit and is especially useful in low concentration environments. At the same time, so far, very few of such sampler concepts have reached the market.

4.1.2. Passive bioaerosol sampling—Passive bioaerosol sampling relies on natural phenomena such as gravity or electrostatic forces to deposit bioaerosol particles onto/into a collection medium and, as a result, does not require air movers or power sources. However, the gravitational settling of particles is affected by their sizes and shapes as well as air motion (Reponen, Willeke and Grinshpun 2011), and larger particles are preferentially captured (Burge and Solomon 1987). The traditional application of passive sampling is the use of settling agar plates (Ghosh, Lal and Srivastava 2015) to determine culturable bioaerosols. While this method is non-quantitative due to the indeterminate air volume and often does not compare favorably against active sampling methods (Buttner and Stetzenbach 1993; Sayer, M. and Wilson 1972; Sayer, Shean and Ghosseiri 1969; Solomon 1975), it is still widely used in contamination sensitive industries, such as food or drug production, industries (Asefa et al. 2009) and health care facilities (Mahida et al. 2018), where exposed agar plates serve as indicators of microbial presence. In order to compare results from different indoor sites, a 1/1/1 scheme has been adopted, where the “1”s refer to the placement of a 90 mm Petri dish at the height of 1 meter, 1 meter away from any wall and 1 hr sampling (Haig et al. 2016). Longer sampling times are not recommended due to agar desiccation.

There have been new technical developments suggesting that passive sampling could serve as a complementary technique to active sampling, or even be a long-term bioaerosol sampling technique on its own right. Due to their light weight, gravity-based passive samplers were combined with released balloon systems to characterize airborne microbial communities at high elevations (Spring et al. 2018). An outdoor study found that settling plates had captured higher biomass compared to an active Button sampler and that the alpha diversity results between the two samplers were similar (Mhuireach et al. 2016). The personal aeroallergen sampler (PAAS) that has a structure resembling a gimbal and is meant to be worn like a necklace. It was designed for coarse particles (Yamamoto et al. 2006) and then successfully applied to measure exposures to pollen (Yamamoto, Matsuki and Yanagisawa 2007). Electrostatic dust fall collectors (EDC) which use electrostatic cloths (Noss et al. 2008) were used to examine two-week long exposures to endotoxin (Liebers et al. 2012). EDC yielded higher levels of fungi and endotoxin compared to dust fall collector (DFC), which is gravity only based device (Frankel et al. 2012). The authors suggested that EDC may be considered as a surrogate for airborne sampling in indoor microbial exposure assessment. However, users should be aware that heating for sterilization diminishes EDC capabilities and its placement significantly affects the detected endotoxin

levels (Kilburg-Basnyat, Metwali and Thorne 2016). The amount of recovered allergen mass seems to be directly proportional to sampling time up to 14 days, but the increase is no longer proportional over longer sampling times (Kilburg-Basnyat, Metwali and Thorne 2014), most likely due to the loss of charge in electrets and electrostatic cloths used in EDCs (Brown et al. 1995). Recently, the Rutgers Electrostatic Passive Sampler (REPS) employing a permanently polarized ferroelectric film has been introduced, where bioaerosol particles are captured by electrostatic attraction, aided by particle settling and diffusion (Therkorn et al. 2017a). The REPS demonstrated equivalent sampling flowrates approaching those of personal filter samplers when enumerating airborne total and culturable bacteria and mold (Therkorn et al. 2017b).

Use of environmental phenomena such as natural deposition via precipitation to investigate bioaerosols could also be considered passive sampling. Here, rain (Kang, Heo and Lee 2015), accumulated snow (Amato et al., 2007) and hailstones (Mandrioli et al. 1973; Temkiv et al. 2012) have been used to investigate the presence of microorganisms and their temporal signatures. Spider webs have also been used to capture bioaerosol particles, including viruses (Mattei et al. 2009; Smither et al. 2011).

4.1.3. Improvement of bioaerosol detection limits—Liquid-based samplers offer compatibility with various analysis methods as well as better protection of the collected bioaerosol particles compared to agar- and filter-based samplers. However, long-sampling durations are often needed to fill the collection liquid with sufficient biomass for reliable and quantifiable detection. This is a serious concern in low bioaerosol concentration environments, especially when fast detection is needed or when long sampling durations are not feasible. A sampler's detection ability could be improved by increasing its Concentration Rate (R_C, t^{-1}) (Han et al. 2015b):

$$Concentration\ rate,\ R_C\left(t^{-1}\right) = \frac{Particle\ concentration\ in\ liquid\left(L^{-1}\right)}{Airborne\ particle\ concentration\left(L^{-1}\right)} \Rightarrow \frac{Q\left(\frac{L}{min}\right)}{v\,(L)}\,\eta, \quad (1)$$

