AIR QUALITY

Bioaerosol Sampling in Animal Environments

AIR QUALITY EDUCATION IN ANIMAL AGRICULTURE

Measuring: Bioaerosols
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This publication discusses different samplers that can be used for collecting bioaerosols from environmental air and considerations for choosing a suitable sampler.

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Bioaerosols in General

Bioaerosols are particles of biological origin suspended in the air. In outdoor air, 30 percent of all particles larger than 0.2 µm appear to be of biological origin.[1] Particle sizes and natural background concentrations of bioaerosols have been reported as following:

Table 1. Bioaerosol sizes and concentrations in natural background[2].

Type of Bioaerosols	Size (µm)	Concentration (#/m³)
Viruses	0.02-0.3	---
Bacteria	0.3-10	0.5 – 1,000
Fungal Spores	0.5-30	0 – 10,000
Pollen	10-100	1 – 1,000

As shown in *Table 1*, bioaerosols cover a wide size range, of which viruses are the smallest. Viruses, bacteria, and fungal spores that are capable of causing disease are referred to as pathogens. Some pathogens are specific for certain hosts, like humans or animals, while others can infect a wide host range that may include humans as well as animals. Those pathogens capable of infecting both humans as well as other animals are called zoonotic pathogens.

Bioaerosol Classification

Depending upon their biological characteristics, bioaerosols can be classified into the following types:

Viruses

Viruses can infect and reproduce only inside a susceptible host cell. As intracellular parasites, viruses never replicate on nonliving substrates in the environment. Several facts about viruses include[2]:

- Naked viruses range in size from 0.02 – 0.3 µm.
- Most airborne viruses are part of droplet nuclei or attached to other particles with a wide range of sizes.
- Airborne viruses are transmitted by direct contact, or by inhalation of aerosolized viruses.
- Aerosolization of viruses may occur by coughing, sneezing, or talking.
- Under favorable ambient conditions (temperature, humidity, etc.), most viruses can survive for weeks.

Bioaerosols are particles of biological origin that are suspended in environmental air.

Bacteria

Bacteria are single-celled organisms that range in size from 0.3 to10 µm. Several facts about bacteria include[2]:

- Bacteria are generally spherical or rod-shaped.
- Bacteria often occur as clusters or chains.
- Under ambient conditions, bacteria colonize water or soil and will be released as aerosols when the water or soil is disturbed.
- In indoor environments, bacteria can survive and colonize in moist environments, such as ventilation systems, and are aerosolized by air currents or vibration.

In addition, bacteria can be classified into two groups based upon their ability to retain crystal violet dye[1]:

- Gram-positive bacteria (retain the dye) have cell walls that consist mainly of peptidoglycan, which can cause adverse health effects.
- Gram-negative bacteria (don't retain the dye) have cell walls that contain an outer membrane composed of lipopolysaccharides (endotoxin), lipoprotein, and other complex macromolecules that can cause adverse health effects.

Specifically, **endotoxins in the outer membrane of Gram-negative bacteria** are chemically stable and heat resistant. Endotoxins are released when the bacterial cells are lysed and will maintain their biological activity (i.e., adverse health effects), even after the bacterial cells are no longer viable. Moreover, they are abundant in agricultural environments, with the highest reported airborne endotoxin levels of 2 to 7 µg/m_3 in cotton mills and are associated with fecal material in agriculture or wastewater treatment. For comparison, ambient background levels of endotoxins in most outdoor environments are in the subnanogram range. The adverse health effects associated with exposure to endotoxins include fever, malaise, changes in white blood cell counts, and respiratory distress.

Fungi

Fungi are a unique group of organisms that can occur as single-celled organisms (e.g., yeasts) or as multicellular branching structures. To date, 70,000 of 1.5 million known fungi have been identified and classified[2]. They are grouped based upon their methods of spore production. Most adverse health effects associated with fungi include allergic reactions and respiratory diseases.

Pollen

Pollen grains, produced by plants to transmit genetic material, are near spherical particles that range in size from 10 to 100 µm, with most between 25 and 50 µm. Although pollen grains are not in the respirable size fraction (can deposit in the lungs), they may contain allergens that lead to adverse health effects in the upper airways (hay fever). In addition, airborne pollen grains are resistant to environmental stresses (e.g., desiccation [drying out], etc.) and may be transported by insects or wind over a wide geographical range.

Bioaerosol Viability and Infectivity

Once released, bioaerosols may travel considerable distances due to atmospheric dispersion. The ability of bioaerosols to cause adverse health effects or to initiate disease depends upon their ability to survive and remain infectious in the environment, as well as their exposure to a susceptible host. The survival or viability of bioaerosols is referred to as their ability to replicate, whereas the infectivity of bioaerosols is referred to as their ability to cause infection. Factors that influence bioaerosol viability and infectivity include:

Relative Humidity and Temperature

Many bioaerosols are hygroscopic (can readily take up and retain water from the environment). After their generation, the viability of bioaerosols are impacted by the rate of water transfer (dehydration or rehydration), which is dependent upon relative humidity (RH) and temperature in their environment. Most bioaerosols tend to lose viability due to desiccation[3] (drying out).

