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# Bioaerosols in the Food and Beverage Industry

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69978>

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## Abstract

Bioaerosol monitoring is a rapidly emerging area of industrial hygiene. Microbial roles in atmospheric processes are thought to be species specific and potentially depend on cell viability. Accumulating evidence suggests that exposure to bioaerosols may cause adverse health effects, including disease. Studies of bioaerosols have primarily focused on chemical composition and biological composition, and the negative effects thereof on ecosystems and human health have largely gone unnoticed. This gap can be attributed to international standards on acceptable maximum bioaerosol loads not being uniform and the lack of uniform standardized methods for collection and analysis of bacterial and fungal bioaerosols. In this chapter, bioaerosol composition, relevance of bioaerosols to the food processing facility, sampling and detection approaches, and complications were discussed.

**Keywords:** bioaerosols, microbial diversity, passive/active sampling, food handler health

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## 1. Introduction

Microbes are ubiquitous in the environment and play key functional roles in nearly all ecosystems [1]. Indeed, environmental bacteria, fungi and viruses are a part of our natural environment, having coevolved with all the other living organisms, including humans. Airborne dissemination is a natural and necessary part of the life cycle of many microbes [2]. Bioaerosols originate from all types of environments, including atmosphere, soil, freshwater and oceans, and their dispersal into air is temporally and spatially variable. Bioaerosols are emerging as important, yet poorly understood players in atmospheric processes. Research on bioaerosols has experienced and continues to experience stellar growth [3].

In 1861, the first measurements of airborne microbes were reported by Louis Pasteur in the *Journal Annales des Sciences Naturelles* [4]. A century later, research into the role of bioaerosols

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in occupation-related diseases mainly focuses on noninfectious diseases. Pepys and coworkers [5] first demonstrated that patients with existing diseases are more likely to suffer attacks of farmer's lung when inhaling spores from thermophilic actinomycetes. Byssinosis among cotton workers was an important research topic during the 1970–1980s. The most likely causative agents for this disease were Gram-negative bacteria, and the endotoxins located in their outer cell wall [6]. The interest in bioaerosol exposure has increased over the last few decades, largely born from the direct association of bioaerosols with a wide range of adverse health effects. These effects can have major public health impacts which include contagious infectious diseases, acute toxic effects, allergies and cancer [7]. Furthermore, bioaerosols could potentially settle on surfaces and equipment and contribute to safety or spoilage risks where food is prepared, processed or packaged [8].

Due to the presence of great amounts of organic matter, the release of bioaerosols can be very high in certain industrial sectors such as agriculture, all types of food industries, waste management facilities, textile and wood industries. Each bioaerosol sample is unique as its composition varies in time and space (abundance and diversity of species, quantity of pro-inflammatory components). This often leads not only to high variation between samples from the same workplace, which can be due to external factors but also to the dynamic evolution of the colonized substrate and the fast multiplication rate of many microbes.

In this chapter, bioaerosol composition, relevance of bioaerosols to the food processing facility, approaches and complications in detection and approaches to sampling bioaerosols will be discussed.

## 2. Bioaerosols composition

An aerosol is a two-phase system of gaseous phase (air) and particulate matter (dust, pathogens), thus making it an important microbial vehicle. Bioaerosols are defined as “aerosols comprising of particles of biological origin or activity which may affect living things through infectivity, allergenicity, toxicity, pharmacological or other processes” [9, 10]. Bioaerosols are a diverse collection of small pieces of material emitted directly from the biosphere into atmosphere [11].

Bioaerosols are globally ever present, in some cases can dominate suspended particle concentrations and comprise a diverse selection of particle types, including whole organisms (bacteria, mold, fungi, yeast and algae), reproductive entities (pollen, spores from fungi, bacteria, ferns and mosses), biopolymers (DNA, chitin, cellulose and other polysaccharides), plant debris, insect parts, and decaying biomass. The components of bioaerosols range in size; pollens from anemophilous plants have typical diameters of 17–58  $\mu\text{m}$ , fungal spores are typically 1–30  $\mu\text{m}$  in diameter, bacteria are typically 0.25–8  $\mu\text{m}$  in diameter, and viruses are typically less than 0.3  $\mu\text{m}$  in diameter. Furthermore, fragments of plants and animals may vary in size. Apart from the fact that bioaerosol particles can span several orders of magnitude in diameter, bacteria may also occur as clusters of cells or may be dispersed into the air on plants or animal

fragments, on soil particles, on pollen or on spores that have become airborne [12]. All these characteristics contribute to making accurate analysis of bioaerosols very challenging.

## 2.1. Microbial component

Microbes are ubiquitous in nature and also present in the air as living cells able to infect or contaminate the surface or tissue it settles in or upon. These airborne bacterial and fungal cells can reach concentrations of  $10^3$  and  $10^5$  cells  $m^{-3}$ , respectively [7]. **Table 1** lists different bacterial, yeast and mold genera detected as bioaerosol components found in food industries from noteworthy research since 2003. The table depicts only data from food-related industries where microbial components were detected and identified to at least genus level. Research focused on viability testing only (total plate counts, total yeast and mold) was not mentioned.