Where Q is sampling flowrate, η is collection efficiency, and v is liquid sample volume. Traditional liquid samplers, such as AGI-30, have an R_C of a few thousand. A new generation of liquid samplers - e.g., the BioGuardian Air Sampler (InnovaTek, Inc., Richland, WA) which operates at flow rates from 100 to 1000 L/min and samples into 10–15 mL of liquid; the DSTL MK1 wetted-wall cyclone sampler (Biotrace International, UK) which runs at 800 L/min and concentrates samples into 100 ml of sampling liquid; the portable BioCapture 650 (Thermo Fisher Scientific, Inc.) that can sample up to 200 L/min into cassettes with 10 mL of liquid - have R_C of tens of thousands. In response to the anthrax events of 2001, the Lawrence Livermore National Laboratory (Livermore, CA) has developed a stationary Autonomous Pathogen Detection System (APDS) that operates at flow rates of up to 3750 L/min and samples into 4–5 mL of collection liquid that is automatically maintained; the APDS can achieve R_C of hundreds of thousands (Hindson et

al. 2004; Hindson et al. 2005a; Hindson et al. 2005b; Langlois et al. 2000). However, this sampler has not become commercially available.

Recent sampler concepts also improved R_C by increasing sampling flowrates. A swirling bioaerosol sampler (SAS) collects atmospheric bioaerosols at a flow rate of 167 L/min and showed viability preservation and collection efficiency close to that of BioSampler (Wubulihairen et al., 2015). An impinger capable of operating at 3100 L/min for 120–300 min was shown to retain cultivability, metabolic activity, viability, and ice-nucleation activity of investigated bacteria (Šantl-Temkiv et al. 2017). The coupling of this device with a Cytosense flow cytometer enabled more sensitive detection of bacteria compared to qPCR (Jang et al. 2018).

These and other batch mode collectors provide an integrated sample and can increase the detection capability by increasing sampling time to improve the concentration factor, C_F:

$$Concentration\ Factor,\ C_F = R_C\left(t^{-1}\right) x\ t\ (\min) \tag{2}$$

On the other hand, instruments with sampling liquid flow-through offer an opportunity to investigate temporal bioaerosol signatures. A family of wetted-wall cyclones (WWC) developed at Texas A&M University have a range of flow rates from 100 to 1250 L/min with the continuous liquid outflow of 1 mL/min and average collection efficiency of ~85% (McFarland et al. 2010).

R_C close to a million could be achieved by decreasing the amount of collection liquid down to tens or even single microliters as was demonstrated in an electrostatic precipitator with the superhydrophobic surface (EPSS) (Han, An and Mainelis 2010; Han and Mainelis 2008; Han et al. 2011). This collection method was successfully coupled with microscopy, qPCR, and ATP-based bioluminescence. The high concentration rate combined with ATP bioluminescence allowed airborne microorganism detection outdoors ~40x more rapidly compared to traditional impingement or filtration (Han et al. 2015b). In a similar concept, an electrostatic collector with circulating liquid as a collection substrate was described (Tan et al. 2011). Park et al. (2015) also demonstrated fast bioaerosol detection by combining a novel hand-held electrostatic rod-type sampler operating at 150 L/min with a commercial luminometer (Park et al. 2015). Similar principles were applied by the same group for continuous and near-real-time bioaerosol detection (Park, Kim and Hwang 2016) using liquid flow-through. In another development, a custom-made ESP sampled viruses into a miniaturized collector with a liquid volume of 150 μL (Ladhani et al. 2017). The authors indicated the collection efficiency of 10% and the detection of a minimum of 3721 RNA. The authors stressed, however, that an improved extraction protocol improves the collection efficiency to 47%. Use of flow-through systems, however, could substantially increase the total amount of collection liquid, thus diluting the sample and making the analysis more challenging. Therefore, low-volume flow-through systems, such as the above-mentioned WWC modified to operate at 100 L/min with collection liquid output rates of 25–50 μL/min (Hubbard et al. 2011) avoid this issue, while reaching the R_C of 1–2 million. This sampler concept can also operate in a batch mode, where it samples bioaerosols at 400 L/min

into 12 ml of collection liquid (King et al. 2009). However, about 30% of the collected particles were lost to the sampler's walls after 1 hr of operation. The authors of the study demonstrated that the recovery of culturable *E. coli* bacteria was 100x greater compared to a high volume impactor (King and McFarland 2012).

By increasing sampling flowrates, C_F was also increased for other sampler types. An ESP designed to operate at 3,500 L/min was used to collect dust to be analyzed for the presence of mold spores, bacteria, endotoxin, and actinomycetes (Madsen and Sharma 2008). Some agar-based samplers with higher flow rates were mentioned earlier. A relatively new addition is the HighBioTrap agar impactor with d_{50} of ~2 μm and capable of operating at of 1200 L/min (Chen and Yao 2018).