Oxygen

Oxygen can be toxic for some species of microorganisms. Anaerobic airborne bacteria may lose viability due to oxygen toxicity, with their survival dependent upon storage time, oxygen concentration, and RH[3]. However, most bacterial species in animal building aerosols are facultative anaerobes, which means that they thrive under low oxygen conditions but can survive and remain viable over long periods of time when exposed to ambient levels of oxygen in the environment.

Other Pollutants

It has been reported that pollutants such as sulfur oxides and nitrogen oxides have less effect on bioaerosols than ozone. Outdoor bioaerosol survival of many species is much poorer than those indoors due to the presence of ozone in outdoor environments. Because of this, the spread of airborne diseases is more likely indoors than outdoors[3].

Radiation

Energetic radiation (e.g., UV) induces free-radical mediated reactions causing damage of nucleic acid, protein, sugar, lipid, and membranes within microbes. Long wave radiation (e.g., microwave) has much lower energy, and is considered to have limited impact on bioaerosol viability[3].

Bioaerosols can be made up of many different types of particles, including bacteria, viruses, parasites, fungi, and other particles, such as pollen, that may lead to adverse health effects in an exposed population.

Bioaerosols in Animal Environments

In intensive livestock production systems, bioaerosols are often rich in both variety and number[3]. The major sources of bioaerosols are animals, animal wastes, feed, and bedding materials. The nature and concentration of bioaerosols in animal environments may be essential in the etiology of animal diseases. It has been reported that pathogens of livestock transmitted through the air may cause infectious diseases in farm animals, as well as in human farm workers and residents living in close proximity to farms. *Table 2* lists some of the diseases that have been linked with airborne pathogens in animal houses. The common pathogenic bioaerosols identified in poultry and pig houses are listed in *Table 3*.

Table 2. Common infectious disease of farm animals and pathogens[3]

Host	Diseases	Factors implicated in causation	
		pathogens	environment
pigs	Atrophic rhinitis	*Bordetella bronchiseptica* *Pasteurella multocida*	Crowding Poor ventilation
	Enzootic pneumonia	*Mycoplasma suipneumoniae*	Poor drainage, high relative humidity
cattle	Diarrhea Pneumonia	*Rotavirus, E. Coli, etc.* *Mycoplasma bovis, dispar*	Weaning, hygiene, cold Crowding, poor feeding
	Shipping fever	*P. haemolytica, etc.*	High relative humidity, stress
	Environmental mastitis	*E. Coli, strep. uberis*	Contaminated bedding, stage of lactation
horses	Obstructive pulmonary disease	*Mycropolyspora faeni* *Aspergillus fumigatus*	Dusty feed and bedding, poor ventilation

Table 3. Common pathogenic bioaerosols identified in poultry and pig houses[3]

Bacteria	**Fungi**	**Viruses**
Bordetella bronchiseptica	*Aspergillus flavus*	African swine fever
Brucella suis	*Aspergillus fumigatus*	Avian encephalomyelites
Corynebacterium equi	*Aspergillus nidulans*	Avian leukosis
Erysipelothrix rhusiopathiae	*Aspergillus niger*	Foot-and-mouth disease
Escherichia coli	*Coccidioides immitis*	Fowl plague
Haemophilus gallinarus	*Cryptococcus neoformans*	Hog cholera
Haemophilus parasuls	*Histoplasma farcinorum*	Inclusion body rhinitis
Haemophilus pleuropneumoniae	*Rhinosporidium seeberi*	Infectious bronchitis of fowls
Listeria moncytogenes		Infectious laryngotracheitis of fowls
Leptospira pomona		Infectious nephrosis of fowls
Mycobacterium arium		Infectious porcine encephalomyelitis
Mycobacterium tuberculosis		Marek's disease
Mycoplasma gallisepticum		Onithosis
Mycoplasma hyorhinus		Porcine enterovirus
Mycoplasma suipneumoniae		Swine influenza
Pasteurella multocida		Transmissible gastroenteritis of swine
Pasteurella pseudotuberculosis		
Salmonella typhimurium		
Straphylococcus aereus		
Streptococcus suis type II		

The major sources of bioaerosols are animals, animal wastes, feed, and bedding materials.

Bioaerosol Sampling

General Sampling Considerations

The specific characteristics of bioaerosols have great impacts on appropriate methods for sampling. In general, bioaerosol sampling applies the same sampling principles as those used for nonbiological aerosols. However, preservation of biological characteristics (e.g., viability) during and after sample collection makes bioaerosol sampling slightly different from nonbioaerosol sampling. Moreover, sample handling, storage, and analysis are different in bioaerosol sampling from that in nonbioaerosol sampling. The following factors require special consideration when planning to measure bioaerosols:

Objectives of Bioaerosol Sampling

Bioaerosol sampling plans should begin by determining the purpose of sampling. In general, bioaerosol sampling objectives include:

- verification and quantification of bioaerosol present (specific species or total bioaerosols),
- identification of sources that could lead to control and mitigation of bioaerosols, and
- subsequent monitoring to ensure the effectiveness of control measures implemented.