Genus	Occupational environment	Sampling method (sampler)
<b>Bacteria</b>		
<i>Acinetobacter</i>	Milk processing [13]	Active: impaction (MAS-100)
	Abattoir (beef) [8]	Passive: petri plate
<i>Arthrobacter</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Bacillus</i>	Milk processing [13]	Active: impaction (MAS-100)
	Food warehouse (rice grains) [14]	Passive: petri plate
	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Brevibacterium</i>	Milk processing [13]	Active: impaction (MAS-100)
	Abattoir (pork) [8]	Passive: petri plate
<i>Brevundimonas</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Brochothrix</i>	Abattoir (pork) [8]	Passive: petri plate
<i>Cedecea</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Cellulomonas</i>	Abattoir (pork) [8]	Passive: petri plate
<i>Chryseobacterium</i>	Milk processing [13]	Active: impaction (MAS-100)
	Abattoir (pork) [8]	Passive: petri plate
<i>Chryseomonas</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Citrobacter</i>	Abattoir (beef) [8]	Passive: petri plate
<i>Curtobacterium</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Enterobacter</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Escherichia</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Flavimonas</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Frigoribacterium</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Klebsiella</i>	Abattoir (pork) [8]	Passive: petri plate

<b>Genus</b>	<b>Occupational environment</b>	<b>Sampling method (sampler)</b>
<i>Kluyvera</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Kocuria</i>	Milk processing [13] Abattoir (beef/pork) [8]	Active: impaction (MAS-100) Passive: petri plate
<i>Leclercia</i>	Abattoir (pork) [8]	Passive: petri plate
<i>Leuconostoc</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Lysinibacillus</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Macrococcus</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Massilia</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Micrococcus</i>	Noodle manufacturing [15] Milk processing [13] Abattoir (beef/pork) [8]	Active: impaction (MAS-100) Active: impaction (MAS-100) Passive: petri plate
<i>Microbacterium</i>	Milk processing [13] Abattoir (beef/pork) [8]	Active: impaction (MAS-100) Passive: petri plate
<i>Moraxella</i>	Milk processing [13] Abattoir (beef) [8]	Active: impaction (MAS-100) Passive: petri plate
<i>Morganella</i>	Abattoir beef/pork) [8]	Passive: petri plate
<i>Nesterenkonia</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Novosphingobium</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Paenibacillus</i>	Abattoir (beef) [8]	Passive: petri plate
<i>Pantoea</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Pedobacter</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Proteus</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Pseudomonas</i>	Milk processing [13] Food warehouse (rice grains) [14] Abattoir (beef/pork) [8]	Active: impaction (MAS-100) Passive: petri plate Passive: petri plate
<i>Rahnella</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Rhodococcus</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Roseomonas</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Salmonella</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Serratia</i>	Abattoir (pork) [8]	Passive: petri plate
<i>Shigella</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Spingomonas</i>	Milk processing [13]	Active: impaction (MAS-100)
<i>Staphylococcus</i>	Noodle manufacturing [15] Milk processing [13] Broiler chicken barn [16] Food warehouse (rice grains) [14] Abattoir (beef/pork) [8]	Active: impaction (MAS-100) Active: impaction (MAS-100) Active: impaction (MAS-100) Passive: petri plate Passive: petri plate

Genus	Occupational environment	Sampling method (sampler)
<i>Stenotrophomonas</i>	Abattoir (beef/pork) [8]	Passive: petri plate
<i>Streptococcus</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Wautersiella</i>	Milk processing [13]	Active: impaction (MAS-100)
<b>Yeast</b>		
<i>Candida</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<i>Cryptococcus</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<i>Meyerozyma</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<i>Pichia</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<i>Rhodotorula</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<i>Wickerhamomyces</i>	Fruit juice production (unpublished data)	Active: impaction (SAMPL' AIR™)
<b>Molds</b>		
<i>Absida</i>	Wheat flour mill [17]	Active: impaction (RCS)
<i>Alternaria</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Aspergillus</i>	Noodle manufacturing [15]	Active: impaction (MAS-100)
	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Aureobasidium</i>	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
<i>Botrytis</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Cephalosporium</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Cercospora</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Cladosporium</i>	Noodle manufacturing [15]	Active: impaction (MAS-100)
	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Colletotrichum</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Curvularia</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Epicoccum</i>	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Passive: petri plate
<i>Eurotium</i>	Wheat flour mill [17]	Active: impaction (RCS)

Genus	Occupational environment	Sampling method (sampler)
<i>Fusarium</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
<i>Helminthosporium</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Mortierella</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Mucor</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Penicillium</i>	Noodle manufacturing [15]	Active: impaction (MAS-100)
	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Rhizopus</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Wheat flour mill [17]	Active: impaction (RCS)
	Rice mill [18]	Active: impaction (six-stage viable Andersen cascade)
	Cake factory [19]	Passive: petri plate
<i>Stachybotrys</i>	Food warehouse (rice grains) [14]	Passive: petri plate
<i>Trichoderma</i>	Food warehouse (rice grains) [14]	Passive: petri plate
	Cake factory [19]	Passive: petri plate
<i>Verticillium</i>	Food warehouse (rice grains) [14]	Passive: petri plate

**Table 1.** Different microbial genera detected as bioaerosol components in food production, processing and storage environments.