In certain instances, e.g., in low concentration environments or when sampling duration is limited, even high flow rates might not yield sufficiently high sample concentrations. In such cases, sample concentration could be increased post-sampling by using centrifugation, accelerated evaporation, e.g., Smart Evaporator (BioChromato, SanDiego, CA), or filtration and reconstitution, e.g., the Concentrating Pipette (InnovaPrep Inc., Drexel, MO) (Alburty et al. 2010; Hunter et al. 2011). Such systems can substantially increase hydrosol concentration, but the user should be aware of potential particle losses, especially in the earlier version of the instruments (Oh and Mainelis 2017).

4.1.4. Application of microfluidics—

Recently, researchers began incorporating microfluidics into bioaerosol samplers to accelerate their integration with laboratories-on-a-chip for faster and more sensitive bioaerosol detection. MicroSampler operating at flow rates from 0.2 to 0.6 L/min successfully captured particles into a microfluidic channel and reported the superior microbial recovery of *Staphylococcus epidermidis* compared to the BioSampler (Choi et al. 2017). Two electrostatics-based samplers with microfluidic interfaces reported a capture efficiency of approximately 20%, leading to concentrated bioaerosol samples (Ma et al. 2016; Pardon et al. 2015). The device by Pardon et al. captures particles into 300 μL of liquid and is meant to be used for *in vitro* point-of-care (PoC) diagnostic tests.

4.2. New and challenging applications of bioaerosol samplers

4.2.1. Collection of viruses—

Exposures to viruses (Pan et al. 2019), including recent deadly pandemics related to SARS, H1N1, and H5N1 viruses (Fineberg 2014) present a serious public health threat. However, the collection of viral aerosols is challenging, and researchers only recently began to investigate the infectivity of viral bioaerosols in the nanoparticle size range (Walls et al. 2016). An excellent review on the sampling and extraction of viruses has been given elsewhere (Verreault, Moineau and Duchaine 2008), and here only the main points, as well as some developments since the review, will be discussed.

AGI-30 and the BioSampler collectors have been used to collect viruses, but their physical capture efficiency for viruses is low (Hogan et al. 2005). However, BioSampler showed good biological efficiency and was successfully applied to conduct surveillance for novel influenza viruses in pig barns (Anderson et al., 2016) and is often used as a reference tool when investigating other samplers for collection of viruses. The infectivity of influenza A

virus sampled for 60 min with a NIOSH two-stage dry personal cyclone was 15% compared with a BioSampler (Cao et al. 2011). On the other hand, the same personal cyclone successfully recovered viral RNA in the cough aerosols from 32 of 38 influenza-positive patients (Lindsley et al. 2010). Efficient capture of viruses was achieved using ESPs with a novel charging system (Hogan, Lee and Biswas 2004; Kettleson et al. 2009), including for the use of plaque assays and qPCR. An ESP with 150 μL of collection fluid was used to capture influenza viruses for point-of-care applications (Ladhani et al. 2017). A personal ESP was found to capture 10x higher concentrations of MS2 and T3 viruses compared to a BioSampler, while the recovery of T3 was almost 1000x higher (Hong et al. 2016). In fact, as mentioned above, the utility of ESPs for capturing viral particles was described as early as in the 1960s (Gerone et al. 1966).

The earlier-mentioned liquid sampler with a bubbling technique was demonstrated to efficiently capture and maintain the viability of viruses (Agranovski et al. 2002a; Agranovski et al. 2002b; Agranovski et al. 2005), and detect them using real-time PCR (Pyankov et al. 2007). Viral particles are usually smaller than the typical filter pore sizes but can be collected using filters due to diffusion (Burton, Grinshpun and Reponen 2007). Gelatin filters are also used, but their performance could be negatively affected by too-low or too-high humidity (Verreault, Moineau and Duchaine 2008). A comparative study showed that the BioSampler had collection efficiency at 5% for H1N1 influenza A virus compared to 1.5% efficiency of gelatin filters (Li et al. 2018). Capture efficiency of viruses is also affected by their morphology and hydrophobicity. The hydrophilic viruses were captured 10–100x more efficiently than hydrophobic viruses when using traditional samplers such as the Andersen impactor, AGI-30 impinger, gelatin filter, and Nuclepore filter (Tseng and Li 2005). The successful capture of bovine adenovirus serotype 3 virus on polyurethane foam (PUF) within the Airborne Sample Analysis Platform (ASAP) 2800 (Thermo Scientific, USA) at laboratory conditions was reported (Sharma et al. 2015).