Temporal and Spatial Variations in Bioaerosol Concentrations

Bioaerosol concentrations generally have considerable variation temporally and spatially because bioaerosol sources don't necessarily generate bioaerosols continuously. The time and space dependant characteristics in bioaerosol concentrations have great effect on determining the optimal sampling duration and location. In addition, since no single sampling technique is capable of collecting, identifying, and quantifying all of the biological species of interest in the environment, it may require a source inventory or a preliminary microbiological analysis of possible sources to determine an appropriate sampling strategy.

Overall Sampling Performance

Overall sampling efficiency of a bioaerosol sampling system ensures accuracy in bioaerosol sampling and analysis. The overall performance of a bioaerosol sampler is determined by two factors:

- physical factors: inlet sampling efficiency and collection efficiency, and
- biological factors: preserving biological characteristics (e.g., viability) of bioaerosol particles during sampling and accurate biological analysis for identification and quantification of sampled bioaerosol particles.

Bioaerosol Sampling in Animal Environments

Challenges for Bioaerosol Sampling in Animal Facilities

In animal environments, bioaerosols cover a broad range of particle sizes and are rich in microbial species. During the sampling process, bioaerosol particles must be collected from air using an appropriate sampling protocol that will minimize bias in both physical and biological aspects mentioned previously. The following challenges should be addressed when determining an appropriate sampling protocol to fulfill a specific sampling objective[3]:

High Concentrations of Bioaerosols

High levels of bioaerosols present in animal houses may overload some samplers. In some cases, sampling times need to be shortened, or in other cases, a diluter system may be required for the sampling.

Requirement for Multiple Sampling Methods

Many diverse microbial species comprise bioaerosols in animal houses. For some studies, a comprehensive quantitative and qualitative analysis is necessary, which may require the use of multiple sampling and analysis methods.

Practical Constraints

Due to the overall high levels of microbes associated with large numbers of animals being housed within limited spatial restrictions, some practical constraints are always related to bioaerosol sampling in animal houses. Several of these considerations might be: proximity to the animals, proximity to the ventilation systems, and timing related to the life cycle of flocks or herds, as well as other logistical considerations.

Bioaerosol Sampling Processes

In general, bioaerosol sampling involves three main processes:

- **Collecting a Representative Sample**

Bioaerosol sampling aims to take a sample that is physically and biologically representative of the system. This process includes:

Determining location and number of sampling sites,
Selecting an appropriate sampler or sampling system, and
Determining sampling duration and frequency.

- **Conditioning and Transporting the Collected Sample**

Once the bioaerosol sample has been taken, it must be conditioned and transported to a microbiology lab for further analysis. Precautions should be taken so the physical and biological properties of the sample are preserved (i.e., refrigeration, observing sample holding times, etc.).

The specific characteristics of bioaerosols have great impacts on appropriate methods for sampling.

The selection of suitable bioaerosol samplers is guided by the objectives of sampling as well as the types and levels of bioaerosols of interest in the environment to be sampled.

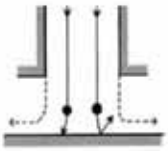
- **Analyzing the Samples for Biological Properties**

Due to the complex nature of bioaerosols in animal houses, no single biological analytic method is perfectly suited to study bioaerosols collected from animal housing facilities. Several different analytical methods may be employed to meet the objectives of the study/sampling.

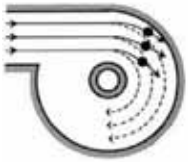
Bioaerosol Samplers

Principles of Bioaerosol Collection

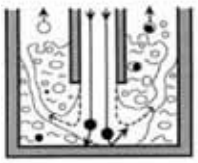
Similar to sampling for general particles in air, bioaerosol sampling also involves physical separation of particles from the air stream. One of the most common particle collection methods for bioaerosol sampling is inertial separation. *Figure 1* summarizes collection mechanisms for different types of inertial bioaerosol samplers.



1. Inertial impaction: the inertial of the particle forces its impaction onto a solid or semisolid impaction surface (a cultural medium or an adhesive surface) that can be examined microscopically. The application of this principle in bioaerosol sampling includes:
   - Single-stage impactors: the surface air sampler (PBI, SPI)
   - Cascade impactors: two or more impaction stages (the Anderson cascade impactor)
   - Slit samplers: the impaction stage consists of one or more slits instead of one or more circle holes (CAS, NBS, BAS cultural plate samplers)



2. Centrifugal inertial impaction: particle separation by centrifugal force in a radial geometry (Reuter centrifugal sampler, BIO)



3. Liquid impingement: the particles are collected by inertial impaction into a liquid, and particle diffusion within the bubbles (the AGI-4 and AGI-30 impingers)

4. Tangential impingement: collects particles by inertial impaction and centrifugation (BioSampler SKC)

Figure 1. Collection principles for different inertial bioaerosol samplers[1]

In addition to intertial separation, filteration is another common type of collection method used for aerosol sampling. For this method, impaction, interception, diffusion, and electrostactic attraction contribute to the deposition of particles onto the filter medium.