Despite the wide diversity detected, not all have been directly indicated as spoilers or contaminants of food or of being the causative agents of disease due to bioaerosol exposure. Furthermore, not all species in a genus are necessarily harmful, which emphasizes using the appropriate sampling technique and identification methods to suite the objective for bioaerosol testing. Although all microbes present in the air may not be harmful as pathogens in vegetative state, their spores, toxins, endospores, LPS and other constituents have been linked to disease and could pose risk.

### 2.1.1. Spores

Bioaerosols contain mostly spores that are tougher, metabolically less active and often better adapted to dispersal. Spores are single or multicellular units surrounded by a rigid cell wall. Each spore is capable of reproducing the entire organism.

Certain bacteria can survive adverse environmental conditions for prolonged periods by producing a thick-walled spore structure called an endospore. Endospores function to protect the bacterial DNA against the conditions or substances in the environment that would lead to the

destruction of nonendospore-forming bacteria [20]. *Bacillus cereus* is one such spore-forming bacterium that naturally occurs in many foods. *B. cereus* form spores that are resistant to heating and dehydration, and when food-containing *B. cereus* spores are in the “temperature danger zone,” the spores geminate and the bacteria grow and produce toxins that cause illness in humans. *B. cereus* can cause vomiting or diarrhea, and, in some cases, both depend on the kinds of toxin it produces [21].

Mold spores are somewhat resistant to destruction, and they are not usually pathogenic to humans. Epidemiological and experimental studies support the fact that *Aspergillus* spp. are highly allergenic molds. These molds are known to cause two allergic diseases of the respiratory system: bronchial asthma and allergic rhinitis. Spore concentrations of above 50 CFU m<sup>-3</sup> have been associated with higher prevalence of sick-building syndrome [22, 23].

### 2.1.2. Toxins

Endotoxins are composed of lipopolysaccharides and lipooligosaccharides associated with proteins and lipids and are part of the exterior cell membrane of Gram-negative bacteria. Endotoxins are either present in the fragments of the cell wall or in the bacterial cell released during bacterial lysis. Endotoxins are nonallergenic, with strong pro-inflammatory properties. They are present in many occupational environments: ambient air and house dust [24]. Induction of airway inflammation and dysfunction can be attributed to the inhalation of endotoxins [25]. Endotoxin exposure has been associated with the occurrence of respiratory disorders, including asthma-like symptoms, chronic airway obstruction, bronchitis, increased airway responsiveness, and byssinosis [26]. Unlike molds, endotoxin has also been recognized as a causative factor in the ethnology of occupational lung diseases, including nonallergic asthma and organic dust toxic syndromes [27, 28].

During the nutrient degradation process, fungi release secondary metabolites called mycotoxins. Mycotoxins are toxic fungal metabolites produced by molds in vegetal matrices and could be potentially detected in bioaerosols because of their adsorption on spores and dust particles [29, 30]. Mycotoxins are nonvolatile compounds and will be found in the air only if the environment in which they are produced is disturbed. These molecules act as defense mechanism against other microbes, including other fungi. A given fungal species may produce different toxins depending on the substrate and local environmental factors. Mycotoxins and their associated health effects through respiratory exposure are not well known. They could be the causal agents of effects reported following exposure to molds. Reported symptoms include skin and mucous membrane irritation, nausea, headache, immunosuppression and systemic effects such as dizziness and cognitive and neuropsychological effects [22, 31, 32].

### 2.1.3. Other

Other bioaerosol components of microbial origin considered nonviable, but bioactive may be present in the air.  $\beta$ -(1-3)-D-glucan is a polymer glucose of high molecular weight found in the cell walls of bacteria, molds, and plants [31]. They consist of glucose polymers with variable molecular weight and degree of branching [24].  $\beta$ -(1-3)-D-glucan is associated with dry cough, cough associated with phlegm, hoarseness and atopy and has been reported in indoor

environments [33]. Part of the components of the cell wall of Gram-positive bacteria consists of peptidoglycans. With the inhalation of Gram-positive bacteria, these peptidoglycans may be potential casual agents of lung inflammation [31].

During bacterial growth or cell death, proteins are normally secreted that are bioactive molecules called exotoxins. Exotoxins are usually associated with infectious diseases such as cholera, tetanus and botulism, but they can also be found on surfaces that can take on an aerosol form and could support bacterial growth [31].

