24 Biological Particle Sampling

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INTRODUCTION

Bioaerosols are particles of variable biological origin, for example, pollen, fungal spores or fragments of fungal mycelium, bacterial cells and spores, viruses, protozoa, excreta or fragments of insects, skin scales or hair of mammals, or other components, residues, or products of organisms, such as bacterial lipopolysaccharides, that is, endotoxins, or fungal mycotoxins. Bioaerosols are ubiquitous in both indoor and outdoor air. Among all particles larger than 0.2 μm in outdoor air, 30% appear to be of biological origin (Matthias-Maser and Jaenicke, 1995). Generally, the collection of bioaerosol particles utilizes the same sampling principles as those used for nonbiological aerosols. However, ensuring the survival or biological activity of the bioaerosol particles during and after collection is an important concern that differs from physical aerosol particle sampling. Furthermore, sample handling and storage, as well as the analysis of the collected biological particles, are considerably different from the procedures used in general particle sampling.

Bioaerosols have specific characteristics that influence their sampling. From the sampling perspective, bioaerosol particles may be

1. Single spores, pollen grains, bacterial cells, or viruses
2. Aggregates of several spores or cells or other biological material (e.g., mammalian allergens)
3. Products or fragments of spores and cells or other biological material
4. Biological material carried by other, nonbiological particles

Many bioaerosol particles, such as fungal spores and pollen, are designed by nature for transmission to other areas and to stay alive during the transmission. This type of hardy bioaerosol is resistant to environmental stresses, such as ultraviolet light, cold, heat, dryness, and toxic gases, and to sampling stresses, such as shear forces acting on them during sampling. In contrast, vegetative cells of bacteria are easily damaged, and their viability may be

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compromised by both environmental and sampling stresses (Cox, 1987). Some bioaerosol particles are bits and pieces of biological origin that are shed by an ecosystem. Bacteria emitted from aerated wastewater are residues of evaporated droplets. Many of the bacteria shed by humans, for example, are residues of the skin. They probably stay viable while airborne because they are adapted to the dry conditions of their environment and protected by their original substrate (i.e., the skin scale). Otherwise, most vegetative cells of bacteria are prone to damage as a result of becoming airborne.

Microbial cells in an aerosol may be viable or nonviable. The definition of the viability of a cell is not explicit (Roszak and Colwell, 1987), but, generally, viable cells are able to reproduce or they have metabolic activity. Nonviable organisms are dead or unable to reproduce. The nutritional requirements of many environmental microorganisms are not known; also, not all microorganisms can be grown on laboratory media. From a natural soil or water sample, for example, it is typically possible to culture less than 1% of the microorganisms present (Atlas, 1988), and this may also be true for airborne microorganisms. Bioaerosols that are not whole cells, such as endotoxins, mycotoxins, or various allergens, may also be present. Therefore, depending on the detection method, the results are commonly expressed as colony-forming units (cfu) when viable organisms are determined or as cells, spores, or pollen grains per unit volume of air when the viability of the organisms is not determined. The results of chemical analyses (e.g., for endotoxins) are expressed in ng/m^3 or mg/m^3.

The density of microbial cells is somewhat variable, depending on the degree of cell hydration, the reserve materials, and the lipid content of the cell (Doetsch and Cook, 1973). Microbial cells consist mainly of water, about 70% of their weight. The rest of the cell material consists of macromolecules, such as nucleic acids, proteins, lipids, carbohydrates, and their combinations. The density of a single microbial cell has been reported to be 1070 to $1090\,kg/m^3$ [1.07 to $1.09\,g/cm^3$] (Hamer, 1985), 900 to $1300\,kg/m^3$ [0.9 to $1.3\,g/cm^3$] (Orr and Gordon, 1956), 1090 to $1240\,kg/m^3$ [1.09 to $1.24\,g/cm^3$] (Bratbak and Dundas, 1984), or $1500\,kg/m^3$ [$1.5\,g/cm^3$] (May, 1966).

Bioaerosol particles cover a wide size range. Viruses are the smallest potentially living particles, about 0.02 to 0.3 μm in length. Bacteria and fungal spores cover a size range from about 0.3 μm up to about 100 μm. Pollen, algae, protozoa, and dander are several tens to hundreds of micrometers in diameter. Fungi and plants are unique in their ability to produce monodisperse spore or pollen aerosol in large quantities. When microbial cells or spores are carried by other materials or when they are present as aggregates, their migration and deposition depend on the overall size of the ensemble.

Although most of the bioaerosols are harmless constituents of normal environments, some bioaerosol particles may be infectious agents or allergens or may carry toxic or irritant components or metabolites. To be infectious, an organism must be viable, but to cause allergenic or toxic effects viability is not a prerequisite. Thus, dead cells as well as cell residues may affect human health (Hirvonen et al., 1997). Host factors, including the genetic and environmental factors affecting the individual immunological response, also play an important role.

Biological characteristics may be utilized in controlling bioaerosol sources. Control measures can be targeted on environmental factors that regulate the growth of microorganisms (e.g., temperature and moisture).

BIOAEROSOL TYPES

Bacteria

Bacteria are generally found in any soils, waters, plants, and animals. In air, bacteria may occur as vegetative cells or endospores. They may be carried by other particles, such as water droplet residues, plant materials, or the skin fragments of animals. Bacteria are single-cell microor-

ganisms that range in size from 0.5 to 30 μm. The aerodynamic size of most indoor air bacteria in relatively clean environments has been reported to be 1 to 3 μm (Nevalainen, 1989; Górny et al., 1999). In homes with high concentrations of other aerosols, such as cigarette smoke, the particle size distribution has been found to have a mean size in the 0.5 to 10 μm range, which suggests that the bacteria are aggregated with other particles (Górny et al., 1999). The shape of bacteria varies from spherical to rod shaped, spiral or filamentous. Many spherical bacteria occur as pairs, tetrads, or clusters (e.g., *Micrococcus* and *Staphylococcus*) or as chains (e.g., *Streptococcus*). The rod-shaped bacteria may occur as single cells or in chains (e.g., *Lactobacillus*). Bacteria can be divided into two groups based on the ability of the cell wall to retain crystal violet dye. Gram-positive bacteria, such as *Bacillus* and *Staphylococcus*, retain the dye, while gram-negative bacteria, such as *Pseudomonas* and *Legionella*, do not retain it. Gram-positive cell wall consists mainly of peptidoglycan, whereas gram-negative cell wall contains relatively little peptidoglycan but contains an outer membrane composed of lipopolysaccharides (endotoxin), lipoprotein, and other complex macromolecules. Both peptidoglycans and lipopolysaccharides can cause adverse health effects, as described below.

Bacterial cells that actively metabolize and divide into new cells are called *vegetative* cells. Endospores are formed within the vegetative cells of certain bacterial genera (e.g., *Bacillus* and *Thermoactinomyces*). Bacterial endospores are dormant forms of cells and are very resistant to cold, heat, radiation, and other environmental stresses. The sizes of bacterial spores range from 0.5 to 3 μm. They are easily carried away by air currents because gravitational settling is fairly insignificant for particles of this size range.

Pathogenic bacteria are known to cause disease in humans, animals, and plants. Pathogens are often specific and cause disease only to a certain species of animal or plant. Most animal and plant pathogens are different from human pathogens. Knowledge about human pathogens is the major focus of clinical microbiology.

Environmental or saprophytic (i.e., living on dead or decaying organic matter) bacteria are found everywhere, and their nutritional and temperature requirements vary. Only a fraction of environmental bacteria appear to be identified and characterized thus far. Some of the environmental bacteria are opportunistic pathogens (i.e., they may attack an individual having weakened immunological response). For instance, some species in the well-known genus *Legionella* are opportunistic pathogens. For reasons presently not known, some *Legionella* species find an ecological niche in manmade warm-water systems, where they may multiply and, if aerosolized, cause serious disease in exposed people (Keleti and Shapiro, 1987).

Actinomycetes are a group of soil bacteria that can both grow in a yeast form and produce mycelium and spores like filamentous fungi. According to the modern taxonomy based on genetic sequencing, they belong to the larger class of *Actinobacteria* (Stackebrandt et al., 1997). Their spores may contribute significantly to occupational exposure in agricultural work situations (Lacey and Dutkiewicz, 1994). In occupational and environmental hygiene, important genera of actinomycetes are *Streptomyces*, which gives soil its characteristic odor, and the thermophilic genera *Thermoactinomyces* and *Faenia*. These organisms cause hypersensitivity pneumonitis. Actinomycete spores may be found in office or residential buildings that have excessive microbial growth due to moisture accumulation within the structure or inside the heating, ventilation, and air conditioning (HVAC) system.

In air, bacteria may occur alone or may be carried by other particles. Bacteria tend to grow in colonies in their natural habitats, such as water and soil, and as biofilms on different surfaces. Therefore, whenever they become aerosolized, bacterial particles often occur as aggregates or microcolonies attached to other materials (Eduard et al., 1990). For example, the skin scales of mammals, which are abundant in indoor air, usually contain colonies of bacteria, such as *Micrococcus* and *Staphylococcus* (Lundholm, 1982; Noble, 1975).