Since the small size of viral particles is one of the challenges in their capture, laminar-flow water condensation method was used to increase the size of MS2 bacteriophage, and it captured 20*x* infective virions compared to AGI-4 impinger (Walls et al. 2016). Another group used a similar concept of a growth tube collector to capture MS2 coliphages and influenza H1N1 virus (Lednicky et al. 2016; Pan et al. 2016). The second generation of this technology was successfully used to determine the size distribution of infectious viruses in aerosol particles (Pan et al. 2019). A new sampler, called the G-II, collects exhaled-breath particles by using a slit impactor to remove particles larger than 5 μm and then grows the remaining particles up to 1 μm to be captured by another slit impactor followed by deposition into a buffer-containing collector (McDevitt et al. 2013). Collection efficiency of G-II for influenza virus was reportedly similar to that of BioSampler.

4.2.2. Collection of bioaerosols for sequencing—The investigation of airborne microbial diversity in various environments is a relatively new but very active area of inquiry, accelerated by the decreased cost of gene sequencing and advances in bioinformatics. At the same time, the efficiency of sampling and recovery of genetic material as well as the results of sequencing, including the number of identified operational taxonomic units (OTUs), depend on the sampling techniques used and it is critical to

examine this dependency as it affects our understanding of air environments (Mbareche et al. 2018). For example, it was found that only 14% of the bacterial OTUs and 44% of the fungal OTUs were shared in four collocated bioaerosol samplers (Hoisington et al. 2014). The same authors indicated that microbial communities were much more diverse in the settled dust and home filters than in the shorter-term bioaerosol samples. This finding underscores the need for long-term integrated sampling of bioaerosols. A preferential collection of some microorganism species by particular samplers was also noted in a study of Mexico city air (Serrano-Silva and Calderón-Ezquerro 2018). Laboratory and field experiments showed that the filter-based Button Aerosol Sampler and the liquid-based BioSampler yield different microbial communities (Zhen et al. 2018). An electrostatic dry filter was found to consistently yield more fungal and bacterial OTUs compared to two liquid cyclonic samplers (Mbareche et al. 2018). A field study in a mountainous area and at a seaside used a filter sampler, impactor, and impinger and indicated a good agreement regarding dominant species but not so for less prevalent species (Fahlgren et al. 2011). While filters from residential environments present a tool to examine long-term exposures, the sample elution protocol affects the recovery of microbial communities (Maestre et al. 2018). A sampler's ability to capture different particle fractions should be taken into account as the microbial diversity in $PM_{2.5}$ and PM_{10} fractions was found to be different (Bowers et al. 2013; Fan et al. 2019). Passive dust collectors have also been explored for use in sequencing (Adams et al. 2015), and the authors found that plastic Petri dishes produced the highest yields, followed by sampling with an EDC. On the other hand, high fidelity recovery of genomic material was reported when using condensational capture (Nieto-Caballero et al. 2019). Given the biases that could be introduced by the sampling method, it has been suggested that a sampler's efficiency and ability to cover microbial diversity be considered when selecting tools to investigate microbial diversity (Maestre et al. 2018). In addition, platform and chemistry selection, data processing techniques, and bioinformatic analyses have not been standardized, therefore comparison among studies needs to be performed with caution (Pauvert et al. 2019).

5. Bioaerosol sampling considerations

The selection and use of a particular bioaerosol sampler could have a profound effect on the study results, and the following section discusses some of the practical aspects of bioaerosol sampling. A more extensive discussion could be found elsewhere (ACGIH 1999; Cox and Wathes 1995).

5.1. Selecting a sampler

A specific sampling technique and analytical method have to be chosen to best fit the objectives of a project (Cox and Wathes 1995; Griffiths and Stewart 1999). When selecting a bioaerosol sampler, one should consider bioaerosol type to be investigated, anticipated bioaerosol particle size range and concentration, sampling environment, analysis type, amount of biomass needed and feasible sampling duration and frequency and then decide upon the best tool for the task. A reverse process, where a sampler is selected first, and then the study parameters are made to fit the sampler, is also feasible but is not always

the best solution. Ease of cleaning or disinfecting the sampler between runs should also be considered to minimize sample contamination or "cross-pollination."

If the culturable analysis is used, then the sampler and collection medium best suited for culturable analysis should be chosen. For sample analysis by multiple techniques, a sampler with a liquid collection medium is preferable. Filter samplers could also be used for this purpose, but particle elution from a filter is an extra step, and it can lead to particle losses. Sampler choice will also be affected by whether an area or personal samples will be taken. If a personal sampler is used, one has to consider which particle fraction(s) will be sampled: total, PM_{10}, $PM_{2.5}$, inhalable, thoracic, respirable, or combination thereof.

5.2. Collection medium

The type and amount of collection medium are typically recommended by the manufacturer or a sampler developer. However, one should be aware of potential issues. For example, when using liquid-based samplers that do not automatically maintain liquid level, liquid lost due to evaporation has to be periodically replenished to extend sampling time (Han et al. 2015a; Simon et al. 2016). Viscous collection fluid, e.g., mineral oil, could also be used to minimize evaporation (Lin et al. 1999; Simon et al. 2016); however, that makes the microorganism extraction somewhat challenging. Mineral oil was shown to be compatible with qPCR when it was loaded into RCS High Flow centrifugal sampler (He and Yao 2011). Automated collection systems, such as SASS-2400 (Research International, Monroe, WA), maintain collection liquid level, but they are more expensive than traditional liquid-based samplers.