### **3. Relevance to the food processing facility**

Airborne particles and bioaerosols are easily transported, transferred and displaced from one environment to the other. Complex mixtures of bioaerosols such as fungi, allergens, and bacteria along with nonbiological particles (e.g., dust, smoke, particles generated by cooking, organic, and inorganic gases) are contained in indoor environments [34]. The bioaerosols and their components could pose an environmental hazard when presented in high concentrations in indoor environments, resulting in spoilage/contamination of food products or occupational health risks [35].

#### **3.1. Food product-related risk: spoilage or contamination**

Before spoilage becomes obvious, microbes have begun the process of breaking down food molecules for their own metabolic needs, resulting in a variety of sensory cues such as off-colors, off-odors, softening of fruits and slime. Firstly, the sugars are easily digested carbohydrates, then plant pectins are degraded, and proteins are attacked and produce volatile compounds with characteristic smells such as amines, ammonia, and sulfides. Early detection of spoilage would be advantageous in reducing food loss because there may be interventions that could halt or delay deterioration. Several methods for determining concentrations of spoilage microbes or volatile compounds produced by spoilage microbes have been devised. Many of these methods are considered insufficient as they are time consuming and/or do not give constant, reliable results and are labor intensive [31].

Food can also be contaminated by the presence of harmful chemicals and microbes which can cause illness when consumed. For this reason, traceability and source determination of contamination remain a relevant topic in food preservation research [36]. Bioaerosols implicated in respiratory-associated hazards have received much attention, but the potential of food-associated microbes and food-borne pathogens in bioaerosols to cause food spoilage needs to be clarified. Evidence exists that pathogenic microbes are found in the air, and that these microbes are present in certain products. However, traceable evidence of bioaerosols as the causative agent of spoilage or contamination of food products is not readily available.

#### **3.2. Food handler-related risk: occupational health**

Exposure to higher risks of biological hazards is characteristic to certain industries such as health care, agriculture, fishery, some food industries, construction, and mining. Workers

employed in these industries have higher prevalence of respiratory diseases and airway inflammation [37]. It is difficult to conduct a comprehensive evaluation of personal bioaerosol exposure in occupational or indoor environments [38], owed to the complex composition of bioaerosols, and the lack of standardized sampling/analysis methods [37]. Without appropriate personal exposure assessment and standardized sampling/analysis methods, establishing dose relationships and relevant exposure guidelines are difficult.

Exposure to bioaerosols in the occupational environment is associated with a wide range of health effects including infectious diseases, acute toxic effects, allergies, and cancer. These possibilities have been studied for the last 20 years; several cases of pulmonary cancers were reported in workers exposed to aflatoxins via respiratory route [39, 40]. In Denmark, an increase in the risk of liver cancer has been reported for workers exposed to aflatoxins in concerns processing livestock feed [41]. Larsson and coworkers [42] have also shown that asymptomatic dairy farmers exposed to airborne mold dust may have signs of immunostimulation and inflammation in their alveolar space. Farmers exposed to mold dust may exhibit signs of alveolitis [42], and severe toxic irritative reactions can occur after a single inhalation of high levels of spores [43]. Studies have suggested that inhalation exposure to mold spores is another cause of organic dust toxic syndrome [44].

Occupational biohazards of biological origin are grouped into (1) occupational diseases of the respiratory tract and skin caused by allergenic/and or toxic agents forming bioaerosols, and (2) agents causing zoonoses and other infectious diseases spread through various exposure vectors [45].

### 3.2.1. Allergenic and/or toxic agents

A wide range of impacts may lead to different types of allergies. Substances such as microbial enzymes for food processing (e.g.,  $\alpha$ -amylase in commercial bakeries) and detergent are potent allergens that can cause asthma and rhinitis [24]. Many fungal species detected in bioaerosols in the food industry, for example, from the genera *Penicillium*, *Aspergillus*, and *Cladosporium* [46, 47], are responsible for respiratory disease and allergies in other environments [48]. Fungi produce copious amounts of spores that are easily dispersed in polluted air and dust [21]. The genera *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Fusarium* are more prone to cause sensitivity. Fungal allergy often appears as type I immediate, IgE-mediated hypersensitivity. In the case of allergic reaction, it can manifest as rhinitis or conjunctivitis, asthma, urticaria, or atopic dermatitis. This is called a type II hypersensitivity reaction as is the case in response to the mannan-polysaccharide of the cell wall of *Candida* and *Aspergillus*. An example of type III hypersensitivity is allergic alveolitis and bronchopulmonary aspergillosis [21]. Allergy to *Aspergillus fumigatus* is common in atopic asthma. In a large part of the population, allergies occur in the form of rhinitis, also accompanied by ocular signs [21]. It is estimated that approximately 2–6% of the general population in developed countries is allergic to fungi.