Bacterial endotoxins are lipopolysaccharides that are specific to the cell wall of gram-negative bacteria. Endotoxins are heat resistant and chemically stable. They maintain their

biological activity after the bacterial cell is no longer viable. Endotoxins cause acute toxic effects, including fever, malaise, and decrements in pulmonary function. They can be abundant in agricultural environments, some industries, and in humidified indoor air and may contribute to symptoms of humidifier fever (Rylander and Haglind, 1984; Rylander and Jacobs, 1994).

Bacterial cell wall also contains peptidoglycan; it comprises 90% of the cell wall of gram-positive bacteria and 10% of the cell wall of gram-negative bacteria. The health effects of peptidoglycan are not as well known as those of endotoxin, but it has been suggested that it has biological activities similar to endotoxin (AIHA, 1996; ACGIH, 1999).

Fungi

Fungi are also omnipresent microorganisms. They are responsible for most of the aerobic decay of natural organic material (Kendrick, 1985). Fungi include yeasts, molds, and mildews, which are often called *microfungi*, as well as large mushrooms, puffballs, and bracket fungi (Burge, 1995). The terms *mold* and *mildew* refer to visible fungal growth on surfaces. Fungi can be unicellular (e.g., yeasts) but are usually multicellular, forming long chains of cells called *hyphae*, which, as an ensemble, are called *mycelium*. Fungi are classified into groups based on their methods of spore production. The primary form of dissemination for this organism is by the release of fungal spores, which are well adapted to airborne transport. The size range of fungal spores, 1.5 to 30 μm or sometimes even larger, allows their transport by winds to long distances. Most of the fungal spores in indoor environments have been reported to be 2 to 4 μm in aerodynamic diameter (Reponen et al., 1994; Górny et al., 1999). Fungal spores are often resistant to various environmental stresses such as dryness, cold, heat, and ultraviolet radiation.

Some fungi can utilize living plant materials and cause crop diseases of great economic importance. A few fungi (e.g., *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Candida*) readily invade living animal tissue and may cause infectious disease. Others are opportunistic pathogens (e.g., *Aspergillus* and *Cryptococcus*), which mainly cause infection only in people with impaired immunity. However, most fungi are saprophytic, provided adequate moisture is present. In buildings, moisture is the main controlling factor for mold growth. The relative humidity of the air (RH) affects the water content of materials in the room. However, it is the available moisture in the substrate, not the RH of the room air, that limits the growth of microorganisms in or on the materials. Virtually no microorganisms found in indoor air are able to grow if the equilibrium RH of the material is below 65% (Flannigan and Morey, 1996).

Most fungal aerosols can cause allergic reactions and diseases, such as asthma, allergic rhinitis, or, in some cases, hypersensitivity pneumonitis. Studies of these allergens usually focus on fungal spores, although metabolites and fragments of mycelium can also become airborne. Although a few genera, such as *Cladosporium*, *Alternaria*, basidiospores, and ascospores, dominate the outdoor aerosols in most of the world, there is some geographical variation. In areas of seasonal variation, the levels of fungal spores are highest in summer and fall and lowest in winter. Massive exposure to fungal spores may occur in farming and food handling occupations as well as in some industries (Rylander and Jacobs, 1994). In office and residential indoor environments, the outdoor air is an important source of fungal spores. The detection of airborne spores, resulting from growth on indoor substrates, can be difficult in the presence of normal background levels of outdoor bioaerosols.

In the cell wall of plants and fungi one finds glucans. The major source of β-(1 $\rightarrow$ 3)-*D*-glucans is the cell wall of fungi (Rylander and Jacobs, 1994). Glucans may play a role in various respiratory diseases and symptoms related to fungi, by themselves or by interacting with endotoxin or other environmental agents.

Some fungi may produce mycotoxins, which are toxic secondary metobolites. Several species of the genus *Aspergillus* produce carcinogenic toxins. These toxins have been studied

primarily with respect to ingestion. There is some indication that aerosol exposure is a hazard as well (Sorenson et al., 1987). Aerosol exposure has been related to trichothecenes, which are mycotoxins produced by, for example, *Fusarium* and *Stachybotrys*. These can cause serious acute effects, including headaches, dizziness, immunosuppression, and pulmonary hemorrhage (Sorenson et al., 1987; Etzel et al., 1998).

Viruses

Viruses differ from other microorganisms because they can reproduce only inside a host cell. Therefore, viruses are intracellular parasites and never grow on nonliving substrates. They may infect bacteria, plants, animals, or humans and are usually targeted toward a specific subgroup. Those viruses that replicate only within bacterial cells are called *bacteriophages*. The smallest of all microorganisms, 0.02 to 0.3 μm, they consist of only one type of nucleic acid, either RNA or DNA, and are, therefore, not able to generate genetic information without a complete host cell. Viruses are surrounded by a protein layer called a *capsid*.

Viruses can be transmitted through air in the absence of the host cell (Gerone et al., 1966). Although individually small, they usually travel in air carried by other materials such as droplets of respiratory secretions. The size of a virus-containing particle may vary greatly, depending on many factors, such as RH of the surrounding air.

Viruses can cause infectious diseases (e.g., influenza, measles, and chicken pox), and the viral sources are almost always other infected humans. The viral agent for hantavirus pulmonary syndrome spreads from infected rodent droppings or urine. There is epidemiological evidence for the transmission of viral infections inside and between buildings (Riley et al., 1978; Donaldson, 1978). There is no evidence of airborne transmission of hepatitis or human immunodeficiency viruses, but there is some concern about blood and tissue aerosols generated during general and dental surgery that might contribute to the transmission of these agents.

Pollen

Pollen grains are produced by plants to transmit the "male" genetic material to "female" flower structures. Many ornamental plants produce pollen grains that are transported through the air by insects. However, many trees, grasses, and weeds produce pollen grains that are dispersed in the air by wind forces without the need of a carrier. To be successfully transmitted, this kind of pollen is usually produced in large amounts. For example, one shoot of hemp may produce over 10^9 pollen grains (Faegri and Iversen, 1989). Airborne pollen grains are usually resistant to environmental stresses such as desiccation, temperature, and light; hence, they tend to resist sampling stresses. Pollen grains from different plants vary in size, surface structure, and, to some extent, shape. The aerodynamic diameters of pollen grains are not well known, but the physical size range is approximately 10 to 100 μm, with many types of pollen grain being between 25 and 50 μm. Thus, pollen grains are not in the respirable size fraction. However, many kinds of pollen contain important allergens, which may be present in the air in smaller fragments (Rantio-Lehtimäki, 1995). Patterns of prevalence for airborne pollen vary with geography and climate. For example, ragweed pollen is considered to be one of the most important allergens in North America, while birch pollen is considered the most important in Northern Europe.

Cat, Dog, House Dust Mite, and Cockroach Allergens

A wide variety of materials derived from mammals contain potentially allergenic material. Cats and dogs are the most common household pets. Cat allergens are found in saliva and skin dander and are carried in small particles, less than 2.5 μm. Dog allergens are found in

saliva, skin dander, and urine. Dog allergens are associated mainly with large particles, >9 μm (Custovic et al., 1997). Cat and dog allergens have been detected in schools and offices where these animals are not kept (ACGIH, 1999), which suggests that the allergens are carried on clothing.

House dust mites (e.g., *Dermatophagoides pteronyssinus* and *D. farinae*) are common allergens in residential environments. Dust mites are most prevalent in mattresses, carpets, and upholstered furniture where human skin scales collect and serve as food. Mites also need high humidity, over 50%, for survival and reproduction. Mite allergens are carried on mite fecal particles and dried body fragments, which are 10 to 20 μm in size (Burge, 1995).

The two most common cockroaches inside homes in temperate climate areas are *Periplaneta americana* and *Blatella germanica*. The source for cockroach allergens is not completely clear: Potential sources include cast skins, whole bodies, egg shells, fecal particles, and saliva. Cockroach allergens are found on particles over 5 μm in size (ACGIH, 1999).

Although the exposure route for allergens is assumed to be through air, most of the allergens are difficult to detect in air samples due to their relatively large size. Therefore, allergen samples are usually collected with a vacuum cleaner directly from the reservoir (e.g., from mattresses and carpets). Immunochemical methods are widely used for allergen analyses. The amount of dust mite allergens in a dust sample can also be determined indirectly through colorimetric indication of the amount of guanine, which is a component of dust mite excreta (von Bischoff, 1989). Specialized knowledge is needed to visually identify mites in samples.

SOURCES OF BIOAEROSOLS

Many indoor bioaerosols originate outdoors. The surfaces of living and dead plants are probably the most important sources of airborne fungal spores and bacteria. Therefore, the mechanical movement of plants or soil (e.g., through farming activities or construction) generates bioaerosols together with nonbiological dust. Actinomycetes may become airborne from soil (Atlas and Bartha, 1987). All natural waters as well as anthropogenic waters, such as sewage lagoons and cooling water systems, contain large numbers of microorganisms. For example, gram-negative bacteria, actinomycetes, and algae are common constituents of water ecosystems. Therefore, droplets resulting from rain, splashes, or bubbling processes may contain bioaerosols, which may remain airborne after evaporation of the liquid.