The collection liquid used in bioaerosol sampling typically is deionized and autoclaved water (Liu et al. 2012), NaCl solution or phosphate buffer saline (PBS) solution with or without surfactants such as Tween-20, Tween-80, Triton X-100, or an antifoaming agent (Chang, Ting and Horng 2019; Dungan and Leytem 2016). PBS provides neutral pH for the organisms and is often the preferred medium when sampling bacteria. The use of Tween, however, was shown to increase the amount of cell membrane damage (Zhen et al. 2013). Specific substances could be added to improve the recovery of target microorganisms (Pan et al. 2019). PBS containing surfactant collected fungi better than that without surfactant (Chang, Ting and Horng 2019).

While the amount of agar in impactors is not standardized, typically Petri dishes are filled with 25–40 mL of agar. Since the amount of agar affects the jet-to-plate distance and the strength of air jets when they reach the agar plate, the agar amount was found to affect sampler performance (Therkorn and Mainelis 2013). Thus, standardization of agar amount used in agar-based impactors should be considered by the bioaerosol sampling community. Given the different impactor designs, the standards could be sampler specific. The choice of agar type depends on the analysis protocols. Trypticase soy agar (TSA) is often used as a general medium for bacteria while sabouraud dextrose agar (SBX) or malt extract agar (MEA) is commonly used for the mold. For the latter, antibiotics are often added to inhibit the growth of bacteria.

5.3. Physical bioaerosol losses inside samplers

Internal losses of particles during sampling should be considered. For liquid-based samplers, such losses may occur due to particle bounce and reaerosolization (Grinshpun 1997) or due to particle losses to internal sampler surfaces (Han and Mainelis 2012; Kesavan 2012). In some cases, such losses could exceed 50%, especially when low amounts of collection fluid are used (Han and Mainelis 2012). Innate losses could be minimized post-sampling by washing a sampler's interior surfaces multiple times coupled with vortexing and/or sonication. Particle losses due to bounce and reaerosolization could be reduced by using viscous collection fluid (Lin et al. 1999; Simon et al. 2016). Temperature-controlled sampling with impingers also was shown to mitigate particle bounce (Springorum, Clauß and Hartung 2011).

Particle bounce is also a potential issue in agar impactors, especially when the sampling process creates high-velocity air jets or agar desiccates due to prolonged sampling time (Zhen et al. 2009). The bounce in impactors could be minimized by using mineral oil to cover the plates, application of which also enhanced recovery of culturable organisms (Xu et al. 2013). A spin-coating by silicon oil reduced bounce in a microscale cascade impactor (Kang et al. 2012).

Overall, sampler operators should take steps to minimize particle losses during sampling. If the extent of losses is known based on literature or preliminary investigations, they should be taken into account when analyzing and presenting the results. However, since the extent of losses typically depends on particle size, which is not known a priori, an estimate of losses is difficult and rarely performed.

Since particle losses affect the accuracy of sampling, the bioaerosol research community should work toward developing standard sampling protocols to minimize losses during sampling and also establish procedures to take losses into account during data analysis and presentation.

5.4. Use of filters

The main advantage of using filters for bioaerosol collection is ease of use and potentially long sampling times. However, the latter can also become a disadvantage as air passing through filters desiccates the collected particles (Grinshpun et al. 2015; Jensen et al. 1992; Lundholm 1982; Thorne et al. 1992; Wang et al. 2001) minimizing sample recovery via culture methods. Gelatin filters or wetted porous foams could be used to reduce the effect of desiccation (Chang and Chou 2011; Chang and Hung 2012; Kenny et al. 1999; Wu, Shen and Yao 2010; Yao and Mainelis 2007a). Gelatin filters can be dissolved in a buffer (Cox and Wathes 1995) or placed directly on agar for culturable analysis(Yao and Mainelis 2007a). Gelatin filters have also been used to collect viruses (Li et al. 2018). However, gelatin filters or wet foams eventually desiccate, offering only limited protection, especially when sampling for a longer time in dry environments (Li et al. 2018). Gelatin filters also have been reported to have some inhibitory effect on PCR analysis (Yamamoto et al. 2010).

Sample loss during elution from filters should also be considered (Dabisch et al. 2012). The particle loss during elution could be especially pronounced when using fibrous or other

"in-depth" filters (Adams et al. 2015). However, elution efficiencies of 80% or higher can be achieved with properly designed elution protocols (Therkorn et al. 2017b; Wang et al. 2001). The losses could also be minimized by using polyethersulfone (PES) membrane filter, such as Supor®, as it dissolves in a buffer eliminating the elution step which is convenient to use with molecular techniques (Rahav et al. 2019; Zhen et al. 2018).