Sampling Efficiency of Bioaerosol Samplers

The overall sampling efficiency of a bioaerosol sampler is dictated by the following components[1,2]:

- the inlet sampling efficiency, which is the same as for non-bioaerosol sampling and depends on the size, shape, and aerodynamics of the particles being sampled,
- collection/deposition efficiency onto glass slides or a semisolid culture medium, and
- the biological aspects of sampling efficiency, which depend on collection of biological particles without altering their viability or biological activity and use of robust microbial analytical methods to identify and quantify the biological particles present in the air parcels sampled.

Bioaerosol Concentration Determination

Some bioaerosol particles, such as bacteria and fungi, can grow and replicate to form a colony after being collected on a nutrient or solid surface. Counts of these colonies are expressed as "colony-forming units," or cfu. For bioaerosol analyses, the term "cfu" is used to quantify numbers of viable microbes, with the concentration of bioaerosols defined as cfu per unit volume of air sampled, e.g., cfu/m^3.

During the sampling period t, the volume of sampled air can be calculated by the following equation:

$$V = Q * t \quad (1)$$

Where V is the volume of sampled air in m^3, Q is the flow rate of the sampling system in m^3/min, and t is the sampling time in minutes.

The average concentration of bioaerosols is determined by the following equation:

$$C = \frac{N}{Q * t} \quad (2)$$

Where C is the average concentration of bioaerosols in cfu/m^3, N is the number of viable bioaerosol particles collected on the impaction substrate, in cfu.

Optimal Sampling Time Determination

In animal environments, the concentrations of bioaerosols vary with time. Sufficiently long collection times or multiple samples with short collection times may be required during periods of changing concentration so that collected sample(s) may properly represent the average environmental concentration over some time period[1].

As shown in *Figure 2*, the bioaerosol concentration varies in a sampled air volume (V) during the sampling period t (from t_s to t_f). During this sampling process, the number of particles per unit area varies with bioaerosol particle concentration in the sampled air. This results in a change in surface density (δ) of the sample, which equates to the number of particles on the surface per viewing area (A), i.e., microbial colonies on a petri dish. The surface density of a bioaerosol sample is determined by the following equation[1]:

$$\delta = \frac{N}{A} = \frac{C * Q}{A} * t \quad (3)$$

Where δ is the surface density of a bioaerosol sample in cfu/m^2, A is the viewing area (i.e., petri dish) in m^2.

The selection of suitable bioaerosol samplers is guided by the objectives of sampling as well as the types and levels of bioaerosols of interest in the environment to be sampled.

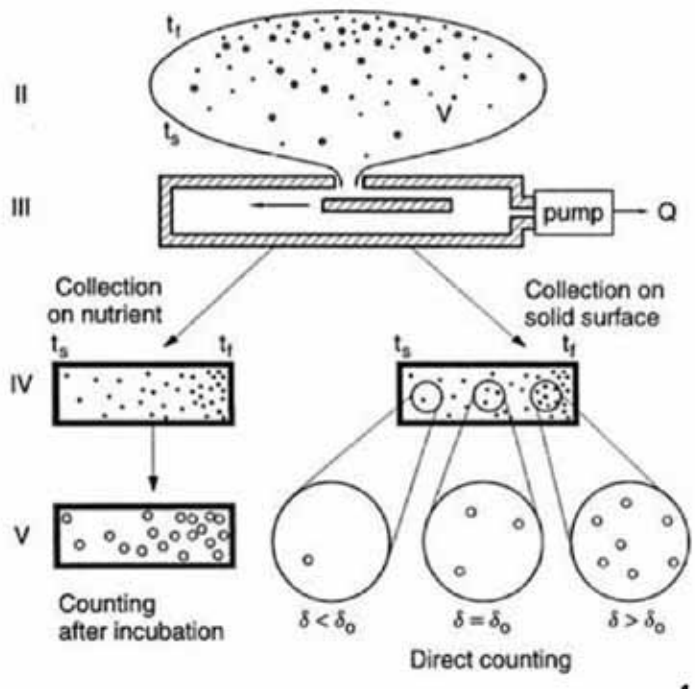


Figure 2. Bioaerosol sampling process[1]

In general, post analyses of bioaerosol samples include viewing, counting, and identifying the particles within the sample. This can occur following collection by viewing the collected particles under a microscope, or it may occur after an incubation period, which allows the colonies to grow to sufficient size so they can be counted without magnification. An accurate quantification of bioaerosols in a sample may only be obtained if the surface density of organisms is optimal, δ_o. If the sample surface density is very low, $\delta << \delta_o$ sampling and counting errors may be high. As a result, the calculated concentration may not be accurate and may misrepresent the true concentration in the original air sampled. On the other hand, if the sample surface density is very high, $\delta >> \delta_o$, the particles may be located in close proximity to each other, whereby the collected organisms may grow together or may inhibit each other's growth such that accurate counting and identification may not be possible.