Strong sources of bioaerosols may be present in various occupational environments when organic material is handled, such as plants, hay, straw, wood chips, cereal grains, tobacco, cotton, organic waste, wastewater, or metalworking fluids. In agricultural and horticultural environments, exposure to fungal and actinomycete spores may be severe (Kotimaa, 1990; Lacey and Dutkiewicz, 1994; Rylander and Jacobs, 1994). Avian and rodent droppings can be a source for viral and fungal agents.

In industrial and nonindustrial situations, specific bioaerosol sources may develop due to microbial growth in a building's HVAC systems or in the building's structure itself. Generally, the prerequisite for microbial growth is excessive and accessible moisture. Standing water is always a good reservoir for microbial growth and therefore a potential source of microbial aerosols when disturbed (Keleti and Shapiro, 1987; ACGIH, 1999). In addition to water, microorganisms only need minute amounts of nutrients, which are available in the water or in building materials, such as cellulose, wood, or concrete. Therefore, a source of spores or other bioaerosol material may develop wherever water is leaking or condensing inside a building.

In nonindustrial indoor environments, the most important source of airborne bacteria is usually the presence of humans. Air with a high concentration of human bacteria is not nec-

essarily a health hazard, but may indicate the presence of humans and their physical activities. It may also signal inefficient ventilation. Although pathogenic bacteria or viruses are often present in these air environments, their presence may be difficult to verify by conventional bioaerosol sampling techniques. Therefore, the concentration of normal human skin bacteria in air is sometimes used as an indicator of indoor air quality.

Recently, the threat of biological warfare and terrorism has gained increased attention. Agents of concern include pathogenic microorganisms, such as *Bacillus anthracis* and smallpox virus, as well as microbial toxins such as botulinum toxin.

GENERAL SAMPLING CONSIDERATIONS

The purpose of bioaerosol sampling is usually to verify and quantify the presence of bioaerosols for exposure assessment, to identify their sources for control, or to monitor the effectiveness of control measures. Dose–response relationships are poorly known, and therefore exposure guidelines have not been established for acceptable healthful levels of any bioaerosol (ACGIH, 1999). Bioaerosol concentrations have time-dependent variations ranging over several orders of magnitude. For example, bacteria and fungal spore concentrations on the order of 10 to 10^3 cfu/m^3 can be found in residential homes or occupational environments with moderate sources (e.g., tobacco processing, sanitary landfills, or biotechnical industries; Macher et al., 1991; Martinez et al., 1988; Rahkonen et al., 1990; Verhoeff et al., 1990; Reponen et al., 1992). Lower concentrations of $\leq 10^2$ cfu/m^3 can be found in well-ventilated facilities without significant bioaerosol sources, such as offices, laboratories, clean rooms, and operating rooms in hospitals. High concentrations of bacteria and fungal spores, with peak concentrations from 10^4 to as high as 10^{10} cfu/m^3, can be found in environments such as textile mills, sawmills, some agricultural exposure situations, and in seriously contaminated homes and offices (Kotimaa, 1990; Eduard et al., 1990; Lacey and Dutkiewicz, 1994). In most of these environments, the bioaerosol concentrations vary considerably in time and space. This is partly because bioaerosol sources do not necessarily generate particles continuously. For example, spore production and spore release from fungal mycelium may occur in bursts under certain air humidity and velocity conditions.

Sampling Strategy

No single sampling method can collect, identify, and quantify all of the bioaerosol components existing in a particular environment. Therefore, a source inventory is important and useful. It may include a preliminary microbiological analysis of the water reservoirs and a contact sample from a surface with assumed fungal growth. In industrial exposure situations, the type and location of sources are frequently evident. In nonindustrial environments, the sources are often less obvious, which necessitates a complex sampling strategy.

Sampling Efficiency of Bioaerosol Samplers

The overall sampling efficiency of a bioaerosol sampler can be divided into three components:

1. The inlet sampling efficiency is a function of the sampler inlet's ability to extract particles from the ambient air environment; it usually depends on the size, shape, and aerodynamic behavior of the particles being sampled.
2. The removal efficiency is determined by the sampler's ability to remove the particles from the air stream of the sampling inlet and deposit them into or onto the collection medium.

3. The biological aspect of sampling efficiency depends on the sampling and removal of the biological particles without altering their viability or biological activity and on the conditions for the organisms to form colonies or to be otherwise detected as biological particles.

The physical (inlet sampling and removal) and the biological factors must be separated in order to quantify their effects on the overall sampling performance.

None of the presently available samplers for culturable bioaerosols can be considered as a reference method, although the all-glass liquid impinger (AGI-30; ***AGI***, ***HAM***, ***MIL****) and the six-stage Andersen impactor (***AND***) have been suggested for that purpose (Brachman et al., 1964). Few of the currently available samplers have been adequately characterized as to their sampling performance. The results of reported field comparison studies are not easily comparable to each other, partly because the samplers, sampling times, and sampled volumes have varied within and between studies and partly due to the different operational principles and parameters of each of the samplers. Eduard and Heederik (1998) have summarized the results of several field comparison studies performed with different bioaerosol samplers. They found that some samplers are more efficient collectors of viable bioaerosol particles than others.

Bioaerosol particles must be collected from the ambient air with as little bias as possible. For minimal bias over a broad particle size range, aspiration into the inlet should occur under isokinetic conditions, that is, at the inlet air velocity equal to the ambient air velocity and the inlet is oriented facing the ambient air flow. Long sampling lines after the inlet may cause significant wall losses of large particles. Dependence of the inlet efficiency on the particle size as well as on wind and sampling conditions can cause significant over- or underestimation of the concentration. For example, theoretical calculations have shown that the Burkard Portable Air Sampler (***BUR***) overestimates the aerosol concentration of 10 μm particles by 2.5 times when positioned horizontally parallel to the wind direction at wind speeds of 0.5 m/s. If the sampler is placed vertically with all other conditions the same, particles larger than 5 μm will not be able to enter the sampler at all, while the concentration of particles of about 2 to 5 μm is considerably underestimated (Grinshpun et al., 1994). Nonisokinetic sampling is discussed in detail in Chapter 8.

The physics of particle motion in air and removal from it is common to all airborne particles. Therefore, physical principles can be applied to the sampling of bioaerosols. These principles determine the amount of sample collected and the sampling time needed for proper analysis.

A bioaerosol sampler has a high biological efficiency if it retains the biological properties that are used for analyzing the biological particles. For example, the sampling should not change the culturability of microorganisms if culture analysis is subsequently used. In the case of microscopic analysis, the sampling should not change the key morphological details of the particles. Table 24–1 shows that in the selected bioaerosol samplers (utilizing impaction or impingement principles) the impact velocity U_0 ranges from about 1 to about 265 m/s. High impact velocity can result in metabolic and structural injuries of the collected microorganisms. Survival of bacteria has been found to decrease when the impaction velocity increases; bacterial survival also depends on the degree of embedding of bacteria into collection media (particularly for impactors) and the sampling time (Stewart et al., 1995; Terzieva et al., 1996; Lin et al., 1999a). The effect of mechanical impaction stress is more pronounced for the higher impaction velocity; the desiccation stress increases with the sampling time increase and when embedding is insufficient. The survival of bioaerosol particles depends also on their internal characteristics. Pollen grains and microbial spores are generally more protected against environmental stresses than vegetative cells (Cox and Wathes, 1995).

* See Appendix I for full manufacturer addresses referenced to the italicized three-letter codes.

TABLE 24–1. Sampling Parameters of Selected Bioaerosol Samplers

Bioaerosol Sampler[a]	Collection Medium	Q (10^{-4} m^3/s [L/min])	U_o (m/s)	Nozzle					d_{50} (μm)	
				Shape	W (mm)	L (mm)	A (mm^2)[b]	Number	Calculated[c]	Reported
AGI-30	Liquid	2.1 [12.5]	265.2	Round	1.0		0.79	1	0.31	<0.3[d]
Air-O-Cell	Adhesive	2.5 [15.0]	16.5	Rectangle	1.055	14.4	15.2	1	2.3	2.6[e]
Andersen, first stage	Nutrient	4.71 [28.3]	1.08	Round	1.18		1.09	400	6.61	7.0[f]
Andersen, sixth stage	Nutrient	4.71 [28.3]	24.02	Round	0.25		0.05	400	0.57	0.65[f]
Burkard Personal Sampler	Adhesive	1.67 [10.0]	11.90	Rectangle	1.0	14	14	1	2.52	2.3–2.4[g]
SAS	Nutrient	30 [180]	17.34	Round	1.0		0.79	219	1.45	1.9[h]
MK-II	Nutrient	5 [30.0]	51.42	Rectangle	0.35	28	9.8	1	0.67	

[a] AGI, All-Glass-Impinger; SAS, Surface Air Sampler; MK-II, Casella Airborne Bacteria Sampler.
[b] Area per nozzle.
[c] Equation 10–4, T_{air} = 293 K [20°C], P_{air} = 1 atm, $\eta_{air} = 1.81 \times 10^{-4}$ g/cm s [1.81×10^{-4} poise], ρ_p = 1000 kg/m^3 [1.0 g/cm^3].
[d] Lin et al. (1997).
[e] Trunov et al. (2000).
[f] Andersen (1958).
[g] Aizenberg et al. (2000).
[h] Lach (1985).

Furthermore, aggregation of bacteria has been shown to increase their survival rate (Lighthart and Shaffer, 1997).