The filtration process can negatively affect not only bioaerosol culturability but also the structural integrity of the microorganisms, even leading to DNA leakage (Zhen et al. 2013). Long sampling durations offered by filtration allow direct bioaerosol examination by microscopy, but the user should be aware of potential filter overloading and contamination by non-biological particles.

5.5. Sampling duration and frequency

Properly selected sampling duration and frequency are essential elements of any successful bioaerosol sampling campaign. Sampling duration is typically dictated by the purpose of the study, bioaerosol concentration, selected or available bioaerosol sampling tools, collection medium, and intended analytical technique. For example, personal samples should be taken for the duration of targeted activity or the length of a work shift; for environmental sampling, sampling duration should be sufficiently long to result in a representative sample. Sampling frequency will depend on how rapidly the bioaerosol concentration is anticipated to change (Cox and Wathes 1995). For fast changing levels, it is advisable to collect multiple samples, especially when the peak exposures are a concern; for long-term integrated samples, longer sampling times or integration of several samples are preferred. However, the selection of sampling times is often a challenge due to the particular limitations of sampling tools.

As mentioned above, the use of filters allows for long sampling durations; however, that may result in damage to bioaerosol particles. When using agar impactors, one has to choose the sampling duration especially carefully or better yet take several samples of different length: if the sampling time is too short, there will be too few colonies for a reliable or a quantifiable result; if sampling time is too long, the number of available "spots" in multi-nozzle impactors will be filled before sampling is completed leading to concentration underestimates. Thus, for multi-nozzle impactors, some studies limit the duration of individual samples to 5 min (Levetin 2004). Desiccation of agar due to prolonged sampling also leads to decreased culturable counts (Mainelis and Tabayoyong 2010). A description of how to calculate an optimal sampling time has been presented elsewhere (Nevalainen et al. 1992).

Sampling duration is also a potential issue for spore traps, where the impaction area is limited and can be easily overloaded. Thus, the sampling duration for spore traps is typically limited to 15 min (Codina et al. 2008). A useful sampling strategy is to take spore samples of different durations.

Liquid collection medium is rarely overloaded, and liquid-based samplers could be used in high concentration environments. On the other hand, as discussed above, the collection fluid

evaporates over time and has to be periodically replenished, which requires additional effort or personnel; here one can use samplers with automated liquid volume control.

The effect of sampling duration on the quality of samples collected by ESPs remains a subject of debate. There is a concern that long sampling times can negatively affect collected bioaerosols due to prolonged exposure to moving air, charging ions, and high collection voltage. On the other hand, some developments show successful sampling for several hours without a significant negative effect on sample viability compared to a reference sampler (Han, Thomas and Mainelis 2018). Use of ESPs with a flow-through collection system largely avoid this issue, provided the bioaerosol was not negatively affected before being transferred into liquid.

There is an increased interest in passive bioaerosol sampling (Adams et al. 2015; Therkorn et al. 2017b; Yamamoto et al. 2011) as that allows sampling for several days and weeks without the operator's attendance. The effect of sampling duration on passive sample quality and representativeness remains a subject of investigation (Viegas et al. 2018a).

5.6. Use of blanks

The use of blanks and controls is an essential element of a sound Quality Assurance/Quality Control (QA/QC) protocol. When using agar plates either for sampling or sample analysis, some blank agar plates should be set aside and incubated. Any observed microbial growth should be subtracted from the actual sampling results. If the observed growth in the blanks is substantial and comparable to the sampling results, then that particular agar batch should be discarded and the experiment repeated. A similar approach should be used with all collection media in order to determine its contribution to the analytical signal.

Another vital element of QA/QC is the use of the field, or dynamic, blanks. Here the collection media and a sampler are handled in a regular way but stopping short of taking an actual air sample. Instead, before taking a sample, the collection media are removed from the sampler and analyzed. Any observed analytical signal is subtracted from the actual sample signal. It is recommended that the field/dynamic blanks constitute ~10% of the total sample number. If the total sample number is small, then at least 1–2 field blanks should be included. For a more exhaustive discussion on QA/QC, the reader should consult existing literature on (ACGIH 1999) and follow best practices and established protocols.

5.7. Sampler placement

Sampler placement can affect the quality and representativeness of samples. These issues are discussed more extensively in other manuscripts of this special issue focusing on indoor and outdoor bioaerosol studies (Cox and Lindsley 2019; Temkiv and Huffman 2019). Briefly, when sampling indoors, samplers are typically placed approximately 1.1 m above the floor (USEPA 1994). Other best practices include avoidance of strong airflows at the point of sampling (Haig et al. 2016). If the goal of sampling is to determine a representative airborne concentration for an indoor space, then a sampler(s) should not be placed near conspicuous bioaerosol sources. Of course, the opposite is correct when a source strength is the target of the investigation, e.g., bioaerosol emissions due to coughing (Lindsley et al. 2010). Also, when sampling in indoor spaces, the sampler aspiration efficiency requirements for calm

air should be met (Hinds 1999), and the sampler's inlet should be placed horizontally to minimize sampling bias (Haig et al. 2016). Study personnel should consult the literature for best sampling practices in specific environments, such as healthcare (Haig et al. 2016), or when sampling for specific bioaerosols, e.g., viruses (Verreault, Moineau and Duchaine 2008) or endotoxin (Duquenne, Marchand and Duchaine 2013).