### 3.2.2. Infection

Recently, infectious diseases are being considered the most frequently occurring occupational diseases. Occupational biohazards are infectious agents or hazardous biological materials

that exert harmful effects on workers' health, either directly through infection or indirectly through damage to the working environment, and it can also include medical waste or samples of a microbe, virus, or toxin from a biological source [45]. Most of the agents responsible for respiratory infections are spread through the air, primarily from person to person (anthroponoses), from living (zoonoses), the abiotic environment (e.g., soil and water), and decaying plant or animal matter (saproponoses) [24]. Inhalation is the most important and efficient route by which infectious agents enter the human body, and infections contracted by this route are the most difficult to control. Transmission by air allows an infectious agent to reach a larger number of potential hosts than would be possible if infected individuals had to come into direct contact to transfer microbes from person to person [24].

#### 4. Legislation

Insufficient occupational exposure limits (OELs) set by regulatory organizations and the diversity of agents in occupational environments often complicate proper risk assessment of exposure to bioaerosols. Regulatory OELs have been adopted for cotton, grain, wood, flour, organic dust, and subtilisins (**Table 2**) [49, 50]. However, these limits are based on dust levels only and do not take specific components present in the dust into consideration. With the exception of subtilisin, even the OEL for "particulates not otherwise regulated" serves as reference where OELs are not specified [49]. Furthermore, the scientific evidence for certain set of exposure limits, such as  $\approx 100$  cells  $\text{m}^{-3}$  allowed for fungal and actinomycetes, can be difficult to access [51, 52]. In some cases, the risk of infectious agents and guidance on health surveillance and containment levels are provided [53], but no limits are specified for either infectious or noninfectious biological agents.

Specific OELs are required to protect workers' health. However, bioaerosol research has thus far only resulted in proposed exposure limits for endotoxins and fungal spores. A criteria document based on inflammatory respiratory effects [51] proposed a lowest observed effect level (LOEL) of  $10^4$  spores  $\text{m}^{-3}$  for nonpathogenic and nonmycotoxin-producing fungal species. Several organizations have also proposed guidelines for fungi in indoor environments, but the criteria were developed for assessing indoor mold problems and are not health based [54, 55]. For other agents, risk assessment may be based on exposure–response associations

Agent	ACGIH	Norway
Raw cotton dust	0.2 mg $\text{m}^{-3}$	0.2 mg $\text{m}^{-3}$
Grain dust (oat, wheat, barley)	4 mg $\text{m}^{-3}$	None
Flour dust	0.5 mg $\text{m}^{-3}$	3 mg $\text{m}^{-3}$
Wood dust	0.5 mg $\text{m}^{-3}$	1–2 mg $\text{m}^{-3}$
Organic dust	None	5 mg $\text{m}^{-3}$
Particulates not otherwise regulated	10 mg $\text{m}^{-3}$	10 mg $\text{m}^{-3}$

**Table 2.** Regulatory occupational exposure limits (OELs) for cotton, grain, wood, flour, organic dust and subtilisin.

found in relevant epidemiological studies, e.g.,  $\beta$  (1→3)-glucans and allergens, but lack of standardization of measurement methods represents a great challenge [56, 57].

There are no uniform international standards available on levels and acceptable maximum bioaerosol loads (**Table 3**) [22]. The American Conference of Governmental Industrial Hygienists (ACGIH) stated that “a general threshold limit value (TLV) for culturable or countable bioaerosol concentrations is not scientifically supported” based on the lack of data describing exposure-response relationships [71]. New revised ACGIH will be released early 2017. Furthermore, no uniform standardized method is available for the collection and the analysis of bacterial and fungal bioaerosols, which makes the establishment of exposure limits challenging. Still, neither air sampling techniques nor identification and cultivation methods have been internationally standardized, impeding, therefore, the prospect of data comparison.

Country	Number of culturable organisms as CFU m <sup>-3</sup>			References
	Bacteria	Yeast	Total Bioaerosols	
Brazil		750		[58, 59]
Canada		150		[60]
China	2500–7000 (location dependent)			[61]
Finland	4500			[62]
Germany	10,000	10,000		[63, 64]
Korea			800	[65]
Portugal		500		[66]
Netherlands	10,000		10,000	[67]
Russia		2000–10,000 (species dependent)		[68]
Switzerland	10,000 (aerobic mesophilic) 1000 (Gram-negative)			[69, 70]
USA		1000		[71, 72]
European Union	10,000 (private home) 2000 (nonindustrial indoor location)	10,000 (private home) 2000 (nonindustrial indoor location)		[73]

**Table 3.** Acceptable maximum bioaerosol loads allowed for indoor air quality in different countries.

## 5. Bioaerosol detection: approaches and complications

Bioaerosol monitoring is a rapidly emerging area of industrial hygiene [74]. Measurements include especially microbes in both indoor (e.g., industrial, office, or residential) and outdoor (e.g., agricultural and general air quality) environments [7]. It is necessary to evaluate

their presence quantitatively (by a count or a determination) and/or qualitatively (by identifying the genus and species) [31]. Each bioaerosol sample is unique, as its composition varies in time and space (abundance and diversity of species, quantity of inflammatory components such as endotoxins and  $\beta$ -D-glucans). This often leads not only to high variation between samples from the same workplace, which can be due to external factors, but also to the dynamic evolution of the colonized substrate and fast multiplication rate of microbes [11].