PRINCIPLES OF BIOAEROSOL COLLECTION

Bioaerosol sampling involves separating the particle trajectory from the air streamline trajectory. To achieve this, different physical forces are applied as illustrated in Figure 24–1.

In Figure 24–1A, the inertia of the particle forces its impaction onto a solid or semisolid impaction surface, usually either a culture medium or an adhesive surface that can be examined microscopically. This principle is used in single-stage impactors (e.g., the Surface Air Sampler; ***PBI***, ***SPI***) and in cascade impactors with two or more impaction stages (e.g., the Andersen cascade impactor). If the impaction stage consists of one or more slits instead of one or more circular holes, the impactor is frequently referred to as a *slit sampler* (e.g., Burkard spore traps and Casella [***CAS***], New Brunswick [***NBS***], and Mattson-Garvin [***BAS***]

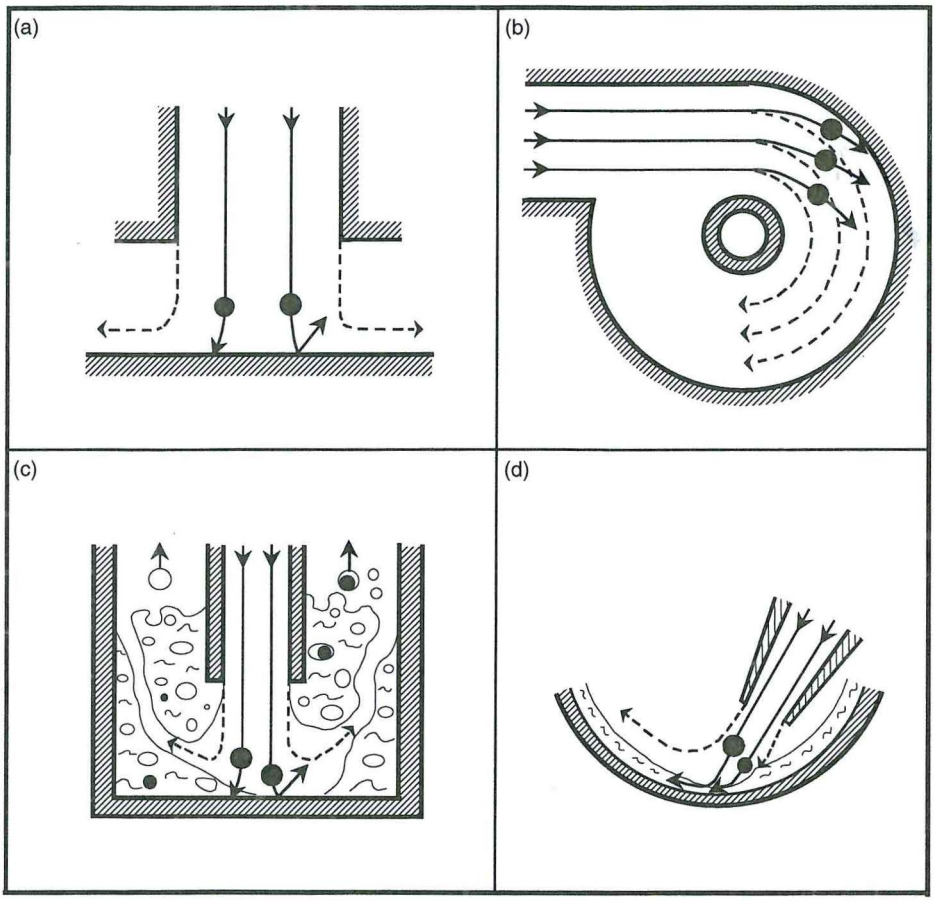

Fig. 24–1. Comparison of collection mechanisms for different inertial aerosol samplers. **A**, Inertial impaction. **B**, Centrifugal impaction. **C**, Liquid impingement. **D**, Tangential impingement. (From Willeke et al., 1998, reproduced by permission of American Association for Aerosol Research.)

culture plate samplers; more information is given below). Inertial gravitational and centrifugal particle collection techniques are described in Chapter 10.

The principle of particle separation by centrifugal force is shown in Figure 24–1B. It also uses the inertial behavior of the particle, but in a radial geometry. The Reuter centrifugal sampler (***BIO***) is of this type.

When filtration is the collection mechanism, inertial forces are also responsible for separating particles from the air stream. However, other mechanisms, such as interception, diffusion, and electrostatic attraction, also contribute to the deposition of particles onto the filter material.

In liquid impingement (Fig. 24–1C), the particles are collected primarily by inertial impaction into a liquid, but also by particle diffusion within the bubbles. Several versions of liquid impingers are currently available (e.g., the AGI-4 and AGI-30 impingers). In a multistage impinger, several impingement stages remove successively smaller particles (May, 1966). Figure 24–1D illustrates the sampling principle of a tangential impinger, which collects particles by inertial impaction, and centrifugation. This sampling principle is used in the BioSampler (***SKC***) (Willeke et al., 1998).

Particles may also be removed from the air stream by externally applied forces, such as electrical forces on charged particles, or thermal forces on an aerosol flow, which has a thermal gradient perpendicular to its flow. An electrostatic sampler has been used for virus sampling (Gerone et al., 1966) and has also been explored for bacterial sampling (Mainelis et al., 1999).

Aspiration of the airborne organisms into an inlet and subsequent collection is the preferred method for bioaerosol sampling. An exception to this is the rotating arm device, for example, the Rotorod sampler (***SAM***) that is commonly used to sample pollen. Settling plates rely on gravity to deposit airborne microorganisms onto a culture plate. The gravitational settling of particles is highly particle size dependent and is strongly influenced by the air motion in the room. Therefore, the colony-forming units counted on a settling plate after incubation or the spores counted on a settling slide cannot be related to the concentration of biological particles in the air. At the same time, gravitational settling methods are suitable to identify the larger microorganisms present in the air environment because they all settle during relatively short time (regardless of their size and air motion).

Particles can bounce when they strike the impaction surface or other previously collected particles. The latter often occurs when the sample is overloaded. Bouncing of larger particles can lead to undersampling in single-stage impactors when the particles bounce away from the collection substrate. Particle bounce can affect the size distribution obtained with cascade impactors when large particles bounce to the next collection stage. The collection efficiency, especially for larger particles, gradually decreases as the sampling surface becomes loaded. In a bioaerosol impactor, the collection efficiency may also decrease because the initially soft agar surface dries up with time, encouraging particle bounce (Juozaitis et al., 1994). This effect has not been accurately examined for commercially available bioaerosol samplers. However, it is a common practice to apply a thin film of grease to the impaction surface to reduce the bounce effect, hence, to increase the collection efficiency. The bouncing phenomenon is further discussed in Chapter 10.

In all of the sampling methods mentioned above, the bioaerosol particles are collected and analyzed in two steps: First the particles are removed from the air, and then they are analyzed and identified. Direct-reading aerosol instruments are used to study aerosols without separating them from the air. These can be used for studying bioaerosol particles in the laboratory, when other particles are eliminated (e.g., Qian et al., 1995). There is increased interest nowadays—particularly among military and antiterrorist agencies—in developing direct-reading instruments that can distinguish the particles of biological origin from other particles. One commercially available, but very expensive, device, the Ultraviolet Aerodynamic Particle Size Spectrometer (UV-APS; ***TSI***), simultaneously measures the aerodynamic

particle size, the light-scattering intensity, and the fluorescence intensity of the particles passing through the instrument. See Chapter 17. The fluorescence is measured between 400 and 580 nm upon exitation of the particles by a UV-laser beam. This fluorescence is related to the presence of life-indicating biomolecules, such as NAD(P)H (nicotinamide adenine dinucleotide phosphate) and riboflavin (Hairston et al., 1997).

Particle Removal by Inertial Impaction

Inertial impaction is the most widely used mechanism for particle removal in bioaerosol samplers. In an impactor, the particle-laden airflow is pulled or pushed through a nozzle, which accelerates the air and the particles to high speed. The air jet emerging from the nozzle is deflected by the impaction surface and makes a sharp turn. Particles with sufficient inertia continue their forward movement toward the collection surface and impact. The impaction process depends on the particles' inertial properties, such as size, density, and velocity, and on the impactor's physical parameters, such as the nozzle dimensions and airflow paths.

The non-dimensional Stokes' number, *Stk*, is used to relate the particle trajectories to the air streamlines in an impactor (see Eqs. 4–38 and 4–39). The *Stk* is defined as a ratio of a particle's stopping distance (see Eq. 4–36) to the dimension of the nozzle. *Stk* can thus be used to predict whether a particle will impact on the collection surface. Stk_{50} defines the numerical value of *Stk* for which 50% of the particles are collected and 50% pass through the impaction stage. For a round nozzle, Stk_{50} is approximately one fourth, and for a rectangular nozzle it is approximately one half.

Similarly, the "cut-off size", d_{50}, designates the particle diameter for 50% removal. Because most impaction stages have sharp cut-off characteristics, almost all the particles larger than that size are collected. Therefore, d_{50} is generally assumed to be the size above which all particles larger than that size are collected. The cut-off size d_{50} is an important characteristic of any bioaerosol sampler. To evaluate the sampling performances of different samplers, the cut-off size can be calculated (see Eq. 10–4 and Example 24–1).