As shown in equation 3, the surface density of a bioaerosol sample collected on a substrate is linearly related to sampling time. To avoid insufficiently-loaded samples ($\delta << \delta_o$) and overloaded samples ($\delta >> \delta_o$), the sampling time should be adjusted accordingly. The optimal sampling time for a given bioaerosol concentration depends upon sampler flow rate and collection surface area as demonstrated by the following equation[1]:

$$t_o = \frac{A}{Q * C} * \delta_o \qquad (4)$$

The calculated optimal sampling times for several commercially available bioaerosol samplers are illustrated in *Figure 3* (left). Impinger samples are not sensitive to under- or overloading during sampling because the liquid sample can be diluted or concentrated following sample collection, depending on the concentration of collected bioaerosol particles in the liquid. However, evaporation of sampling liquid and reaerosolization of prior-collected particles limit the sampling time for most impingers. *Figure 3* (right) also illustrates optimal sampling time for impingers at a sampling flow rate of 12.5 l/min.

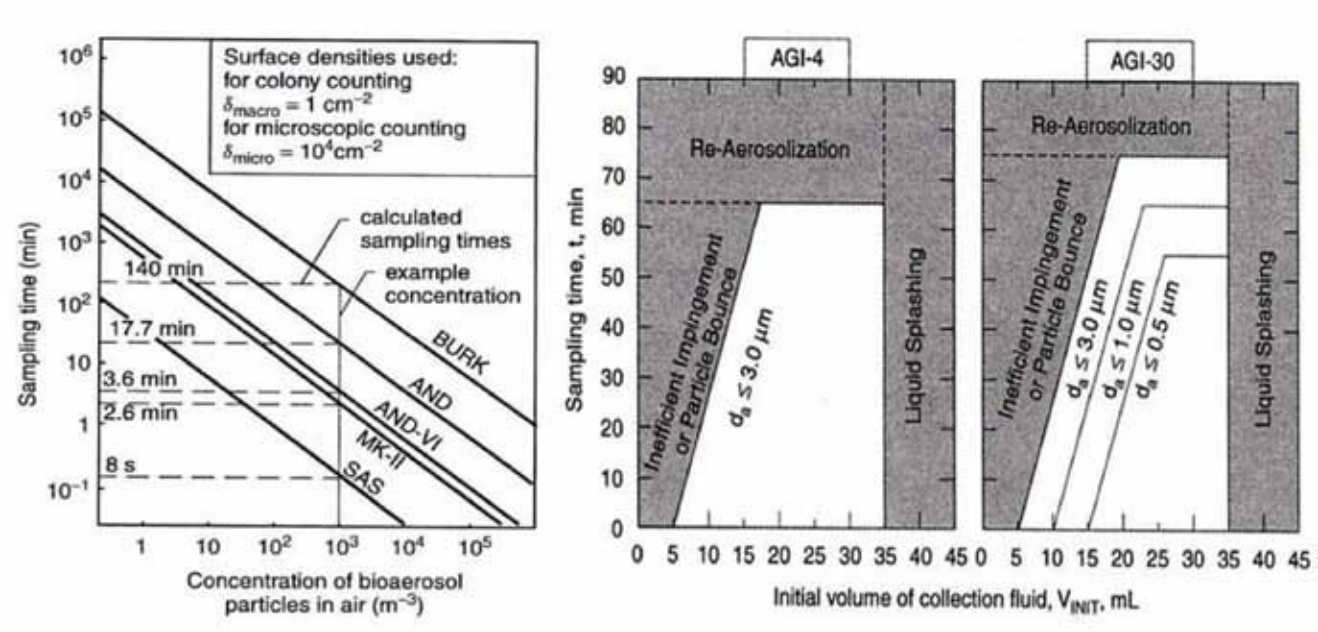


Figure 3. Optimal sampling times for selected bioaerosol samplers[1]

Table 4. Commonly used and commercially available bioaerosol samplers[1]

Sampler	Manufacturer/ Supplier	Notes
Inertial impactors Air-O-Cell® Allergenco Air Sampler (MK-3) Andersen Samplers, 1-, 2-, or 6-stage Burkard Sampler Casella Airborne Bacteria Sampler (MK-II) Mattson-Garvin Slit Sampler Rotorod Surface Air Sampler (SAS)	 ZAA/SKC ALL AND BUR CAS BAR SAM PBI/SPI	• Air-O-Cell and Burkard samplers — the slit impactors, on microscope slides or tape • SAS amplers — portable one-stage, multiple-hole impactors • The Reuter centrifugal sampler (RCS) — portable — d_{50} = 3.8 µm • The AGI-30 and the AGI-40 can only be used with water-based collection fluids • The BioSampler can be used with nonevaporative liquids (mineral oil) — it permits long sampling time. This type of sampler utilizes several sampling principles, including centrifugal forces and tangential impingement
Centrifugal impactors BioSampler Multistage Liquid Impinger Reuter Centrifugal Sampler (RCS)	 SKC BUR BIO	
Impingers AGI-4, AGI-30 BioSampler	 AGI/HAM/MIL SKC	
Filter samplers 37-mm Cassette Button Sampler	 CCO/MIL/SKC SKC	

The ability of bioaerosols to cause adverse health effects or to initiate disease depends upon their ability to survive and remain infectious in the environment, as well as their exposure to a susceptible host.