### 5.1. Available sampling methods

A wide variety of bioaerosol sampling equipment are available, and no standardized protocols have yet been established. There are two primary methods for microbial air sampling, namely passive and active monitoring. Passive monitoring, also referred to as settle plates or petri plates, requires petri dishes containing agar or Petrifilm™ that are opened and exposed to the air for specified periods of time. Microbes that settle out of the ambient air can then be determined qualitatively. The passive approach offers lengthy sampling periods at low cost but does not take into account air movement or airborne populations per volume of air and may miss critical microbes [75]. Active monitoring requires a microbial air sampler to force air onto or into collection media at a specific rate over a specified time period. This approach is less time consuming and better for areas with low microbial loads and allows for both quantitative and qualitative analyses. However, vigorous air movement may cause injury to vegetative cells [76]. Three approaches can be used for active monitoring: impaction, impingement, and filtration.

Impaction involves the use of an air pump to capture air over the surface of a petri dish containing agar. The airflow over the agar is controlled by slits or holes that are arranged to distribute the airflow evenly over the agar surface. Sampling equipment is easy to use, and the consumable costs are relatively low. Different sampler options are summarized in **Table 4**. Drawbacks may include loss of microbial cells viability due to impact stress and loss of recovery efficiency due to the failure of microbes to adhere to agar surfaces. Competition for growth and the influence of selective media choices should also be considered when planning a monitoring strategy [92]. Impaction is often the preferred active monitoring approach for bioaerosol sampling in the food processing environment.

Impingement of microbes in a liquid matrix requires particulate laden air to accelerate as it is drawn through the cassettes tapered inlet slit and directed toward a small slide containing the collection media, where the particles become impacted, and the airflow continues out the exit orifice. With this approach, it is possible to measure both the culturable and the nonculturable components of bioaerosols and is ideally suitable for aeromicrobiology studies because the liquid matrix can be divided for various analyses. Sampler options are listed in **Table 5**. Collection vials are often constructed from glass and can be easily damaged or broken. This approach tends to be expensive and may also present low capture rates, loss of collection fluid to evaporation and violent bubbling, low capture rate of virus-sized particles, and loss of cell viability [101].

Sampler	Information	Difficulty to use	Flow rate	References
Single-Stage Viable Andersen Cascade Impactor	<ul style="list-style-type: none"> <li>• N6 microbial impactor</li> <li>• Meet the specifications of latest ACGIH Bioaerosol Committee</li> <li>• EPA, OSHA and FDA referenced</li> <li>• Sharp cutoff diameter of 0.65 <math>\mu\text{m}</math></li> </ul>	Easy to use	28.3 L $\text{min}^{-1}$	[31, 59, 77–81]
Two-Stage Viable Andersen Cascade Impactor	<ul style="list-style-type: none"> <li>• Multi orifice cascade impactor</li> <li>• Whenever size distribution is not required</li> <li>• When only respirable segregation or total counts are needed</li> <li>• 95–100% of viable particles above 0.8 <math>\mu\text{m}</math></li> </ul>	Easy to use	28.3 L $\text{min}^{-1}$	[82]
Six-Stage Viable Andersen Cascade Impactor	<ul style="list-style-type: none"> <li>• Multi-orifice cascade impactor</li> <li>• Measure the concentration and particle size distribution of aerobic bacteria and fungi Viable particles can be collected on a variety of bacteriological agar</li> <li>• Calibrated to collect all particles (physical size, shape or density)</li> <li>• Can be directly related to human lung deposition</li> </ul>	Easy to use	28.3 L $\text{min}^{-1}$	[31, 61, 83–90]
Mattson Garvin Slit-to-agar	<ul style="list-style-type: none"> <li>• Accurate and quantitative</li> <li>• Sampling even the smallest of viable particles</li> <li>• Collection on 150 mm <math>\times</math> 15 mm disposable culture plate</li> <li>• No dilution or plating steps are required</li> <li>• Results are expressed as viable particles per unit of air</li> <li>• Time-concentration relationship may be determined</li> </ul>	Self-contained	cu ft $\text{min}^{-1}$	[31]
SAS Super 180	<ul style="list-style-type: none"> <li>• Considered the international standard for portable air microbiology sampling</li> <li>• Pharmaceutical, food industry, hospital sector and indoor air quality</li> <li>• Used onboard the International Space Station</li> </ul>	Easy to use	60–100 L $\text{min}^{-1}$	[86, 91, 92]
Biotest RCS	<ul style="list-style-type: none"> <li>• Evaluate microbiological quality of ambient air, functionality of air treatment equipment and systems, effectiveness of decontamination measures</li> <li>• Collection on agar media strip</li> </ul>	Pushbutton operation Remote control	50 L $\text{min}^{-1}$	[31]