Numerical Estimation of Cut-Off Sizes

The design and performance characteristics of six commonly used bioaerosol samplers are compared in Table 24–1. These samplers have a single impactor nozzle or several in parallel. The range of their volumetric flow rate, Q, is from 1.67×10^{-4} m^3/s to 3×10^{-3} m^3/s [10 to 180 L/min]. For the six-stage Andersen impactor, data are given for the first and sixth stages. As seen, particle cut-off diameters (d_{50}) are close to the experimentally determined values. The small differences between calculated and experimental values are due to the dependence of Stk_{50} on the impaction nozzle geometry of each sampler and the air flow pattern in the impaction stage that does not exactly match the pattern used in the theoretical calculations.

The d_{50} value of an impactor can be used to determine which microorganism species are likely to be collected in the impactor if the mean aerodynamic sizes of the bioaerosol particles are known. The aerodynamic diameter of a particle equals the diameter of a spherical particle with density 1000 kg/m^3 [1 g/cm^3] (= density of water) that has the same gravitational settling velocity as the particle in question (see also Chapter 3 and Example 3–2). For bioaerosol particles with a density close to that of water and a shape close to spherical, the physical and aerodynamic diameters are approximately equal to each other. However, for rod-shaped particles, or for particles with density other than that of water, the aerodynamic diameter predicts the particle behavior more accurately than the bioaerosol particle's geometrical dimensions. The physical sizes (length and width as measured under a microscope) of bioaerosol particles are better known than their aerodynamic sizes. Table 24–2 summa-

Example 24–1

An Andersen N-6 sampler (i.e., the sixth stage of a six-stage cascade impactor) is operated at a volumetric flow rate of 20 L/min instead of the required 28.3 L/min (1 cfm). This is a single-stage sampler with 400 nozzles of 0.25 mm diameter each, that is, 400 jets of aerosol flow are directed toward the nutrient medium below them. (1) Calculate the cut-off diameter d_{50} for the reduced flow. What is the smallest particle collected? (2) The N-6 is operated at the required flow rate of 28.3 L/min, but 40 of its 400 nozzles are plugged. What is the smallest particle collected?

Answer: (1) The air velocity, U_0, and, therefore, the approximate particle velocity in each of the n jets of cross-sectional area A_j are

$$U_o = \frac{Q}{nA_j} = \frac{Q}{n\left(\frac{\pi W^2}{4}\right)} = \frac{\left(20\times10^3\,\frac{\text{cm}^3}{\text{min}}\right)\left(\frac{\text{min}}{60\text{s}}\right)}{400\left[\frac{\pi(0.025\text{cm})^2}{4}\right]} = 1697\,\frac{\text{cm}}{\text{s}}$$

To determine the cut-off diameter, d_{50}, use Eq. 10–4. At normal pressure and air temperature (1 atm and 20°C), the viscosity of air, η, equals 1.81×10^{-4} poise (1.81×10^{-4} g/cm s). The density of bioaerosol particles ranges from 900 to 1500 kg/m^3 [0.9 – 1.5 g/cm^3] and is assumed to equal 1000 kg/m^3 [1 g/cm^3] for this example. For the round nozzles, the value of Stk_{50} is approximately one fourth.

$$d_{50} = \sqrt{\frac{9\eta W Stk_{50}}{\rho_p U_o C_c}} = \sqrt{\frac{9\times1.81\times(10^{-4}\text{g/cms})(0.025\text{cm})\times0.25}{(1\,\text{g/cm}^3)(1697\text{cms})\times1}} = 7.74\times10^{-5}cm = 0.77\mu m$$

Therefore, a 0.77 μm particle is approximately the smallest one collected. In the above calculation, the Cunningham correction factor, C_c, is assumed to be 1. It is actually somewhat larger than 1 for the calculated d_{50} (see Eqs. 4–23 and 4–24, Table 4–2, and Appendix F). Through an iteration procedure, the d_{50} is therefore calculated to be somewhat smaller than indicated. At the recommended flow rate of 28.3 L/min, d_{50} is 0.57 μm (see Table 24–1).

(2) If 40 of the nozzles are plugged, the air velocity through the 360 remaining nozzles at a flow rate of 28.3 L/min is 2668 cm/s. For this velocity and $C_c = 1$, the new cut size is $d_{50} = 0.62$ μm. Because $C_c > 1$ for this particle size, the actual cut size is somewhat smaller.

rizes data on the measured aerodynamic sizes of several fungal and bacterial species. It also shows the aspect ratios for some of the species. Aspect ratio is defined as the ratio of length to cross-sectional area diameter of a particle and can influence the collection efficiency of particles sampled onto a filter.

The aerodynamic particle sizes shown in Table 24–2 can now be compared with the particle cut-off sizes in Table 24–1. As seen in Table 24–1, the cut-off sizes for the various samplers or sampler stages range from less than 0.5 μm to over 5 μm. A particle is collected by an impactor if its aerodynamic size is higher than the cut-off size of the impactor. For example, the aerodynamic size of *Aspergillus fumigatus* spores has been reported to be 2.0 to 2.1 μm (see Table 24–2). Thus, the Burkard sampler and the first stage of the Andersen sampler do not effectively collect these fungal spores. The differences in the cut-off sizes of the various samplers partly explain the differences between sampler performances that have been reported in the literature (e.g., Eduard and Heederik, 1998).

TABLE 24–2. Aerodynamic Sizes and Aspect Ratios of Microorganisms as Measured in Laboratory Experiments

Microorganism	Aerodynamic Size (μm)	Aspect Ratio
Fungal spores		
Aspergillus flavus	3.6[a]	
Aspergillus fumigatus	2.0[b], 2.1[a,c]	
Aspergillus versicolor	2.4[c]	
Cladosporium cladosporoides	1.8[c,d], 2.4[a]	1.9[e]
Paecilomyces variotii	2.6[a]	
Penicillium brevicompactum	2.1[c], 2.3[d]	1.3[e]
Penicillium chrysogenum	2.8[a]	
Penicillium melinii	2.7[c], 3.1[d]	1.1[e]
Penicillium minioluteum	1.7[a]	
Scopulariopsis brevicaulis	5.3[a]	
Bacterial spores		
Bacillus subtilis var. *niger*	0.9[d]	
Faenia rectivirgula	1.1[a]	
Saccharomonospora viridis	1.3[a]	
Streptomyces albus	0.8[d], 0.9[c], 1.2[a]	1.2[e]
Thermoactinomyces vulgaris	0.6[f]	1.2[e]
Bacterial vegetative cells		
Pseudomonas fluorescens	0.8[g]	3.0–3.1[g]
Micrococcus luteus	1.0[h]	
Bacillus subtilis	0.8[i]	
Bacillus megatherium	1.0[i]	2.6[g]
Mycobacterium smegmatis	1.2[e]	
Mycobacterium bovis	0.9[e], 0.8–1.0[k]	3–8[j]

[a] Madelin and Johnson (1992).
[b] Pasanen et al. (1991).
[c] Reponen et al. (1996).
[d] Aizenberg et al. (2000).
[e] Reponen, Willeke, Grinshpun (unpublished data).
[f] Reponen et al. (1998).
[g] Willeke et al. (1996).
[h] Stewart et al. (1995).
[i] Qian et al. (1997).
[j] Schafer et al. (1998).

COLLECTION TIME

An essential part of the sampling strategy is to define the sample collection time. Bioaerosol concentrations vary greatly with time, which is graphically shown in Part I of Figure 24–2. Typically, periods of low concentrations (e.g., t_1 to t_2) are followed by periods of high concentrations (e.g., t_3 to t_4) and vice versa. These large fluctuations are best represented on a logarithmic concentration scale, as shown. In this time plot, the average concentration, c_a, is 1000 bioaerosol particles/m^3. Bioaerosol concentrations of this order of magnitude are common in outdoor air and in many indoor situations. The ambient concentrations rarely remain stable within a narrow concentration range unless the time period is relatively short (i.e., minutes) or the atmosphere is undisturbed, such as in an unventilated, nonpopulated, closed room.