Selection of Bioaerosol Samplers

The selection of suitable bioaerosol samplers is guided by the objectives of sampling as well as the types and levels of bioaerosols of interest in the environment to be sampled. Some commonly used and commercially available bioaerosol samplers are listed in *Table 4*.

Inertial Impactors

Andersen viable impactors are probably the most common inertial impactors used for bioaerosol sampling in animal houses. This type of sampler consists of (1) a top inlet "cone," (2) the sampling impactor stage(s), utilizing hundreds of precision-drilled holes to selectively allow for deposition of bioaerosols within a certain size range, and (3) a base section that holds the agar media (petri dish) on which the particles collected from air are deposited, as shown in *Figure 4*.

The sampler is held together by three spring clamps and sealed with two O-ring gaskets. When air is drawn through the sampler, multiple jets of air direct size-selective airborne particles toward the surface of the agar collection surface (petri dish). Andersen samplers are available as a single-stage collection system or with multiple stages. For multiple stage samplers, larger holes, and, therefore, larger particles, are deposited in the upper stages with subsequently smaller and smaller holes, and, therefore, smaller particles, deposited in the lower stages. Each stage has precisely etched holes that will allow for deposition of bioaerosols within a relatively narrow size range.

Impingers

A second type of sample collection device, the impinger, is commonly used for collecting bioaerosols in a liquid or nutrient broth. An impinger operates as an impactor, with the exception that its jet is immersed in liquid collection media. Large particles are captured by an inertial mechanism and suspended in the liquid contained within the impinger. Collecting the bioaerosol samples in liquid may prevent sample desiccation; however, the shear forces in the jet and in the turbulent liquid may have adverse impacts on the viability of sampled bioaerosols. In general, impingers provide less damage to bioaerosols and preserve viability better than impactors.

All glass impingers, including AGI-30 and AGI-4, are the most commonly used impingers for collection of bioaerosols. The cutoff diameter of an AGI-30 impinger is 0.3 µm, above which all particles are collected during sampling. In addition, size fractionating impingers (multistage impingers) have also been used for bioaerosol sampling. As shown in *Figure 5*, the multistage liquid impinger (MSLI) collects bioaerosols and separates them into three size fractions by utilizing three different sampling flow rates.

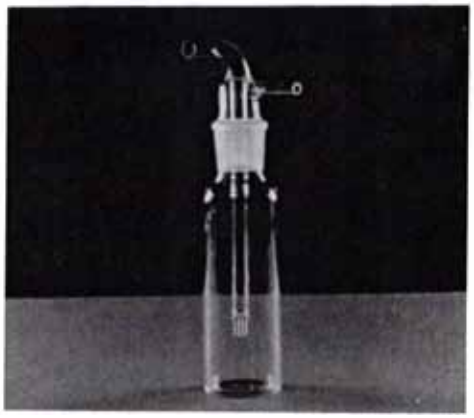
Anderson single-stage viable impactor

Anderson two-stage viable impactor

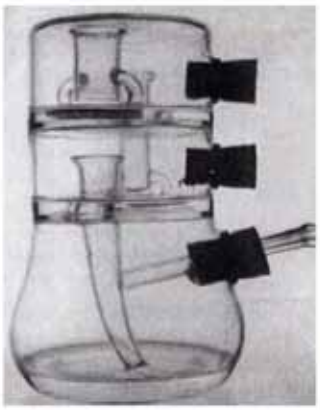
Anderson six-stage viable impactor

Stage with petri dish

Figure 4. Examples of inertial impactor—Anderson viable samplers

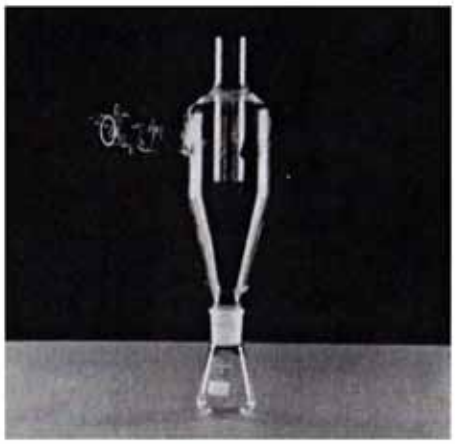


All glass AGI-30 liquid



Multistage all glass liquid

Figure 5. Examples of impingers[3]



Aerojet® cyclone sampler

RSC Biotest® centrifugal sampler

Figure 6. Examples of centrifugal samplers[3]

The Centrifugal Sampler

The Reuter centrifugal air sampler or the Biotest RCS® biocollector are two commonly used types of centrifugal samplers[3]. *Figure 6* illustrates two examples of this type of sampler. Each of these samplers uses centrifugal force to "spin" bioaerosols in the sampled air along a cone, with air pressure decreasing as the air parcel moves lower in the sampler, such that particles are deposited in the lower chamber of the sampler.