Sampler	Information	Difficulty to use	Flow rate	References
IOM Sampler	<ul style="list-style-type: none"> <li>• Reusable two-part filter cassette with specified 25-mm filters</li> <li>• Collection of inhalable airborne particles</li> <li>• Available in conductive plastic or stainless steel</li> <li>• Stainless steel model ideal for sampling vapor-phase isocyanates followed by chemical analysis</li> <li>• Sample culturable and nonculturable</li> <li>• Collection on membrane filters</li> </ul>	Difficult to use	2 L min <sup>-1</sup>	[37, 93]
SKC BioStage®	<ul style="list-style-type: none"> <li>• Single stage</li> <li>• Viable cascade impactor</li> <li>• Meets NIOSH requirements and ACGIH recommendations</li> <li>• Collection on standard-size agar plates</li> <li>• SureLock positive seal ensures sample integrity</li> </ul>	Easy to use	28.3 L min <sup>-1</sup>	[31, 90, 92, 94]
SAMPL' AIR™	<ul style="list-style-type: none"> <li>• 99% microbial collection rate</li> <li>• High efficiency, even with the smallest particles</li> <li>• Ideal for regular, thorough air quality control</li> </ul>	Easy to use	100 L min <sup>-1</sup>	[92]
MAS-100eco	<ul style="list-style-type: none"> <li>• Sieve impaction systems</li> <li>• Accurately regulates airflow in real time</li> <li>• Collection media: 90–100 mm petri dish or 55–60 mm contact plate</li> </ul>	Flexibility Remote control	100 L min <sup>-1</sup>	[95–99]
RCS	<ul style="list-style-type: none"> <li>• Rotary centrifugal air sampler</li> <li>• Lightweight and portable</li> <li>• Collection on agar strips</li> </ul>	Easy to use	40 L min <sup>-1</sup>	[34]

**Table 4.** Available impaction-based bioaerosol sampling devices.

Filtration involves pumping air through a porous membrane filter to capture bioaerosols. This method can be used to detect both culturable and nonculturable components and has proven highly efficient in trapping of microbes larger than the chosen pore size of the filter surface. It does, however, require expensive sampling equipment and sample processing, and data analysis may require a high level of expertise [102]. Available cassettes for filtration sampling of bioaerosols are listed in **Table 6**.

Sampler	Information	Difficulty to use	Flow rate	References
All-Glass (AGI-30) Impinger	<ul style="list-style-type: none"> <li>• High velocity impinger</li> <li>• Can be used in heavily contaminated environments</li> <li>• Sampling times up to 30 min (dilute impinge solution prior to use)</li> </ul>	Easy to use	12–13 L min <sup>-1</sup>	[31]
Burkard May-Impringer	<ul style="list-style-type: none"> <li>• Since 1966</li> <li>• Fractions collected gently into liquid where clumps separate into viable units</li> <li>• Little danger of sample overload</li> <li>• Subsamples permit the use of a variety of culture methods</li> <li>• Particle fractions (&gt;10 µm, 10–4 µm, &lt;4 µm)</li> </ul>	Difficult to use	20 L min <sup>-1</sup>	[31]
BioSampler®	<ul style="list-style-type: none"> <li>• Collection time up to 8 h with sonic-flow Vac-U-Go Sampler</li> <li>• Recommended for: infection control investigation in hospitals and veterinary clinics, biological research, infectious disease investigations in public buildings, and safety concerns in the food handling industry</li> </ul>	Easy to use	12.5 L min <sup>-1</sup>	[31]
Air-O-Cell® cassette	<ul style="list-style-type: none"> <li>• Use with any standard off-the-shelf area sampling pump (15 LPM open flow)</li> <li>• Unique design for the rapid collection of a wide range of airborne aerosols including mold spores, pollen, insect parts, skin cell fragments, fibers (e.g., asbestos, fiberglass, cellulose, clothing fibers, etc.) and inorganic particulate, e.g., ceramic, fly ash, copy toner and so on).</li> <li>• Collects both viable and nonviable sample specimens</li> <li>• Direct microscopic analysis can be performed immediately</li> <li>• Collection media compatible with a wide range of biological stains and refractive index oils</li> <li>• Direct quantitative analysis of organic and inorganic particulate possible</li> <li>• Suitable for use in confined or restrictive spaces</li> </ul>	Easy to use	15 L min <sup>-1</sup>	[100]
Micro-Orifice Uniform Deposition Impactors™ (MOUDI™)	<ul style="list-style-type: none"> <li>• 18 µm cut-point inlet stage</li> <li>• Additional stages to size-fractionate aerosols particles: 8-stage (0.18 µm) and 10-stage (0.056 µm)</li> </ul>	Difficult to use	30 L min <sup>-1</sup>	[101]