Sampling during periods of changing concentration must be sufficiently long, or many short samples must be combined to properly represent the average environmental concentration. Part II of Figure 24–2 reflects how the concentration varies in air volume, v, being

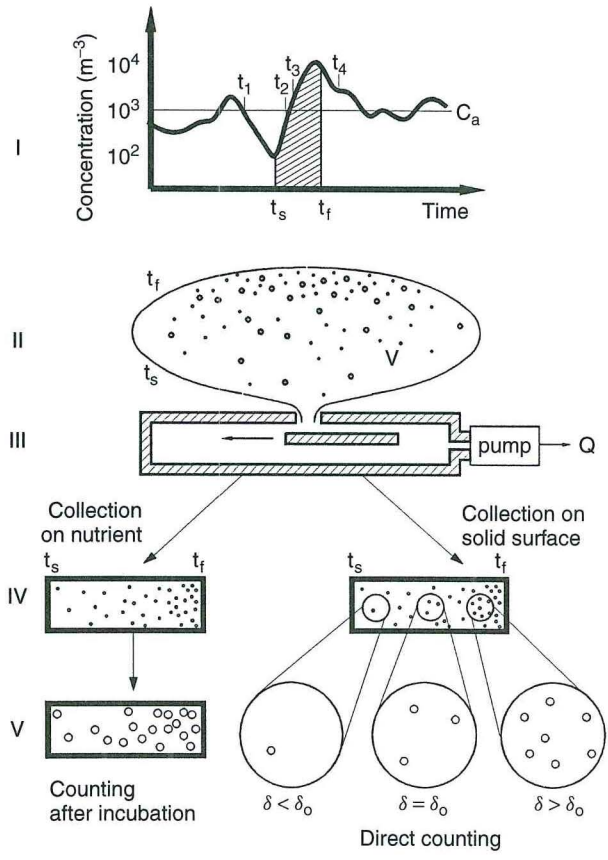

Fig. 24–2. The process of bioaerosol sampling. (From Nevalainen et al., 1992, with permission of Elsevier Science.)

sampled during the sampling period from starting time, t_s, through the finish, t_f. The volume of air equals the product of sampling flow rate, Q, and sampling time, t:

$$v = Qt \tag{24–1}$$

The sampling process actually integrates the instantaneous concentrations within the sampled period with respect to time. The number of particles, N, collected on the impaction substrate, Part III of Figure 24–2, equals the product of the average particle concentration, c_a, and the sampled air volume, v (Eq. 24–1):

$$N = c_a Qt \tag{24–2}$$

Surface Density of Collected Particles

Part IV of the sampling process in Figure 24–2 shows the bioaerosol particles being collected on a nutrient or solid surface that moves to the left during sampling, resulting in varying numbers of particles per unit area according to the changing bioaerosol particle concentration in the sampled air volume. The number of objects on the surface per viewing area, A—

microbial colonies on a Petri dish or microscopic particles on an adhesive surface—is referred to as the *surface density* of a sample. This surface density, δ, is

$$\delta = \frac{N}{A} = \frac{c_a Q}{A} t \tag{24–3}$$

Part V of Figure 24–2 presents the postcollection phase when the collected material is analyzed. For example, this can occur either immediately upon collection by viewing the collected particles directly with a microscope or after an incubation period when the colonies are sufficiently developed to be viewed and identified by visual inspection. Viewing, counting, and identifying the particles in a sample—either optically or otherwise–is facilitated if the surface density is optimal, δ_o. If the sample surface density is very low, $\delta << \delta_o$, the sampling and counting errors may be high, and the calculated aerosol concentration may not accurately reflect the true airborne concentration. If the sample surface density on a microscopic slide is very high, $\delta >> \delta_o$, the particles may be located too closely to each other and pose resolution problems in counting and identification. In addition, the bioaerosol particles may be covered and masked by dust particles. If $\delta >> \delta_o$ on a nutrient surface, the collected organisms may grow together or inhibit each other's growth. This is especially important for fungal spores, which often release substances that inhibit germination of adjacent spores. Nonbiological particles that impact on nutrient surfaces along with the microorganisms may not cause viewing problems, but may inhibit growth.

Optimal Sampling Time for Solid Surface Samplers

As seen in Eq. 24–3, the surface density of microorganisms collected on a substrate is linearly proportional to sampling time. The other parameters in Eq. 24–3—c_a, Q, and A—are normally beyond the control of the investigator. Thus, insufficiently loaded samples ($\delta << \delta_o$) and overloaded samples ($\delta >> \delta_o$) can be avoided only by adjusting the sampling period.

By inverting Eq. 24–3, the sampling time can be calculated for each sampler by using the desired value of surface density and assuming a value for the airborne bioaerosol concentration:

$$t = \frac{\delta}{c_a} \frac{A}{Q} \tag{24–4}$$

The optimal sampling time for a given bioaerosol concentration is different for each sampler, depending on the sampler's flow rate and collection surface area. For purposes of this discussion, we assume an ambient concentration of 10^3 bioaerosol particles/m^3 and a sample collection efficiency of 100%. We also assume that $\delta_{macro} = 1$ particle/cm^2 is the ideal surface density for colony counting on a culture plate and that $\delta_{micro} = 10^4$ particles/cm^2 is the ideal surface density for microscopic particle counting on a sample slide. The latter equals three particles per microscopic field of 200 μm diameter. Using these assumptions and Eq. 24–4, we have calculated the optimal sampling times for several of the bioaerosol impactors/impaction stages listed in Table 24–1. The surface density δ was determined as the density of countable objects on the sampling surface. The definition of the sampling surface area A is specific for each sampler design. For a slit sampler with bioaerosol particles impacting onto an adhesive surface (e.g., the Burkard sampler), A is assumed equal to the area of the slit nozzle. For slit-to-agar impactors and sieve-type impactors, such as the Andersen impactor with 400 jets or the Surface Air Sampler (SAS) with 219 jets, the sampling surface is postulated to be equal to the sum of all nozzle cross sections. In culture plate samplers, the sampled particles are allowed to develop into colonies before viewing, and, therefore, the area of the sampling surface A is assumed equal to the area of the nutrient plate. For the

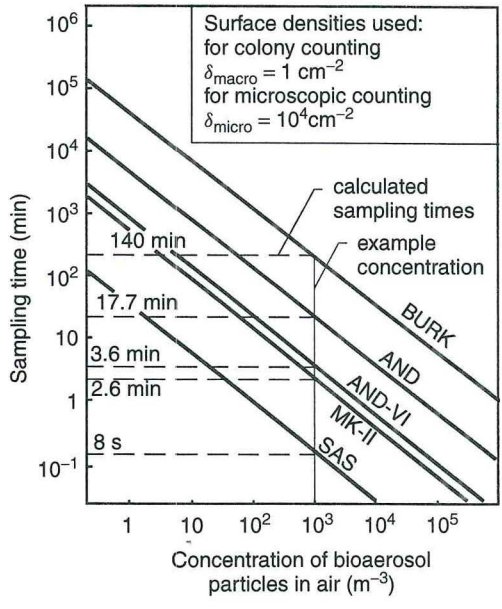

Fig. 24–3. Collection times for selected bioaerosol samplers. (From Nevalainen et al., 1992, with permission of Elsevier Science.)

Andersen six-stage impactor (referred to as AND below), it is assumed that bioaerosol particles impact onto each of the six stages. Therefore, the total area of all six stages is used in calculating the sampling time. This assumption is valid when the particle size distribution produces an equal number of particles deposited on each of the six stages. When the sixth stage is used as a separate sampler, annotated here as the AND-VI and often referred to as the N-6, only the surface area of one Petri dish is used.

It can be seen in Figure 24–3 that for $c_a = 10^3$ particles/m^3 and optimal surface density, the sampling time for the SAS (utilizing colony counting) is 8 s, while the Burkard personal air sampler has a sampling time of 140 min (utilizing microscopic counting). This represents a difference of three orders of magnitude. Lengthening the sampling time of a culture-plate sampler may result in a more representative air sample, but is likely to cause overloading, leading to counting errors and inhibition effects. Therefore, many culture-plate samplers are best suited for environments where a very low bioaerosol concentration is expected (e.g., $\leq 10^2$ cfu/m^3). In the latter case, the sampling periods can be longer, or several short-term samples can be taken over a long period of time.

As seen in Figure 24–3, the calculated sampling time for the Burkard personal sampler is 140 min for an expected average bioaerosol concentration of 10^3 bioaerosol particles/m^3. However, in most environments this would result in a sample that is grossly overloaded with nonbiological particles. Therefore, the assumed or expected values of c and δ can be modified in order to obtain a sampling time more in line with realistic expectations. For instance, for a higher bioaerosol concentration of 10^4/m^3, the sampling time would be as short as 14 min.

Optimal Sampling Time for Impingers

Impinger samples are not sensitive to overloading or undersampling because the liquid sample can be either diluted or concentrated, depending on the concentration of collected

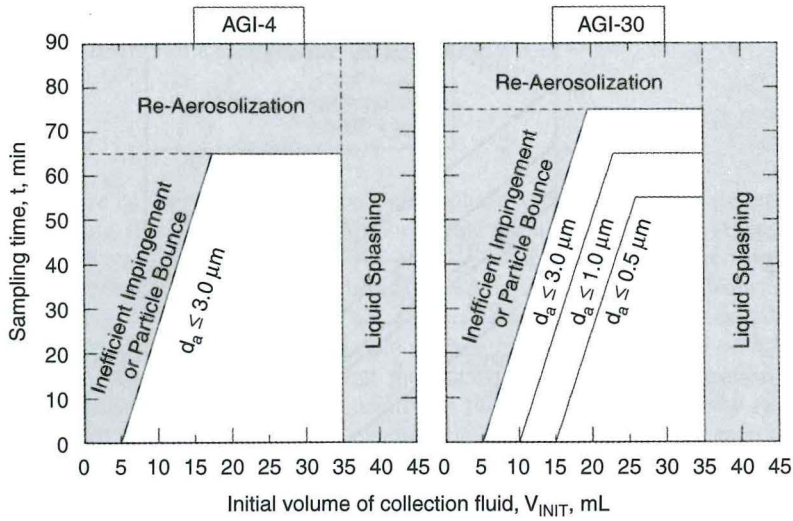

Fig. 24–4. Permissible sampling parameter ranges for less than 10% change in collection efficicency with the AGI-4 and AGI-30 impingers when operated at a sampling flow rate of 12.5 L/min. (Copyright American Industrial Hygiene Association, from Lin et al., 1997.)

bioaerosol particles in the liquid. However, evaporation of the sampling liquid and reaerosolization of already collected particles limit the sampling time in most impingers (Lin et al., 1997). Figure 24–4 shows the permissible sampling times and initial collection fluid volumes for sampling with AGI-4 and AGI-30 impingers when less than about 10% change

EXAMPLE 24–2

The Surface Air System (SAS) sampler is used to collect bioaerosols at an expected concentration level of 500 particles/m^3. The microbiologist who will analyze the results would like to see an average of 50 colonies on each 55 mm diameter Rodac plate sample (nutrient dish). The SAS sampler has 219 sampling jets and a flow rate of 180 L/min. Calculate the optimal sampling time.