Filter Samplers

Simple filtration methods using membrane filters have also been used for bioaerosol sampling. Basically, an air parcel is pulled through the membrane filter, and bioaerosols larger than the pore diameter of the filter are collected on the filter surface. Bioaerosol particles collected onto filters can be directly examined using an optical microscope. Bioaerosol particles also may be cultured before examination by placing the filter on a culture medium to enumerate viable organisms.

The filtration method is easy to use. However, the filtration process may cause significant desiccation of collected bioaerosols, which may lead to a significant loss in viability. This method has been used to sample robust or microscopically identifiable bioaerosols in highly contaminated environments[1,2,3]. Filtration also can be used for sampling endotoxins, (1-3)-bata-D-glucan, and dusts for DNA extraction where viability may not be an issue.

Biological Analysis

Many different assay methods to detect and quantify microbes in the collected bioaerosol samples are described in the literature. Traditional methods for microbiological analyses include microscopic counting and culture-based analyses. Some of the more advanced methods currently available include biochemical and molecular biological assays.

Selection of a method for analysis should be based upon objectives of the study and availability of methods. If a study is evaluating the concentration of viable bioaerosols for public health in general, analysis should be performed without emphasis on specific identification of a particular microorganism. In this case, a traditional cultivation method with no further differentiation of microbes is sufficient. However, a wide range of studies requires identification of species that may be present in a sampling environment as well as determination of viability or infectivity of sampled bioaerosols. In these circumstances, more advanced analytical methods may be required for the study.

Microscopy

Bioaerosol particles, collected on a glass slide, tape, or appropriate filter, can be enumerated using microscopic methods. Large particles (e.g., pollen grains) are readily enumerated using a light microscope. However, smaller aerosols (e.g., bacteria cells) may be masked by other particles, and some of the smaller bacteria cells may not be visible using an optical microscope. In certain cases, collected bioaerosols can be stained to better distinguish them from background materials and then be enumerated using conventional light microscopic techniques. One of the limitations of microscopic methods is that they do not distinguish culturable or viable bioaerosols[1].

Some of the commonly used microscopic techniques include[3]:

Microscopy

- mono-objective bright field light microscopy,
- dark field illuminated microscopy,
- phase contrast microscopy,
- polarizing microscopy,
- Differential-interference Contrast microscopy (DIC),
- fluorescence light microscopy (using antibodies specific to target organisms to differentiate them from background materials),
- confocal laser-scanning microscopy,
- scanning electron microscopy, and
- transmission electron microscopy.

Image capture and analyses, which utilize image analysis software to capture images of microscopic fields for subsequent particle analyses and counting.

Culture-based Assays[1]

(adapted from reference 1)

Culture-based, or cultivation, methods are used to detect viable organisms and allow for bacteria or fungi to be collected directly onto agar plates or transferred onto agar plates from a liquid or a particle-laden filter sample. Following collection, sampled bioaerosols are cultured under controlled laboratory conditions optimal for growth, and colonies are enumerated after a prescribed incubation period. The results of this method are usually given in colony-forming units per cubic meter of sampled air (cfu/m^3).

For general bioaerosol sampling, a nonselective agar medium is often used to detect the varied organisms that may be present in air sampled. Nonselective culture media provides the basic nutrients for growth of the most common microorganisms. However, in some cases all viable microorganisms will not be cultured on the same medium due to significant differences in growing needs for the different organisms in samples.

A variety of targets, methods for sampling, and methods of analyses are available for detection and quantification of bioaerosols in animal environments.

A wide range of nonselective as well as selective media are commercially available. Selective media are available for specific types of microorganisms. By changing the composition of the medium, or using specific nutrients, selected microorganisms can be cultivated or excluded. Bioaerosol samples for culturable microbes are incubated in an incubator at specific temperatures, depending upon the types of microorganisms that are to be detected. In some instances, temperature alone can be selective for a specific group of microorganisms.

Identification of fungal colonies is based on the morphology of the colonies, spores, and hyphae, whereas identification of bacteria colonies is based on the morphology and staining properties of the cells, with further confirmation using physiological and biochemical tests. A number of easy-to-use, commercially available kits are useful for identifying bacteria in collected bioaerosol samples.

General classification of colony types or genus identification may provide enough information to draw conclusions for sampling results to meet certain general project objectives. However, environmental bacteria generally differ from the clinically significant species. Therefore, identification of environmental microorganisms requires special experience and expertise. An in-depth discussion about the principles of identification and classification of microorganisms isolated from the environment are presented in several bacteriological handbooks[3-5].

Although the culture-based methods have commonly been used to quantify the concentrations of airborne bacteria from environmental bioaerosol samples, these methods miss 99 percent of the total microbial diversity since only 1 percent of airborne bacteria are culturable[6].