**Table 5.** Available impingement-based bioaerosol sampling devices.

Sampler	Information	Difficulty to use	Flow rate	References
Burkard Spore Trap (1,7-Day)	<ul style="list-style-type: none"> <li>• Particles sizes 1–10 <math>\mu\text{m}</math></li> <li>• Continuous sampling</li> <li>• Spores are impacted on adhesive coated transparent plastic tape (Melinex)</li> <li>• Sensitive to small changes in wind direction</li> </ul>	Reliable and simple operation	10 L $\text{min}^{-1}$	[31]
Button Aerosol Sampler	<ul style="list-style-type: none"> <li>• Porous curved-surface inlet</li> <li>• Particles sizes 100 <math>\mu\text{m}</math></li> </ul>	Easy to use	4 L $\text{min}^{-1}$	[31, 37]
Buck BioAire™ Model B520	<ul style="list-style-type: none"> <li>• Compact, lightweight, controlled flow sampling pump</li> <li>• Uses Allergenco-D™ or Air-O-Cell™ cassettes</li> <li>• Unattended timed programming</li> <li>• 5 h of continuous operation</li> </ul>	Easy to use	15 L $\text{min}^{-1}$	[103]
Zefon 37 mm Clear Styrene Air Sampling cassettes	<ul style="list-style-type: none"> <li>• Meet all applicable NIOSH, OSHA and EPA air sampling standards</li> </ul>	Easy to use	4 L $\text{min}^{-1}$	[37]
NIOSH Personal Bioaerosol Cyclone Sampler	<ul style="list-style-type: none"> <li>• Tube wall impaction</li> <li>• Third stage filtering</li> </ul>	Convenient Easy to use	4 L $\text{min}^{-1}$	[37, 93]

**Table 6.** Available filtration-based bioaerosol sampling devices.

## 5.2. Complications and considerations related to bioaerosol detection

It is important to emphasize that bioaerosols are ubiquitous environmental contaminants and in the majority of cases, not an integral part of the process. It would therefore be inappropriate to “sample-to-see-what-is-in-the-air” since the presence of microbes in the air can be expected. The field is dominated by lack of consistent data and an abundance of speculation [7]. The lack of standard methods, environmental guidelines, and databases complicates the interpretation and comparison of results [92]. Also, since no single method can fully characterize all bioaerosols components [7], it is imperative to do a proper evaluation/investigation before choosing a sampling method or initiating a sampling protocol. The following questions summarize important aspects to address when planning a bioaerosol monitoring approach and can be used as guidelines.

Why sample? Formulate the objectives for sampling clearly. It is important to establish whether sampling bioaerosols is necessitated by baseline monitoring for compliance or to confront an existing quality (product) and/or safety (food handler health) problem for which bioaerosols as causative agent need to be ruled out.

Where to sample? The notion of sampling before doing a critical assessment of the facility is a current shortcoming. This approach can even be misleading because it produces information

that is difficult to interpret, might create unnecessary concern, and may lead almost inevitably to the sampling having to be repeated professionally/by external consultants. Foci for the assessment should include environmental factors, factory design/layout, equipment, product type, and food handlers (health, shifts/placement, skills level, training, behavior) [76]. Certain environmental factors such as temperature, airflow, and relative humidity can be associated with bioaerosol levels [104]. Heating, air-conditioning, or ventilating systems may provoke fluctuations in temperature and relative humidity. Detectable bacterial and fungal levels can also be affected by these factors, since they require specific environmental conditions to grow and propagate. Sampling sites to consider include areas with negative air pressure, raw material area where a lot of dust is generated, under air vents, areas where water spraying or misting can occur, active floor drains and areas with higher worker activity or other movement.

Which bioaerosol component to measure? Information from the evaluation/investigation should be able to establish which bioaerosol component is of interest: viable microbial components (culture dependent) or nonviable but still bioactive (culture independent) component. Although culture-dependent methods are by far the most widely used procedures for assessing the microbiological content of bioaerosols (**Table 1**); it is now widely accepted that such methods significantly underestimate the total quantity of microbes present. Plate count media describe the well-known problem that only a small fraction (10%) of airborne microbes forms colonies on a typical culture media, thus leading to a significant underestimation of the actual viable airborne bioaerosol concentration. The vast remaining number of airborne microbes can be described as viable but nonculturable, indicating very low metabolic activity or resting dormant state. Dead airborne bacteria or fungi debris or toxins retain their allergenic or toxic properties and are therefore also relevant to any occupational health assessment.

Which air sampler to use? Impingement sampling devices (**Table 5**) can be used to detect both viable and nonviable bioaerosol components. Either viable or nonviable components can be assessed using impaction (**Table 4**) or filtration (**Table 6**), respectively. Choosing a sampling device will also depend on availability, level of expertise and funding.

How often and when to sample? In a new program for compliance monitoring, it is advisable to start with more frequent data collection as this will allow for baseline establishment. When the data are available to show that the bioaerosols in a system/area are stable enough, the number of data collection points can be reduced. Microbial results can differ depending on the activity in a specific area. Sampling times should include both “dynamic” and “static” conditions monitoring.

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