Answer: The area for the 55 mm diameter Rodac plate equals 23.8 cm^2. Thus, the surface density for colony counting (Eq. 24–3) is

$$\delta = \frac{N}{A} = \frac{50 \text{ particles}}{23.8 \text{ cm}^2} = 2.1 \frac{\text{particles}}{\text{cm}^2}$$

To determine the optimal sampling time, use Eq. 24–4:

$$t = \frac{\delta A}{c_a Q} = \frac{(2.1 \text{ particles/cm}^2)(23.8 \text{ cm}^2)}{(500 \text{ particles/m}^3)(180 \times 10^{-3} \text{ m}^3/\text{min})} = 0.55 \text{ min} = 35 \text{ s}$$

This sampling time may be too short for an environment with variable concentrations of bioaerosols. Several consecutive samples should be taken, or a different sampler, which allows a longer sampling period, should be chosen (see Fig. 24–3).

in the collection efficiency is permitted. The AGI-4 is an All-Glass-Impinger with its impingement nozzle 4 mm above the bottom of the liquid; in the AGI-30, this distance is 30 mm, while the liquid height is usually about 20 mm, that is, the top surface of the liquid—when there is no airflow—is 10 mm below the impingement nozzle. If the volume of the collection fluid is over 35 mL, a significant amount of liquid splashes out of the impinger. On the other hand, a minimum volume of collection fluid is required for each sampling time so that the collection efficiency does not change by more than 10% during the sampling. The upper particle size limit of 3 μm is due to decreasing inlet efficiency when increasing particle size in the AGI-4 and the AGI-30 (Grinshpun et al., 1994). When the sampling time exceeds 65 min with the AGI-4 and 75 min with the AGI-30, a significant amount of already collected particles is reaerosolized.

EXAMPLE 24–3

In planning bioaerosol sampling in a hospital you are told that cleaning of the postoperative patient room will take 20 min. Previous reports indicate an average bacterial aerosol concentration of about 10^2 cfu/m^3. Which sampler would you choose to sample for 20 min in this patient room?

Answer: In Figure 24–3, the concentration of 10^2 bioaerosol particles/m^3 can be located on the x axis and followed up to the 20 min level on the y axis. At this intersection, the MK-II sampler and the Andersen N-6 sampler are indicated as being suitable for surface density $\delta_{macro} = 1\ cm^{-2}$ when the collected bioaerosol particles are counted as colonies after incubation.

SELECTION OF SAMPLER

The selection of a suitable bioaerosol sampler depends on the types and levels of bioaerosols of interest in the environment to be sampled. For example, when evaluating allergens, total rather than viable counts are important. Also, when selecting a cultural sampler one must consider the vulnerability of the bioaerosol to the sampling forces because some samplers affect viability more than others. The most commonly used instruments are discussed briefly in order to provide the user with the basis for deciding which sampler to use and how it operates. The names and manufacturers of some of the commonly used instruments are given in Table 24–3. More sampler information is given by Willeke and Macher (1999).

The Andersen six-stage sampler has been used in many studies (e.g., Reponen et al., 1992; DeKoster and Thorne, 1995; Górny et al., 1999). It allows characterization of bioaerosols in specific size ranges. Two-stage and one-stage versions are also commercially available. One usually expects that the ambient bioaerosol concentration is low enough so that by the end of the sampling period bioaerosol particles have passed through only about 10% of the 400 nozzles in each impaction stage. When the bioaerosol concentration is high, several bioaerosol particles may deposit below each impaction nozzle. Only one of the organisms may grow, preventing the development of others into visible colonies. In some cases, two or more organisms may grow together into a single colony. When a high percentage of the impaction spots below the nozzles of a multiple-nozzle impaction stage develops colonies, the actual concentration of bioaerosol particles can be statistically calculated by the positive-hole conversion method described by Andersen (1958) and Macher (1989). The masking effect has been studied theoretically and experimentally by Chang et al. (1995a,b).

TABLE 24–3. Company Information about Bioaerosol Samplers

Sampler	Manufacturer/ Supplier[a]
Inertial impactors	
Air-O-Cell	***ZAA/SKC***
Allergenco Air Sampler (MK-3)	***ALL***
Andersen Sampler, 1-, 2- or 6-stage	***AND***
Burkard Sampler	***BUR***
Casella Airborne Bacteria Sampler (MK-II)	***CAS***
Mattson-Garvin Slit Sampler	***BAR***
Rotorod	***SAM***
Surface Air Sampler (SAS)	***PBI/SPI***
Centrifugal impactors	
BioSampler[b]	***SKC***
Multistage Liquid Impinger	***BUR***
Reuter Centrifugal Sampler (RCS)	***BIO***
Impingers	
AGI-4, AGI-30	***AGI/HAM/MIL***
BioSampler[b]	***SKC***
Filter samplers	
37-mm Cassette	***CCO/MIL/SKC***
Button Sampler	***SKC***

[a] See key to company abbreviations in Appendix I.
[b] the BioSampler utilizes several sampling principles, including centrifugal forces and tangential impingement.

The SAS samplers are portable one-stage multiple-hole impactors that are commercially available in three models. These samplers utilize 55 mm diameter contact plates filled with nutrient medium and also require statistical adjustments for multiple impactions.

Several models are available among the slit impactors. Some rotate a Petri dish below the inlet nozzle (e.g., the Mattson-Garvin slit sampler); others impact particles on a microscope slide or tape (e.g., the Air-O-Cell and the Burkard samplers). The rotating culture plate impactors are especially useful for determining temporal changes in viable bioaerosol concentrations. This feature can be used to implicate a specific source of bioaerosol when used before, during, and after an emission episode. The Burkard recording spore traps yield time discrimination for sampling periods from 1 hour to 1 week.

The Reuter centrifugal sampler (RCS, ***BIO***) is portable and inconspicuous and, therefore, causes minimal disturbance to a room's occupants. However, the results obtained with the earlier version, the Standard RCS, must be interpreted carefully because the d_{50} is about 3.8 μm (i.e., higher than for the other inertial impactors), and the device cannot be easily calibrated. The newer version, the RCS Plus, has a lower cut-off size, 0.82 μm (Buttner et al., 1997), and a built-in calibration system.

Filtration is an easy-to-use method to sample robust or microscopically identifiable bioaerosols in heavily contaminated environments (Palmgren et al., 1986; Eduard et al., 1990). High physical collection efficiency can be achieved when the filter sampler is used with an appropriate filter. For example, porous membrane filters with a pore size of 5 μm collect 0.3 μm particles with an efficiency of 95% or higher, whereas capillary pore membrane filters must have a pore size of 0.6 μm to reach the same efficiency (Eduard and Heederik, 1998). However, due to desiccation of bioaerosol particles by the air flow through the filter after collection, filtration is not a suitable method for evaluating the levels of vegetative cells if used in combination with culture analysis (Näsman et al., 1999; Wang et al., 2000). Gelatin

filters (Li et al., 1999) and wetted porous foams (Kenny et al., 1999) have been used to decrease the desiccation effect. Filter samplers are usually small and can mostly be used with personal sampling pumps, thus allowing the collection of personal samples. Recently, filter samplers have been adapted for the sampling of inhalable bioaerosols (Kenny et al., 1999; Aizenberg et al., 2000).

The liquid of an impinger-type sampler can be used for serial dilutions and subsequent cultivation or microscopic analysis. Collection in a liquid can also be used for endotoxin determinations (Milton et al., 1990), as well as for immunological, genetic, and viral analyses. Conventional impingers, such as the AGI-30 and the AGI-4, can only be used with water-based collection fluids. These fluids evaporate quickly and are not efficient for the collection of hydrophobic particles, such as fungal and bacterial spores, due to the particle bounce and the reaerosolization of already collected particles (Grinshpun et al., 1997; Lin et al., 2000; Lin and Li, 1999). The BioSampler can be used with nonevaporative liquids, such as glycerol or mineral oil, which do not evaporate and thus permit long sampling times. The viscosities of glycerol and mineral oil are up to three decades higher than that of water, thus decreasing the potential for the reaerosolization of hydrophobic particles (Lin et al., 1999a,b). Mineral oil maintains the viability of the collected microorganisms and thus can be used with culture analysis (Lin et al., 1999; Lin et al., 2000).