Biochemical Analysis

Morphological evaluation and viability assay methods for organisms in or on various media are classical techniques for detection and identification of bioaerosols in environmental air samples. However, no single test can provide a definitive identification of environmental isolates, and these classical methods tend to be relatively expensive and time consuming.

Biochemical analytic methods for detecting and identifying microorganisms measure certain biological molecules (biochemical components of unique chemical structure) in bioaerosol particles to distinguish biological from nonbiological materials. These methods are capable of providing information about the organisms collected to the genus and often to the species levels.

Biochemical methods use the following high-end techniques for rapid measurement of specific chemical properties of the microorganisms collected[3]:

- gas-liquid chromatography,
- mass spectrometry,
- laser spectroscopy,
- fluorescence and luminescence spectroscopy,
- infrared and Raman spectroscopy,
- flow cytometry, and
- electrochemical techniques.

Molecular-Biological Assays

Molecular assays can be used to detect and enumerate specific organisms, such as pathogenic organisms, or to look more generally at a collected sample to identify all of the bacteria within the population collected. A commonly used molecular assay procedure for bioaerosol analysis is polymerase chain reaction (PCR) with subsequent genetic hybridization potentially followed by genetic sequencing. PCR can be used to detect known and specific nucleic acid sequences isolated from bioaerosols for specific organisms.

In this method, areas of the genetic material (i.e., DNA or RNA) in specific microorganisms are replicated, and then detected. If the genetic sequence is not detected from the amplified sample, the specific organism was not present in the original sample. This method is useful for the rapid detection of microorganisms that are difficult or impossible to culture, especially pathogenic microorganisms. DNA-sequencing can be used to better understand specific genetic sequences within an unknown population. When compared to a known sequence database, it can be used to identify the organisms present in the original sample.

A second type of molecular detection for identifying unknown organisms in a sample is ribotyping. In this method, the total genetic material of a population collected is extracted, and a series of restriction enzymes are used to cut the DNA or RNA at specific sequences. The samples are then analyzed using computer software to compare with the genetic banding patterns of known groups. In this way, all of the bacteria within the population of the collected sample can be identified. A limitation of this method is that it cannot be used to predict the relative concentrations of each group identified from the original population.

Molecular methods are extremely useful for environmental detection of microorganisms collected from the environment. They are generally cheaper, considerably faster, and can be more specific than conventional culture methods. However, they are plagued by other materials co-concentrated in the samples that can inhibit detection and provide no information about the viability or infectivity of microorganisms collected. Implementing molecular detection for environmental isolates requires a relatively high level of training and expertise to provide accurate and meaningful results.

Summary

Bioaerosols are particles of biological origin that are suspended in environmental air. Bioaerosols can be made up of many different types of particles, including bacteria, viruses, parasites, fungi, and other particles, such as pollen, that may lead to adverse health effects in an exposed population. When designing a study to detect and quantitate particles that make up bioaerosols in outdoor environments, it is important to understand the sampling methods that are currently available, along with their limitations, so that the best techniques can be utilized to collect information that will best meet project objectives.

For any bioaerosol sampling plan used for outdoor environmental samples, one must consider:

- Which bioaerosol or group will be targeted?
- What sampler will be used?
- What is a suitable sample time that will optimize the concentration of particles collected?
- What methods will be used to detect and quantify collected bioaerosols?

A range of different samplers can be used for collecting bioaerosols from environmental air. These include simple filters, impactors, impingers, and other advanced types of samplers, such as centrifugal samplers. Considerations for choosing a suitable sampler include whether viability of collected organisms will be measured, and if the goal is to collect total particles, or to separate bioaerosols based on size fractions.

Once a suitable sampler is chosen that will meet project objectives, it is extremely important to choose a suitable sample time and plan. For most of the filter and impactor type samplers, it is possible to under- or overload the samplers. Bioaerosol concentrations are generally high in animal environments so this information should be considered when choosing an appropriate sampler and sampling time. An advantage for impingers is that this under/overloading factor can be overcome following sample collection.

In addition to sample time, it is important to design a suitable sample plan that will account for the variability in sampled environments over time. Because bioaerosols are generally not released on a consistent basis, the sample site, duration, and timing will

be important. For example, animals may be more active during certain times of the day and during certain seasons. Bioaerosol release will generally be higher during these times, and the sampling plan should be adjusted accordingly.

Finally, once samples are collected, it is important to choose a suitable analytical technique that will provide suitable information to meet project objectives. Methods are available to detect viable microbial contaminants and to quickly detect certain targeted organisms, such as particular pathogens, or molecular methods for determining the total population contained within a sample.

Molecular detection methods are useful for detecting specific targets as well as for determining the total population. However, they do not provide information regarding the concentrations of viable microbial contaminants in a sample. Viability and infectivity are important factors to consider from a public health standpoint because this portion of the total population sampled is capable of leading to adverse health effects following exposure to a susceptible population.

A variety of targets, methods for sampling, and methods of analyses are available for detection and quantification of bioaerosols in animal environments. When designing a suitable sampling plan, each of the available techniques should be considered to optimize the information that is collected.

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