Sampling outdoors presents its own set of challenges as wind, and its direction and speed may affect a sampler's aspiration and collection efficiencies (Li, Lundgren and Rovell-Rixx 2000). The aspiration efficiency of bioaerosol samplers has been explored for calm air sampling (Grinshpun 1993) but not sufficiently yet for environments where strong air currents are encountered. Certain samplers, such as the Button Aerosol Sampler, have a dome-shaped inlet which minimizes their sensitivity to the wind (Kalatoor 1995). During sampler comparison, the effect of wind conditions is taken into account when the relative physical and biological efficiencies are calculated. However, the obtained result will be valid for just this specific set of conditions and the samplers being compared. Thus, the performance evaluation of new and existing bioaerosol samplers should include wind tunnel or similar tests at different air velocities (Su et al. 2012b) and directions (Su et al. 2012a).

6. Goals for the community to work towards

6.1. Harmonization of reporting guidelines

The high number of available bioaerosol samplers and a variety of their applications have resulted in diverse sampling and reporting protocols. The lack of standard sampling and reporting protocols often make it difficult to compare results from various studies thus slowing down our ability to develop a holistic understanding of bioaerosol presence, their role in the environment, and our exposures to bioaerosols and resulting health effects. Harmonized sampling protocols would also improve the reproducibility of bioaerosol studies. Several excellent references are available to help select bioaerosol sampling protocols (ACGIH 1999; Jensen et al. 1994; Reponen, Willeke and Grinshpun 2011), but more work is needed to develop standard bioaerosol sampling and reporting guidelines, especially given rapid advances in sampling technologies and analytical tools. At a minimum, a bioaerosol study report should include the following aspects: 1) sampler or samplers used, including their d_{50}, sampling flow rate, and any other relevant operating parameters; 2) sampling duration, frequency, and number of samples; 3) sampling media, including its type and preparation protocol; 4) environmental conditions, including temperature, relative humidity (RH), wind speed and direction, season and time; 5) presence of any conspicuous sources near the sampling site(s); 6) sample handling and storage procedures; and 7) sample analysis details.

6.2. Harmonization of bioaerosol sampler testing protocols

Another vital aspect of harmonized reporting is the description of sampler testing and comparison studies. This is an active area of research, and authors often choose their individual testing approaches. Having harmonized sampler testing and comparison guidelines will help move the field forward, including gaining a better understanding of sampler performance in various environments and conditions. Testing of bioaerosol

samplers can be typically divided into the investigation of their physical collection efficiency and biological performance. Below are some suggestions regarding the reporting on both aspects.

6.2.1. Determination of physical collection efficiency—When a sampler's physical collection efficiency is evaluated, the authors should report all relevant technical parameters, including a sampler's operating conditions, test particle type and size, particle generation parameters, as well as analytical techniques used to quantify the test particles. The manuscript or report should also describe how the collection efficiency was determined and what the reference point or sampler was.

Depending on the testing protocol, the measurement of collection efficiency can often be divided into a) total collection efficiency, which is determined by comparing particle concentration upstream and downstream of the sampler and does not differentiate between the particle losses inside the sampler and the actual collection efficiency; and b) the actual collection efficiency, which is a quantity of test particles or substance that ended up in the collection medium vs the reference quantity, which is typically determined through isokinetic sampling in the test chamber, or using a reference sampler. Both of these metrics should be reported as they describe different aspects of sampler performance. They allow determining the extent of particle losses within the sampler and may lead to a further improvement of the sampling technology. If the post-sampling process involves eluting particles from a collection medium, then the elution efficiency should be reported.