CALIBRATION

No matter how efficient a sampler is, unless the air flow through the device is properly calibrated, reliable bioaerosol quantification will not be possible. Chapter 21 on instrument calibration discusses various flow calibration procedures. In addition to precisely knowing the volume of air sampled for the quantification of the sampled bioaerosol concentration, accurately calibrated air flow rates are especially critical for impactors because they are designed to operate at specific air flow rates. Improperly adjusted air flow rates will alter an impactor's cut-off size and, thus, its ability to collect an air sample that represents the ambient bioaerosol size distribution. When sampling with impactors, the distance from the inlet to the surface of the collection medium must also be correct. If the impactor is equipped with a movable stage, as in slit-to-agar impactors, the adjustment of the nozzle-to-medium distance is fairly easy. If the sampler is not equipped with an alignment device, the nutrient medium must be poured into the Petri dish to a predetermined depth.

The performance characteristics of bioaerosol samplers can be evaluated in the laboratory using aerosolized test particles. Inert test particles simulating the aerodynamic size of the bioaerosol particles in question can be used to evaluate the physical sampling efficiency of bioaerosol samplers, whereas testing of the biological sampling efficiency requires the aerosolization of the specific biological particles of interest. Bioaerosols can be aerosolized in the laboratory using various wet or dry dispersion methods (Griffiths et al., 1996; Reponen et al., 1997). Ideally, the aerosolization method simulates the natural release of the bioaerosol particles. For example, fungal spores are released from moldy building materials as dry particles by air currents.

CONTAMINATION

To avoid contaminating and, therefore, compromising bioaerosol samples, the principles of aseptic techniques must be followed. Aseptic technique means that a systematic practice is maintained to prevent undesired microorganisms and spores from contaminating the sample. In the case of ambient air sampling, for example, both the microorganisms on the human skin and within the respiratory system are among the nondesirable particles.

All surfaces, including washed hands, contain bacteria and spores unless they are specifically sterilized. Sterilization means total destruction of both cells and spores. This can be done by autoclaving or flaming the objects or materials in question. For example, all microbial culture media are sterilized before use. Not all objects can be sterilized, and therefore disinfection is used to remove the majority of microorganisms from the surfaces and materials. Disinfection treatment with an oxidizing chemical or alcohol destroys pathogenic organisms and most other vegetative cells. Although it does not destroy all spores, disinfecting sampling equipment is usually enough to prevent significant contamination of the air sample.

Most samplers are reusable and should be thoroughly cleaned before each use and decontaminated either by autoclaving or by disinfection via a chemical soak or wipe-down. Special care should be given to samplers that have convoluted inlets and pathways leading to the collection media; contaminating organisms and debris can accumulate within the sampler, making it difficult to disinfect. This creates the potential for compromised successive samples. When mounting the nutrient medium dishes or slides into samplers, touching of the nutrient surfaces by hand or other nonsterile objects should be avoided. This includes falling droplets and settling dust (e.g., from inside a ventilation system). A set of control nutrient dishes should be present during the same sampling period and be incubated with the samples in order to confirm the sterility of the medium. Once used, the Petri dishes should be sealed and transported gently with the sampling surface facing down.

SAMPLE ANALYSIS

The sample analysis method should be selected as part of the sampling plan. There are different ways to detect and quantify the collected bioaerosol particles. Traditional methods include microscopic counting and cultivation analysis. However, limitations of these traditional methods have led to the development of other methods, such as biochemical, immunological, and molecular biological assays.

Microscopy

Bioaerosol particles can be enumerated under the objective lens of the microscope after collection on a glass slide, tape, or appropriate filter. This method does not distinguish between culturable and nonculturable bioaerosol particles. Large bioaerosol particles, such as pollen grains and fungal spores, are readily enumerated under the light microscope by an experienced microscopist. Pollen grains can be identified based on their morphology, but identification of fungal species is limited with the microscopic techniques. Smaller bioaerosol particles, such as bacterial cells, are easily masked by other more numerous particles. Also, bacterial cells are not visible with a light microscope unless stained. Particles containing biological material can be detected by staining their nucleic acids with a fluorescent stain (e.g., acridine orange) and counting them with an epifluorescence microscope. Labeled antibody stains can be used to identify selected microorganisms, for example, *Legionella* (AIHA, 1996). Various microscopic analysis methods have been discussed in detail by Morris (1995).

Culture Methods

Culture-based assays are used for bacteria and fungi by collecting them directly onto a nutrient agar or transferring them onto an agar from a liquid or a filter sample. When microorganisms are cultured into countable colonies, the incubation conditions and the medium should be suitable for the organisms of interest, ideally for all viable microorganisms. In most cases, however, one cannot culture all viable microorganisms on the same medium because of the great differences in growing needs for the different organisms present. With this type of sample analysis, one cannot determine the absolute number of bacterial cells or fungal

spores in the sampled air because the bioaerosol particles usually contain aggregates of two or more cells or spores (Eduard et al., 1990). The results are given in colony-forming units per cubic meter of air (cfu/m^3).

For air sampling purposes, a nonselective medium is most often used. Many media provide the basic nutrients needed for the growth of the most common environmental microorganisms. These nutrients usually include carbon, nitrogen, phosphate, sulfate, iron, magnesium, sodium, potassium, and chloride ions. Many broad-spectrum media are commercially available (e.g., tryptone-glucose-yeast agar, nutrient agar, or casein-soy-peptone agar for bacteria and malt-extract agar for fungi). By varying the composition of the medium or using very specific nutrients, selected microorganisms can be cultivated or excluded. Selective media are available for many types of microorganisms (e.g., eosin-methylene blue agar for gram-negative bacteria and dichloran glycerol agar for xerophilic fungi, which grow on media with low water content). Fungi and bacteria may prevent each other's growth. Therefore, it is sometimes beneficial to use a fungicide in the bacterial medium and an antibiotic against bacteria in a fungal medium. A commonly used fungicide for this purpose is cycloheximide (~ 500 mg/L). Streptomycin or chlortetracycline (~40 mg/L) are used as antibiotics.

Bioaerosol samples for culturable counts are usually incubated at room temperature (21° to 25°C) or at 28°C or 35°C in an incubator. These temperatures may favor the growth of some microorganisms more than others, but generally most environmental strains grow well at temperatures below 30°C. A temperature of 35°C is generally used when monitoring food and water. As a general rule, it is important to incubate all the samples, including the reference samples, at the same temperature. Exceptions to this are selective media, for which the incubation temperature is usually given by the manufacturer. If thermophilic organisms are of interest, they are usually differentiated from other species by using a much higher incubation temperature (e.g., 55°C).

Identification of fungal colonies is based on the morphology of the colonies, spores, and hyphae as well as on different physiological tests. Although a number of handbooks are available that allow identification of fungi to the genus level (e.g., Kendrick, 1985), this remains an elaborate task that requires specific expertise in environmental mycology.

Identification of bacterial colonies is based mainly on the morphology and staining properties of the cells and different physiological and biochemical tests. Easy-to-use test kits are available that identify the bacteria with a certain probability when supported by morphological characteristics. Identification of environmental bacteria requires special experience because these organisms differ from the clinically significant species. The principles of identification and classification are presented in bacteriological handbooks, such as Bergey's *Manual of Systematic Bacteriology* (Holt, 1984) and the handbook of Truper and Kramer (1981). In most cases, general classification of the colony types or genus identification provides enough information to draw conclusions from the sampling results. For instance, the colony type may be a gram-negative rod versus a gram-positive coccus, while the genus identification differentiates between *Pseudomonas* and *Staphylococcus*. In some cases, however, species identification is needed. For example, it may be important to know whether pathogenic *Pseudomonas aeruginosa* or *Staphylococcus aureus* is present.

Other Analysis Methods

Biochemical methods measure certain biological molecules in bioaerosol particles, such as endotoxin, mycotoxins, β-glucans, and fatty acids. Depending on the agent, the analysis requires gas chromatography, mass spectrometry, high-performance liquid chromatography, or spectrophotometry. In an immunoassay, antibodies are bound to a specific target antigen, such as dust mite, cockroach, animal allergen, or fungal allergen. The major limitation of immonuassay is that specific antigens for microorganisms are difficult to define and standardize (Buttner et al., 1997). Polemerase chain reaction (PCR) is a molecular biological

assay method in which selected nucleic acid sequences contained in bioaerosol particles are replicated and detected. It is especially used for the rapid detection of organisms that are difficult or impossible to culture in the laboratory, such as *Mycobacterium tuberculosis* and *Histoplasma capsulatum* (Schafer et al., 1998).

These analysis methods are usually applied to filter or liquid samples. Although many of the new methods are promising, most are still research techniques. If one of these techniques is to be used, arrangements must be made with an appropriate research laboratory. Various analysis methods are discussed in detail elsewhere (AIHA, 1996).

Data Analysis and Interpretation

There are no guidelines for acceptable or harmful levels of bioaerosols. Therefore, it is necessary to decide in advance the criteria that will be used to determine whether or not an environment is contaminated. For this purpose, reference data on the range of bioaerosol concentrations and the airborne microbial flora in outdoor air and in indoor air in nonproblem environments should be collected with the sampler and the analysis method to be used. For example, for fungal spores in nonindustrial indoor environments, the levels should be lower than those outdoors except during the periods of snow cover, when the outdoor concentrations are close to zero. Furthermore, the composition of the fungal species in the environment under study can be compared with that in control environments. The data interpretation is discussed in more detail elsewhere (ACGIH, 1999).

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AEROSOL MEASUREMENT

Principles, Techniques, and Applications

SECOND EDITION

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