6.2.2. Determination of biological sampler performance—The biological performance, or bioefficiency, of a sampler, could be understood as its ability to maintain specific biological properties of the sample, such as culturability, viability, and membrane integrity, among others. A sampler's biological performance depends on its design, sampling mechanism, sampling duration, operating parameters, bioaerosol type, and environmental parameters such as temperature and RH. As discussed above, to some degree, all samplers negatively affect bioaerosol particles. Since the absolute biological performance is hard to establish, the relative biological performance is determined by comparing the output of a sampler being investigated to a reference sampler. Such comparative studies are typically specific to the samplers and the test bioaerosol species used, testing environment, and analytical technique. Because of differences in testing conditions, which could be subtle, the results from seemingly similar comparative studies can often be different. Thus, there is a strong need to standardize bioaerosol sampler comparison protocols and reports as that would help integrate results from various studies and help improve the reproducibility of such comparisons. In addition to technical sampler parameters, the bioefficiency testing report should specify, at a minimum, test bioaerosol species used, their origin, preparation, and analytical method. The authors should also determine and report the initial condition of the test bioaerosol, e.g., viability, culturability, and other relevant metrics. Aerosolization of test species is another critical aspect of sampler comparison because it may negatively affect bioaerosol properties before it even reaches the test samplers. Aerosolization of bioaerosols is addressed in another manuscript in this special issue (Thomas et al. 2019). Also, the

author would like to encourage more sampler comparisons in diverse field environments as that represents the actual environments where the samplers will be used.

It would also be beneficial to develop a searchable database on bioaerosol comparison studies and comparison metrics used. The database would help improve the reproducibility of comparison studies and help users to select the best tools for their applications. It is evident that the development and maintenance of such a database would be a substantial undertaking. However, the author believes that the benefits of such a database would outweigh the burdens of its development.

Another step toward harmonizing the testing and reporting guidelines could be a series of active, hands-on workshops, where multiple investigators jointly perform sampler evaluation and comparison in the laboratory and/or the field. Such workshops would be especially useful when investigating the performance of new sampling technologies or exploring new sampler applications. Such instrument calibration workshops have been successfully performed in other fields, including particle measurements (Cross et al. 2010).

7. Grand challenges in the field

The bioaerosols are very diverse in terms of their sizes, species, biological properties, and requirements for their detection and quantification. Their diversity is one of the reasons that a universal bioaerosol sampler does not exist and, short of a Tricorder-type device featured in Star Trek, probably cannot exist. On the other hand, tremendous strides in bioaerosol sampling technology have been achieved over the past couple of decades, and many more promising technologies are under development. Yet, a number of challenges remain, and many of them have been discussed in the Grand Challenges Forum at the 2018 International Aerosol Conference (St. Louis, MO). These challenges could be broadly grouped as the need to understand personal exposures, examine the spread of microbial vectors, and investigate the role of bioaerosols play in environmental and climate processes. All of these areas of inquiry require the development and adaptation of advanced sampling tools.

To better understand personal bioaerosol exposures and their connection with long-term health effects, sampling tools are needed that allow us to differentiate between personal bioaerosol exposures and ambient bioaerosol concentrations. A personal bioaerosol sampler responding to these challenges has to be lightweight, unobtrusive, and easy to wear, capable of sampling for prolonged time periods, and with robust and convenient sample removal. Application of such a personal bioaerosol samplers will help us link individual exposures with health effects. As discussed above, the work in this area is already ongoing.

To be able to answer environmental questions, such as “how to detect and predict the spread of microbial vectors of disease” or “what the climatic and environmental effects of bioaerosol are,” most likely, multiple sampling technologies will be required. At a minimum, the researchers should minimize sampling in limited locations and short durations and seek sampling technologies that provide high spatiotemporal resolutions of various bioaerosol

species and quantities. For determining long-term bioaerosol trends, samplers capable of long-term unsupervised sampling are needed.

There is a renewed interest to investigate bioaerosol presence in various challenging environments, such as at high altitudes (Smith et al. 2018) or extreme temperatures. In many cases, bioaerosol concentrations in such environments are low. Thus, samplers needed to meet those challenges will have to be lightweight to be fitted onto drones or balloons (Spring et al. 2018) yet capable of concentrating bioaerosol samples for reliable detection.

The emergence and application of low-cost sequencing tools have shown great promise to advance our understanding of airborne microbiological environments. At the moment, however, we have a limited understanding of whether and how bioaerosol sampling tools affect the representativeness of airborne microbial assemblages due to preferential sampling and extraction of bioaerosol species. The studies to understand this potential bias have already begun (Mbareche et al. 2018; Zhen et al. 2018), but a great deal remains to be done. A related area of development is the integration of bioaerosol samplers with advanced analytical tools, such as laboratories-on-a-chip and other fast detection technologies. The work in this area has already yielded exciting prototypes (Choi et al. 2017; Pardon et al. 2015). In order to accelerate advances in this area, the bioaerosol community should engage the analytical community so that we are able to “speak each other’s language” and better understand the requirements of our respective technologies.

8. Summary

Bioaerosol sampling is a fascinating and challenging field. Tremendous advances have been made over the past decades; however, much work remains to be done, including the development and adaptation of tools that will help meet the challenge in the field. Another challenge is a better understanding of the performance of bioaerosol sampling tools in various applications and development of harmonized sampling and reporting protocols. The development and adaptation of such protocols will help move the field forward, including improved reproducibility of results. It is hoped that this manuscript will stimulate a discussion within the bioaerosol research community regarding the current research and reporting needs.

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